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Protecting Group Free Total Syntheses of Rubrolide R and S

M. Schacht,[a] G. J. Boehlich,[a] J. de Vries,[a] S. Bertram,[b] G. Gabriel,[b] P. Zimmermann,[c] P. Heisig[c] and N. Schützenmeister*[a]

Dedicated to Professor Dr. Dr. h.c. Lutz F. Tietze in occasion to his 75th birthday

Abstract: The two marine natural products rubrolide R **1** and S **2** were synthesised in only three linear steps starting from commercially available tetronic acid without using protecting group chemistry. Key steps in the syntheses were the Pd-catalysed Suzuki-Miyaura cross coupling followed by a vinylogous Aldol condensation. Both compounds have been tested for their antibiotic and antiviral activities. At concentration of 10 µM rubrolide R **1** and S **2** a 2-log and 1.5-log reduction in virus titre has been detected for a seasonal influenza virus (H3N2) and the pandemic swine influenza virus (pH1N1), respectively.

Introduction

Rubrolides are a marine natural product class that were first isolated from the tunicate *Ritterella rubra* in 1990.[1] Since then more than 19 different rubrolides have been isolated from marine organisms.[2-7] Rubrolides are furanone derivatives, which are substituted with two aromatic, mostly highly brominated, substituents. Their biological activities range from anti-bacterial[1,2,4,6], anti-inflammatory[3] to aldose-reductase inhibition[8] with a so far unknown mode of action. In 2014, two new antiviral rubrolides, R **1** and S **2**, have been isolated from the marine-derived fungus *Aspergillus terreus* OUCMDZ-1925, which was obtained from barracuda intestines, showing superior activity against influenza A (H1N1) compared to ribavirin. [7]

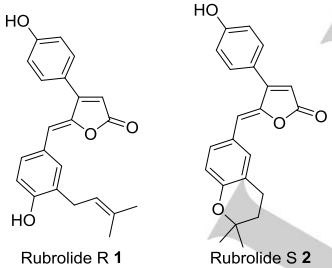

Figure 1. Rubrolide R (**1**) and S (**2**).

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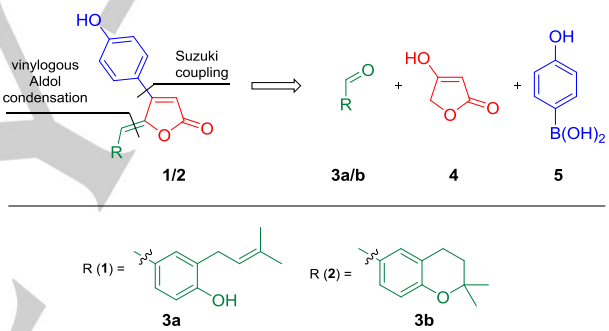
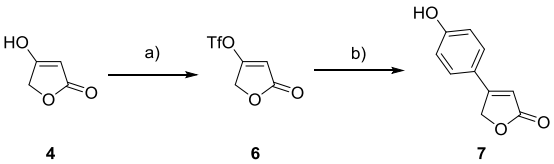
Recently, the first total syntheses of rubrolide R **1** and S **2** have been reported showing that beside the antiviral activity both rubrolides show significant inhibition of the NO production.[9] Herein, we describe the more efficient syntheses of both rubrolides R **1** and S **2** using a Suzuki-Miyaura cross coupling and a vinylogous aldol condensation as key steps starting from tetronic acid (Scheme 1). Due to the lack of protecting groups in this synthetic route[10] the total syntheses were dramatically shortened to only three linear steps starting from commercially available tetronic acid. This route is, compared to earlier total syntheses of rubrolides,[11] highly flexible and can be used to easily synthesise further rubrolides and analogues to contribute to structure activity relationship studies for the determination of the unknown molecular target.



Scheme 1. Retrosynthesis of rubrolide R **1** and S **2**.

Results and Discussion

The retrosynthetic analysis of the two rubrolides **1** and **2** shows the four required building blocks **3a**, **3b**, **4** and **5**. The benzaldehyde derivatives **3a**[12] and **3b**[13] as well as the required triflate **4**[14] can be synthesised using literature known protocols. The commercially available tetronic acid **4**[15] was transferred into triflate **6** in 90% yield (Scheme 2).



Scheme 2. Synthesis of furanone **7**: a) NEt_3 (1.2 equiv.), Tf_2O (1.2 equiv.), CH_2Cl_2, 0 °C→r.t., 2 h 90%; b) **5** (1.2 equiv.), $Pd(PPh_3)_4$ (0.5 mol%), Na_2CO_3 (3.0 equiv.), dioxane, 70 °C, 16 h, 95%.

The introduction of the first aromatic moiety can be achieved via a Pd-catalysed Suzuki-Miyaura cross coupling using commercially available boronic acid **5**. In contrast to earlier

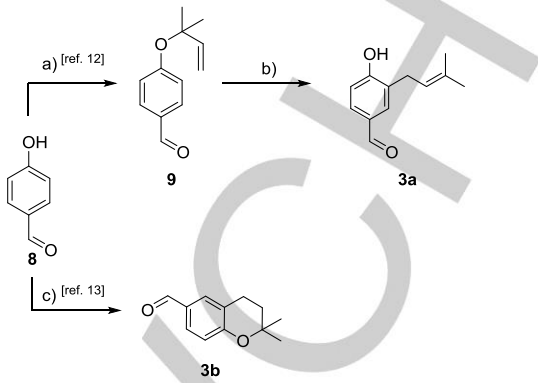
rubrolide syntheses using this strategy [16] a protecting group free boronic acid has been chosen to avoid additional deprotection steps. For the optimisation of the Suzuki-Miyaura cross coupling two different catalysts, $Pd(PPh_3)_4$ and $PdCl_2(PPh_3)_2$, have been investigated. Both catalysts yielded Suzuki-product **7** at 70 °C using Na_2CO_3 as base.

Table 1. Optimisation of the Pd-catalysed Suzuki-Miyaura cross coupling (**6** (1.0 equiv.), **5** (1.2 equiv.), Na_2CO_3 (3.0 equiv.), 70 °C); catalyst **A**: $PdCl_2(PPh_3)_2$; catalyst **B**: $Pd(PPh_3)_4$.

entry	time	catalyst	Loading (mol%)	solvent	yield
1	66 h	A	5.0	1,4-dioxane	72%
2	66 h	A	2.5	1,4-dioxane	54%
3	66 h	A	1.0	1,4-dioxane	33%
4	16 h	A	5.0	1,4-dioxane	21%
5	16 h	A	2.5	1,4-dioxane	29%
6	16 h	A	1.0	1,4-dioxane	29%
7	66 h	B	5.0	1,4-dioxane	72%
8	66 h	B	2.5	1,4-dioxane	54%
9	66 h	B	1.0	1,4-dioxane	33%
10	16 h	B	5.0	1,4-dioxane	45%
11	16 h	B	2.5	1,4-dioxane	68%
12	16 h	B	1.0	1,4-dioxane	81%
13	16 h	B	0.5	1,4-dioxane	95%
14	16 h	B	1.0	toluene	81%
15	16 h	B	1.0	2-MeTHF	69%

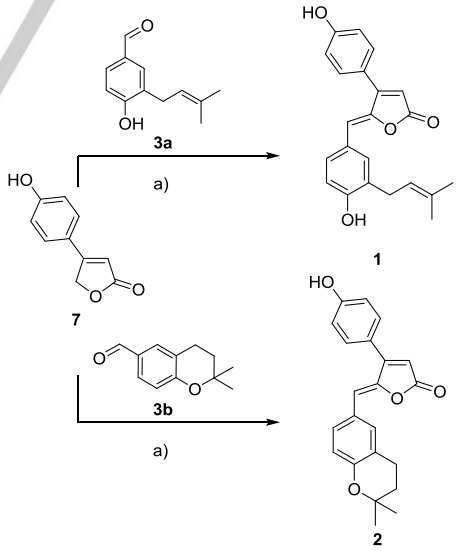
In case of the use of $PdCl_2(PPh_3)_2$ (**A**) the best yield of promising 72% (entry 1) has been achieved with relatively long stirring times of 66 h and a catalyst loading of 5.0 mol%. Reduction of the catalyst loading (entry 2 and 3) or stirring time (entry 4-6) gave lower yields. Using $Pd(PPh_3)_4$ (**B**) as Pd-source yielded after 66 h and 5.0 mol% of the catalyst the same yield of 72%. Reduction of the amount of catalyst led to decreasing yields (entry 8 and 9) as observed in case of the previously used catalyst **A**. A different trend occurred while reducing the catalyst loading at the reduced stirring time of 16 h (entry 10-13) and reached its maximum at 0.5 mol% giving the Suzuki-Miyaura product in 95% yield after purification by crystallisation. Change of the solvent to toluene (entry 14) gave similar results, while use of 2-MeTHF (entry 15) led to decreasing yields. The key intermediate **7** can be used to synthesise further rubrolide analogues without using protecting group chemistry.

The benzaldehyde derivative **3a** was synthesised following a slightly optimised procedure[12] for the Claisen rearrangement using microwave irradiation in DMF at 180 °C to give aldehyde in 88% yield. Building block **3b** was synthesised in one step from 4-hydroxybenzaldehyde **8** [13] (Scheme 3).



Scheme 3. Syntheses of building blocks **3a** and 3**b**: a) *tert*-butyl (2-methylbut-3-en-2-yl) carbonate, $Pd(PPh_3)_4$ (1.0 mol%), THF, 4 °C, 16 h, 80% (79%[12]); b) DMF, microwave, 180 °C, 45 min, 88%; c) isoprene, H_3PO_4, PE, r.t., 16 h, 52% (51.3%[13]).

As last step we employed a vinylogous aldol condensation between our key intermediate **7** and the benzaldehyde derivatives **3a** and **3b**. The condensation led to an *E*/*Z*-mixture of the products of approximately 1:10 for rubrolide R **1** and 1:4 for rubrolide S **2**. Unfortunately, it was not possible to separate the isomers by crystallisation or standard silica gel column chromatography since the *Z*-products are prone to isomerisation to the corresponding *E*-isomer after longer exposure to silica gel. Subsequent, reversed-phase column chromatography yielded rubrolide R **1** in 63% and rubrolide S **2** in 66% as pure *Z*-isomers



Scheme 4. Vinylogous aldol condensation of **1** and **2**: a) TBSOTf, DIPEA, DMF, r.t., 1 h, then **3a** or **3b** in DMF, 2 h then DBU, 120 °C, 5 h, then H_2O, DBU, r.t. 16 h, 63% (**1**) 66% (**2**)

The double bond configuration of **1** and **2** was analysed by NOESY experiments confirming the *Z*-configuration (see supporting information).
In addition, the structure of rubrolide S **2** was unambiguously determined by single crystal X-ray crystallography (Figure 2).[17]

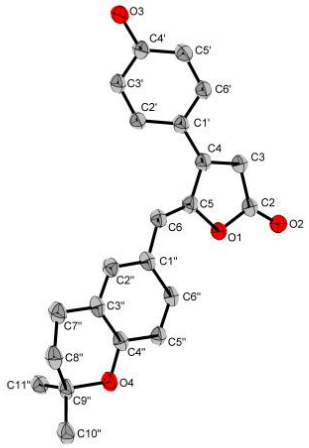


Figure 2. X-ray single crystal structure of rubrolide S **2**. Thermal ellipsoids are shown at 50% probability level. Hydrogen atoms are omitted for clarity.

Biological evaluation

Rubrolide R **1** and S **2** as pure *Z*-configurated and as *E*/*Z*-mixtures, were tested for their antibacterial and antiviral activities. We addressed whether rubrolide treatment would reduce replication of various influenza virus subtypes. Therefore, MDCK cells were infected with H3N2 or pH1N1 influenza viruses and co-treated with ribavirin or rubrolids R **1** and S **2** (supporting information).[18] The viral titer of infected and PBS (Ctr1) or DMSO (Ctr2) treated cells served as a negative control. Rubrolide R **1** and S **2** as *E*/*Z*-mixtures (rubrolide R **1**: 1:9 *E*/*Z*; rubrolide S **2**: 1:4 *E*/*Z*) showed weaker inhibition of H3N2 and no inhibition of pH1N1 virus replication compared to controls (Figure 3A and C). In contrast, the pure *Z*-isomers of rubrolide R **1** and S **2** displayed significant inhibition of H3N2 and pH1N1 virus replication in a concentration dependent manner compared to controls (Figure 3B and D). Furthermore, the pure *Z*-isomers of rubrolide R **1** and S **2** were most effective against H3N2 (Figure 3A and B) compared to pH1N1 (Figure 3C and D) virus replication. Treatment of virus and cells with ribavirin, which is a guanosine analogue and inhibits viral RNA synthesis, resulted in no significant inhibition on influenza virus replication (Figure 3). These data demonstrate that the pure *Z*-isomers of rubrolids R **1** and S **2** are able to inhibit the influenza virus replication of H3 and H1 subtypes more efficiently than the *E*/*Z*-mixtures.

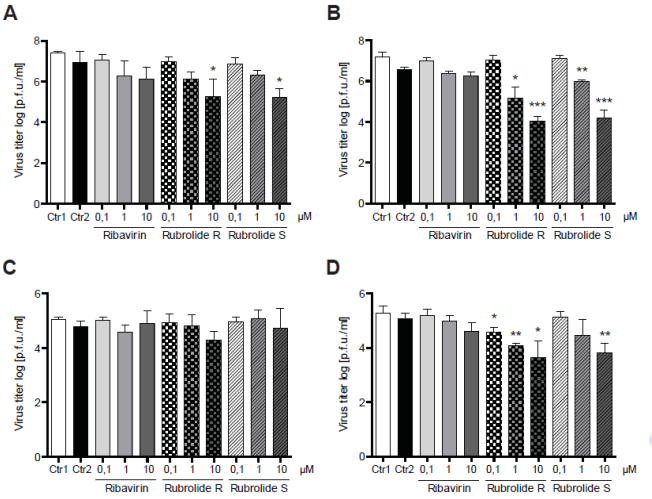


Figure 3. Antiviral effect of rubrolide R **1** and S **2** on influenza virus replication. MDCK cells were infected with either H3N2 (A and B) or pH1N1 (C and D) influenza viruses and co-treated with either an *E*/*Z*-mixture (rubrolide R **1**: 1:9 *E*/*Z*; rubrolide S **2**: 1:4 *E*/*Z*) (A and C) or the pure *Z*-isomer (B and D) of rubrolide R **1** and S **2**.

Furthermore, the pure rubrolide R **1** and S **2** were tested for their antibacterial activity against type strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus.* The preliminary test showed growth inhibition for rubrolide S **2** for the gram-negative *E. coli* and in weaker form for *P. aeruginosa*. This effect could not be confirmed by agar diffusion nor by serial dilution tests.

Conclusions

The dramatically short and protecting group free syntheses of the two most recent isolated rubrolides R **1** and S **2** are reported Over three steps, rubrolide R **1** was obtained in 54% and rubrolide S **2** in 56% total yield as single *Z*-isomers starting from commercially available tetronic acid. Both rubrolides showed inhibition of virus replication of a seasonal influenza virus (H3N2) and the pandemic swine influenza (pH1N1) from 2009.

Acknowledgements

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Keywords: total synthesis • cross coupling • antiviral compounds • natural products • aldol reaction

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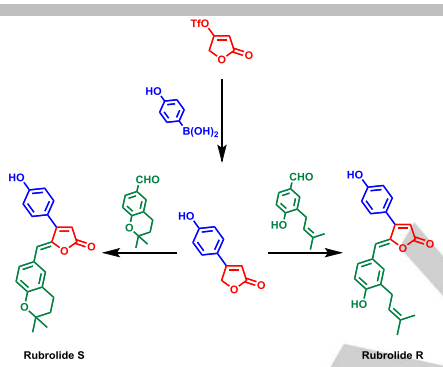
Entry for the Table of Contents

Layout 1:

COMMUNICATION

Rubrolide R and S are two new representatives of a marine natural product class showing activity against the influenza viruses H1N1 and H3N2. Both natural products have been synthesised *via* a concise, protecting group free, three step synthesis. The key steps involve a Suzuki-Miyaura cross-coupling and a vinylogous Aldol condensation.



Natural Product Synthesis

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