A Convergent Total Synthesis of the Telomerase Inhibitor (±)-γ-Rubromycin**

Michael Wilsdorf and Hans-Ulrich Reissig

Dedicated to Professor Axel Zeeck on the occasion of his 75th birthday

Abstract: *The total synthesis of the human telomerase inhibitor γ-rubromycin in its racemic form was accomplished in 3.8% overall yield. The key feature of this synthesis is an efficient acid-catalyzed spiroketalization for the construction of the spiroketal core. The required electronically well-balanced spiroketal precursor was obtained by the convergent assembly of a naphthyl-substituted aldehyde, an α-methoxy-allyl-γ-silyl-substituted phosphonate as the central C_3 building block, and a highly functionalized aryl Grignard reagent. Another key feature is the late-stage construction of the isocoumarin moiety and a simultaneous protodesilylation furnishing the known methyl aryl ether protected precursor of γ-rubromycin.*

The history of the rubromycins started with the isolation of β-rubromycin (**1**) from the mycelia of the actinomycetes strain *Streptomyces collinus* by Brockmann and Renneberg in the 1950s.[1] Shortly after, the structure of the isolated natural product was elucidated by chemical derivatizations, degradation experiments, and NMR spectroscopic studies; a first proposal suggested an *ortho*-quinoid structure.[2] However, in 2000 Zeeck and co-workers could unequivocally confirm a *para*-quinoid structure of the naphthoquinone moiety with the aid of modern NMR methods and ^{13}C-labeling experiments, thus correcting the originally proposed formula.[3] In addition to β-rubromycin, other representatives of this interesting class of natural products have been described over the years, including the structurally closely related γ-rubromycin (**2**), purpuromycin (**3**), and heliquinomycin (**4**, Figure 1).[4] Biological studies reveal, that—in addition to their role as effective antibiotics and HIV-1-RT inhibitors[1,3]—the rubromycins display potent activity against human telomerase, and their high biological activity significantly depends on the presence of the [5,6]-bisbenzannulated spiroketal moiety as a central structural motif.[5,6]

Since the discovery of the rubromycins various synthetic approaches towards [5,6]-bisbenzannulated spiroketals have been developed.[4,7] To date only the total synthesis of the racemic aglycone of heliquinomycin by Danishefsky (2001)[8]
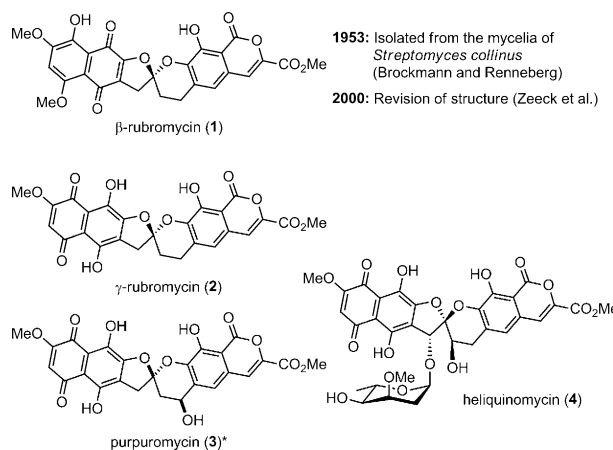
Figure 1. Prominent members of the rubromycin family of natural products. * The absolute configuration of **3** is not known.

and only two total syntheses of (±)-γ-rubromycin by Kita (2007)[9] and Pettus (2011)[10] and the total synthesis of (±)-δ-rubromycin by Li (2013)[11] are known. Notably, none of these total syntheses relies on an obvious acid-mediated ketalization[12] for the generation of the spiroketal core. The failure of this strategy can be attributed to the electronic properties of the spiroketal precursors used, which has been experimentally confirmed by Kozlowski[13] and our group.[14] Thus, the acid-catalyzed spiroketalization is almost completely inhibited in the case of isocoumarin-substituted substrates due to the decreased nucleophilicity of the phenolic oxygen atom.[4,15] The replacement of the isocoumarin fragment by a synthetic equivalent allowed Brimble (2009)[16] and Li (2012)[17] to achieve acid-induced spiroketalizations which led to formal total syntheses of (±)-γ-rubromycin.[18] We here report our route to this natural product, which afforded the target molecule in larger quantities by a convergent approach and highly efficient reaction steps.

From the outset, our concept envisaged an acid-catalyzed spiroketalization as a key step, but it should take place at a late stage of the synthetic sequence. Additionally, according to Brimble's strategy the lactonization for completing the isocoumarin fragment should occur after the spiroketalization. Owing to our experience in previous model studies[14,19] we were confident that rubromycin could be derived from spiroketal precursors such as **5** (Scheme 1). Compounds of this type are attractive due to the presence of all functional groups required for the construction of the isocoumarin fragment and the naphthoquinone oxidation level. Target

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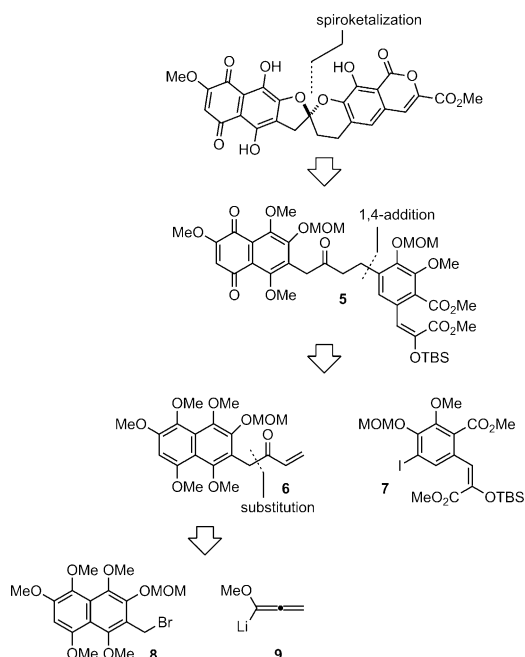
Scheme 1. Retrosynthetic analysis of (±)-γ-rubromycin based on an acid-catalyzed spiroketalization of precursor **5**.

compound **5** was traced back to the pentamethoxynaphthyl-substituted enone **6**, which serves as acceptor for a Normant cuprate, generated from aryl iodide **7**. Enone **6** should be generated by reaction of bromide **8** with lithiated methoxyallene (**9**)[20] (Scheme 1).

Our synthesis started with the preparation of pentamethoxynaphthyl-substituted alcohol **10** which was accessible from 2,4,5-trimethoxybenzaldehyde over 10 steps on a multigram scale following a procedure described by Kozlowski.[21] Unfortunately, we were not able to convert alcohol **10** into a compound with a suitable leaving group such as bromide **8**; due to the extraordinarily electron-rich naphthalene ring the electrophile to be generated is apparently too labile (Scheme 2). As a consequence, the planned strategy could not be applied to the synthesis of enone **6**, although this approach was successful for simpler benzyl-substituted model substrates (e.g. conversion **11**→**12**) employing lithiated methoxyallene **9** as an acyl anion equivalent (Scheme 2).
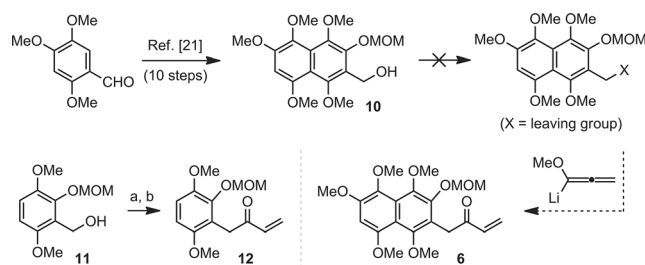
Scheme 2. Attempts to synthesize enone **6** and model reaction **11**→**12**. Reagents and conditions: a) NBS, Ph₃P, CH₂Cl₂, 0 °C, 1 h, 98 %; b) H₂C=C=C(OMe)Li, −78→0 °C, then H₂SO₄ (5 %, aq.), 86 %. NBS = N-bromosuccinimide.

To escape from this dead end we directed our attention to the α-methoxyallyl-substituted phosphonate **13**.[22] The metalation of **13** generates an ambident allyl anion which upon addition to aryl aldehydes reacts in a Horner–Wittig reaction to afford 2-methoxybuta-1,3-dienes. They should be convertible into the corresponding enones by acidic hydrolysis of their enol ether moiety. In our case, we solved the expected problem of α- versus γ-addition by silylation of the γ-position[23] with chloro(4-methoxyphenyl)dimethylsilane[24] to yield phosphonate **14**. The 4-methoxyphenyl-substituted silyl group was chosen since a smoother removal of this group by an acid-induced protodesilylation was expected at a later stage. The metalation of **14** with potassium bis(trimethylsilyl)-amide (KHMDS) and the subsequent addition to naphthyl aldehyde **15**—obtained by oxidation of alcohol **10**—afforded (Z,E)-2-methoxybuta-1,3-diene **16** in good yield. Hydrolysis of the enol ether moiety furnishing the β-silyl-substituted enone **17** was achieved under acidic conditions using trichloroacetic acid in wet dichloromethane (Scheme 3). As illustrated by the sequence **15**→**17**, the reagent **14** provides an interesting alternative to methoxyallene as a C₃ building block and should also be valuable for other synthetic endeavors.
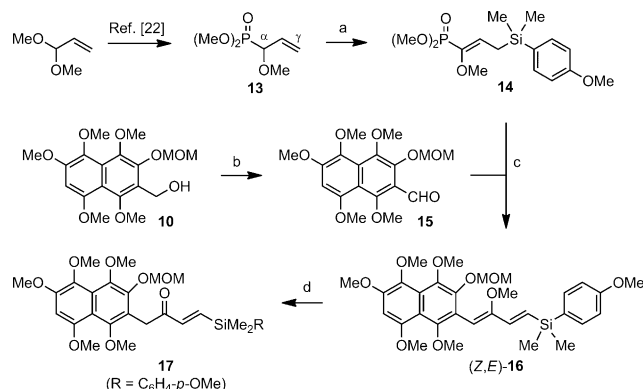
Scheme 3. Reagents and conditions: a) *n*BuLi, −78 °C, 10 min, then chloro(4-methoxyphenyl)dimethylsilane, −78 °C→RT, 64 %; b) IBX, DMF, RT, 1 h, 85 %; c) KHMDS, THF, −40 °C, 15 min, then aldehyde **15**, −78 °C→RT, 82 %; d) TCA, CH₂Cl₂/H₂O (25:1), RT, 45 min, 94 %. IBX = 2-iodoxybenzoic acid, KHMDS = potassium bis(trimethylsilyl)-amide, TCA = trichloroacetic acid.

Enone **17** represents an equivalent of the required building block **6** (Scheme 1), albeit being functionalized with a sterically demanding silyl group whose compatibility with the next steps was uncertain at this point. The second component, the highly substituted aryl iodide **7**, was readily available with standard reactions: starting from vanillin, the aldehyde **18**[25] was prepared in four known steps and subjected as methoxymethyl ether **19** to the carbanion of phosphonate **20**[26] efficiently leading to compound **7** (Scheme 4).

Grignard reagent **21** was generated following a procedure described by Knochel et al.[27] by reaction of **7** with *i*PrMgBr at −40 °C in a mixture of Et₂O/THF (4:1).[28] The quantitative halogen–metal exchange proceeded rapidly (<5 min). The
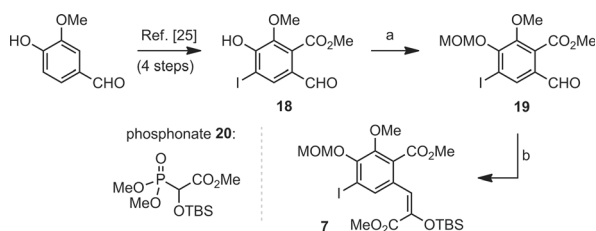
Scheme 4. Reagents and conditions: a) MOMCl, *i*Pr₂NEt, DMF, RT, 1 h, 83%; b) 1.2 equiv **20**, LiHMDS, −78°C, 5 min; then addition of **19**, −78°C→RT, 90%. MOMCl = chloromethyl methyl ether, LiHMDS = lithium bis(trimethylsilyl)amide.

Cu^{I}-catalyzed 1,4-addition[29] of **21** to β-silylated enone **17** furnished the arylated product **22** after acidic hydrolysis of the intermediate silyl enol ether in excellent yield. Remarkably, the highly functionalized aryl iodide **7** proved to be fully compatible with the reaction conditions for the halogen–metal exchange. The generated Grignard reagent displays sufficient stability which is probably due to the complexation of the metal by the methoxymethyl ether group in *ortho*-position. The subsequent oxidation of the pentamethoxy-substituted naphthyl fragment of **22** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone occurred regioselectively affording γ-naphthoquinone **23**. With this substrate, we have prepared a spiroketal precursor that is stable towards oxidation and, in contrast to the originally envisaged target **5**, bears a silyl substituent in β-position (Scheme 5).
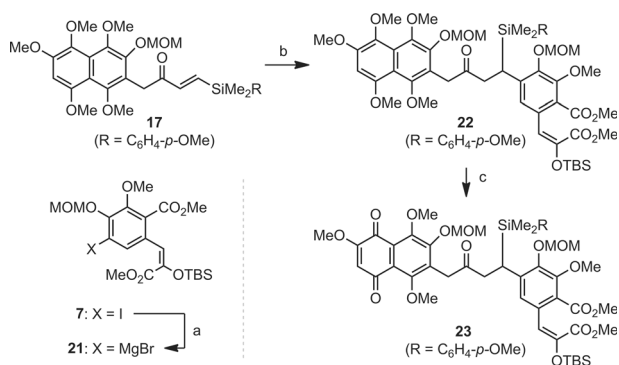
Scheme 5. Reagents and conditions: a) *i*PrMgBr, Et₂O/THF (4:1), −40°C, 5 min; b) 1.4 equiv **21**, 0.15 equiv CuI·2 LiCl (0.20 M in THF), 6.0 equiv TMSCl, Et₂O/HMPA (10:1), −40°C→RT, 30 min; then H₂SO₄ (5%, aq.), THF, RT, 20 min (85%, over 2 steps); c) DDQ, MeCN/H₂O (4:1), 0°C, 20 min, 91%. HMPA = hexamethylphosphoric acid tri-amide, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

The synthesis should now be continued with the pivotal spiroketalization of **23** to compound **24**. In model studies we had already discovered that catalytic amounts of trifluoro-methanesulfonic acid in MeCN efficiently promote the smooth spiroketalization of MOM-protected substrates.[30,31] Ketone **23** was converted into highly functionalized spiroketal **24** in very good yield and with high chemo- and diastereo-selectivity (Scheme 6). The cleavage of the MOM ether was chemoselective while the potentially acid-labile TBS enol ether remained untouched under these conditions (0.2 equiv-
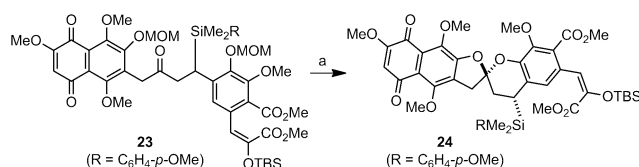
Scheme 6. Reagents and conditions: a) 0.2 equiv TfOH, MeCN, −25°C→RT, 90 min, 80%, d.r. > 97:3. TfOH = trifluoromethanesulfonic acid.

alents TfOH, MeCN, −25°C→RT). The latter was an important prerequisite for the success of this transformation. Remarkably the conversion of **23** to spiroketal **24** occurred with high diastereoselectivity (d.r. > 97:3) and could reliably be conducted even on a 1.0 g scale. Moreover, this is the first example in which the spiroketalization of a rubromycin derivative was successfully achieved at the naphthoquinone oxidation level (Scheme 6).

The final lactonization to the isocoumarin was carried out under acidic reaction conditions:[32] the treatment of **24** with fluoroboric acid etherate (ca. 10 equivalents of HBF₄·Et₂O) in CH₂Cl₂ at 50°C resulted in immediate hydrolysis of the TBS enol ether, followed by formation of the isocoumarin. This process was accompanied by complete dearylation of the silyl substituent (Scheme 7).[33] The treatment of the intermediate fluorosilane **26** with peracids (e.g. with *m*-CPBA), however,
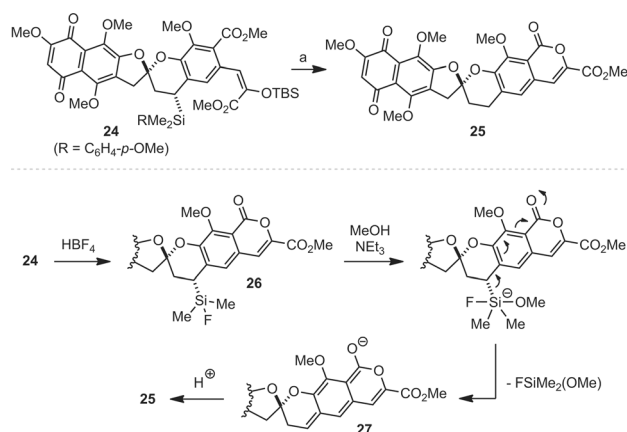

Scheme 7. Lactonization and protodesilylation of compound **24** to γ-rubromycin precursor **25**: Reagents and conditions: a) 10.0 equiv HBF₄·Et₂O, CH₂Cl₂, 50°C, 15 min; then 0°C, MeOH/NEt₃, 0°C→RT, 15 min, 66%.

did not lead to a Tamao–Fleming-type oxidation, but resulted in the rapid protodesilylation to γ-rubromycin precursor **25**. This unexpected behavior may be explained by the electron-withdrawing effect of the isocoumarin fragment facilitating the desilylation by formation of the stabilized enolate **27**.[34] The yield of the transformation of the spiroketal **24** to γ-rubromycin precursor **25** could be significantly increased by employing a one-pot procedure: after acid-induced dearyl-ation, isolation of compound **26** was omitted and the mixture was worked-up under basic conditions (MeOH/Et₃N) result-

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ing in a methoxide-induced protodesilylation. After column chromatography the desired spiroketal **25** was obtained as yellow solid in 66% overall yield and with high purity.

The synthesis of (±)-γ-rubromycin (**2**) was completed by selective O-demethylation of the methylaryl ether moieties of **25** by treatment with an excess of boron tribromide. Hence, the natural product was obtained in several experiments in 40–50% yield (purity approximately 90%)[35] in its racemic form as an intensively red solid (Scheme 8). The ^{1}H NMR spectroscopic data of (±)-(**2**) match perfectly with the data published by Kita,[9] Pettus,[10] and Zeeck.[3] In addition, the acquired ^{13}C NMR data of our synthetically prepared sample are in full agreement with the published data.[3]
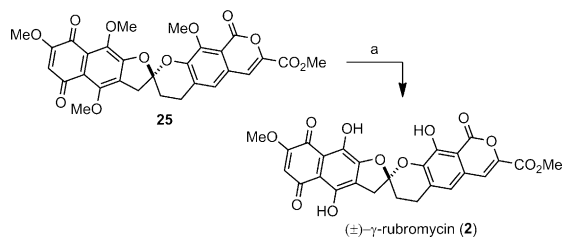
Scheme 8. Demethylation of precursor **25** to (±)-γ-rubromycin (**2**). Reagents and conditions: a) 6.0 equiv BBr$_3$, CH$_2$Cl$_2$, −78→0°C, 90 min, 40–50%.

With this route to (±)-γ-rubromycin (**2**) we have described a new total synthesis of this natural product. The synthesis is convergent and very efficient in its single steps and makes **2** accessible in 18 steps (longest linear sequence) with an overall yield of 3.8%. The key steps of this synthesis are: the chemoselective 1,4-addition of highly functionalized Grignard reagent **21**, the efficient ketalization of intermediate **23** to spiroketal **24**, and the subsequent acid-induced proto-desilylation with concurrent isocoumarin formation to give γ-rubromycin precursor **25**. The developed protocols are very robust and also feasible on a larger scale and should be suitable for the synthesis of analogues of rubromycin. In addition, our strategy should allow an asymmetric synthesis of γ-rubromycin, since enantioselective 1,4-additions of functionalized aryl Grignard reagents to β-silylated enone **17** are known.[36]

[1] a) H. Brockmann, K. H. Renneberg, *Naturwissenschaften* **1953**, *40*, 59–60; b) H. Brockmann, K. H. Renneberg, *Naturwissenschaften* **1953**, *40*, 166–167.

[2] a) H. Brockmann, W. Lenk, G. Schwantje, A. Zeeck, *Tetrahedron Lett.* **1966**, *7*, 3525–3530; b) H. Brockmann, W. Lenk, G. Schwantje, A. Zeeck, *Chem. Ber.* **1969**, *102*, 126–151; c) H. Brockmann, A. Zeeck, *Chem. Ber.* **1970**, *103*, 1709–1726.

[3] C. Puder, S. Loya, A. Hizi, A. Zeeck, *Eur. J. Org. Chem.* **2000**, 729–735.

[4] Review article: M. Brasholz, S. Sörgel, C. Azap, H.-U. Reissig, *Eur. J. Org. Chem.* **2007**, 3801–3814.

[5] T. Ueno, H. Takahashi, M. Oda, M. Mizunuma, A. Yokoyama, Y. Goto, Y. Mizushina, K. Sakaguchi, H. Hayashi, *Biochemistry* **2000**, *39*, 5995–6002.

[6] a) M. Chino, K. Nishikawa, A. Yamada, M. Ohsono, T. Sawa, F. Hanaoka, M. Ishizuka, T. Takeuchi, *J. Antibiot.* **1998**, *51*, 480–486; b) Y. Ishimi, T. Sugiyama, R. Nakaya, M. Kanamori, T. Kohno, M. Enomoto, M. Chino, *FEBS J.* **2009**, *276*, 3382–3391; c) T. Sugiyama, M. Chino, T. Tsurimoto, N. Nozaki, Y. Ishimi, *J. Biochem.* **2012**, *151*, 129–137.

[7] Reviews: a) J. Sperry, Z. E. Wilson, D. C. K. Rathwell, M. A. Brimble, *Nat. Prod. Rep.* **2010**, *27*, 1117–1137; b) M. C. McLeod, D. C. K. Rathwell, Z. E. Wilson, T.-Y. Yuen, M. A. Brimble, *Pure Appl. Chem.* **2012**, *84*, 1379–1390; c) J. C. Green, G. L. Burnett IV, T. R. R. Pettus, *Pure Appl. Chem.* **2012**, *84*, 1621–1631.

[8] a) D. Qin, R. X. Ren, T. Siu, C. Zheng, S. J. Danishefsky, *Angew. Chem.* **2001**, *113*, 4845–4849; *Angew. Chem. Int. Ed.* **2001**, *40*, 4709–4713; b) T. Siu, D. Qin, S. J. Danishefsky, *Angew. Chem.* **2001**, *113*, 4849–4852; *Angew. Chem. Int. Ed.* **2001**, *40*, 4713–4716.

[9] S. Akai, K. Kakiguchi, Y. Nakamura, I. Kuriwaki, T. Dohi, S. Harada, O. Kubo, N. Morita, Y. Kita, *Angew. Chem.* **2007**, *119*, 7602–7605; *Angew. Chem. Int. Ed.* **2007**, *46*, 7458–7461.

[10] K.-L. Wu, E. V. Mercado, T. R. R. Pettus, *J. Am. Chem. Soc.* **2011**, *133*, 6114–6117.

[11] W. Wang, J. Xue, T. Tian, J. Zhang, L. Wei, J. Shao, Z. Xie, Y. Li, *Org. Lett.* **2013**, *15*, 2402–2405.

[12] a) F. Perron, K. F. Albizati, *Chem. Rev.* **1989**, *89*, 1617–1661; b) J. E. Aho, P. M. Pihko, T. K. Rissa, *Chem. Rev.* **2005**, *105*, 4406–4440.

[13] S. P. Waters, M. W. Fennie, M. C. Kozlowski, *Org. Lett.* **2006**, *8*, 3243–3246.

[14] C. Venkatesh, H.-U. Reissig, *Synthesis* **2008**, 3605–3614.

[15] So far, only two examples of an acid-catalyzed spiroketalization with a complete isocoumarin fragment are known. In our studies towards the total synthesis of rubromycins the synthesis of dimethylated (±)-3-hydroxy-β-rubromycin could only be achieved in very low yield (7%) for the spiroketalization step: S. Sörgel, *Dissertation*, Freie Universität Berlin, **2006**; also see Ref. [4]. Moreover, Kozlowski et al. reported a spiroketalization of an isocoumarin-substituted substrate under harsh conditions. However, the envisaged total synthesis of purpuromycin could not be achieved: A. N. Lowell, M. W. Fennie, M. C. Kozlowski, *J. Org. Chem.* **2011**, *76*, 6488–6502.

[16] D. C. K. Rathwell, S.-H. Yang, K. Y. Tsang, M. A. Brimble, *Angew. Chem.* **2009**, *121*, 8140–8144; *Angew. Chem. Int. Ed.* **2009**, *48*, 7996–8000.

[17] L. Wei, J. Xue, H. Liu, W. Wang, Y. Li, *Org. Lett.* **2012**, *14*, 5302–5305.

[18] Both formal total syntheses led to an intermediate of Kita's synthesis of (±)-γ-rubromycins, see Ref. [9].

[19] S. Sörgel, C. Azap, H.-U. Reissig, *Org. Lett.* **2006**, *8*, 4875–4878.

[20] Recent review: R. Zimmer, H.-U. Reissig, *Chem. Soc. Rev.* **2014**, in press; DOI: 10.1039/C3CS60429B.

[21] A. N. Lowell, M. W. Fennie, M. C. Kozlowski, *J. Org. Chem.* **2008**, *73*, 1911–1918.

[22] K. Fettes, L. McQuire, A. W. Murray, *J. Chem. Soc. Perkin Trans. 1* **1995**, 2123–2127.

[23] This approach was elaborated with phosphine oxides by Murray: M. D. Ironside, A. W. Murray, *Tetrahedron Lett.* **1989**, *30*, 1691–1694.

[24] A. Deally, F. Hackenberg, G. Lally, H. Müller-Bunz, M. Tacke, *Organometallics* **2012**, *31*, 5782–5790.

[25] M. Brasholz, X. Luan, H.-U. Reissig, *Synthesis* **2005**, 3571–3580.

[26] a) D. Horne, J. Gaudino, W. J. Thompson, *Tetrahedron Lett.* **1984**, *25*, 3529–3532; b) H. Itoh, T. Kaneko, K. Tanami, K. Yoda, *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3356–3358.

[27] L. Boymond, M. Rottländer, G. Cahiez, P. Knochel, *Angew. Chem.* **1998**, *110*, 1801–1803; *Angew. Chem. Int. Ed.* **1998**, *37*, 1701–1703.

[28] The 1,4-addition of aryl Grignard reagent **21** to benzyl-substituted enone **12** (Scheme 2) proceeded smoothly in THF as solvent. However, for the successful conversion of **21** with naphthyl-substituted enone **17** a change to a mixture of Et$_2$O/THF as solvent was mandatory as in pure THF no 1,4-addition occurred.

[29] a) G. Varchi, A. Ricci, G. Cahiez, P. Knochel, *Tetrahedron* **2000**, *56*, 2727–2731; b) M. T. Reetz, A. Kindler, *J. Organomet. Chem.* **1995**, *502*, C5–C7.

[30] The cleavage of MOM ethers with TMSOTf and TESOTf is known; however, these reactions were carried out in the presence of an excess of base (pyridine or bipyridine): H. Fujioka, Y. Minamitsuji, O. Kubo, K. Senami, T. Maegawa, *Tetrahedron* **2011**, *67*, 2949–2960.

[31] M. Wilsdorf, D. Leichnitz, H.-U. Reissig, *Org. Lett.* **2013**, *15*, 2494–2497.

[32] The use of TBAF surprisingly led to a fragmentation of the TBS enol ether and the formation of aryl aldehydes: M. Wilsdorf, *Dissertation*, Freie Universität Berlin, **2013**.

[33] In the case of a phenyldimethylsilyl substituent no fluorodephenylation of the silyl substituent was observed: M. Wilsdorf, *Dissertation*, Freie Universität Berlin, **2013**.

[34] The oxidation of the silyl group would afford a potential precursor of purpuromycin; however, to date all attempts have been unsuccessful.

[35] We have observed that the natural product slowly decomposes into unknown products at room temperature.

[36] For the enantioselective 1,4-addition of Grignard reagents, see: a) review: T. Jerphagnon, M. G. Pizzuti, A. J. Minnaard, B. L. Feringa, *Chem. Soc. Rev.* **2009**, *38*, 1039–1075; b) Addition to β-silyl-α,β-unsaturated ketones: M. A. Kacprzynski, S. A. Kazane, T. L. May, A. H. Hoveyda, *Org. Lett.* **2007**, *9*, 3187–3190.

Communications

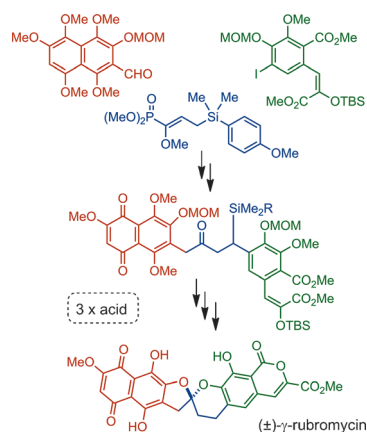## Natural Product Synthesis

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H.-U. Reissig* —————— ▪▪▪▪–▪▪▪▪

A Convergent Total Synthesis of the
Telomerase Inhibitor (±)-γ-Rubromycin

(±)-γ-rubromycin

3 building blocks + 3 acids→natural product: After the assembly of the crucial precursor from three highly functionalized building blocks, the pivotal spiroketalization was achieved with trifluoromethanesulfonic acid, the isocoumarin formation with fluoroboric acid, and the O-demethylation with the Lewis acid boron tribromide. (±)-γ-Rubromycin was thus synthesized in 18 steps with an overall yield of 3.8% from commercially available precursors.