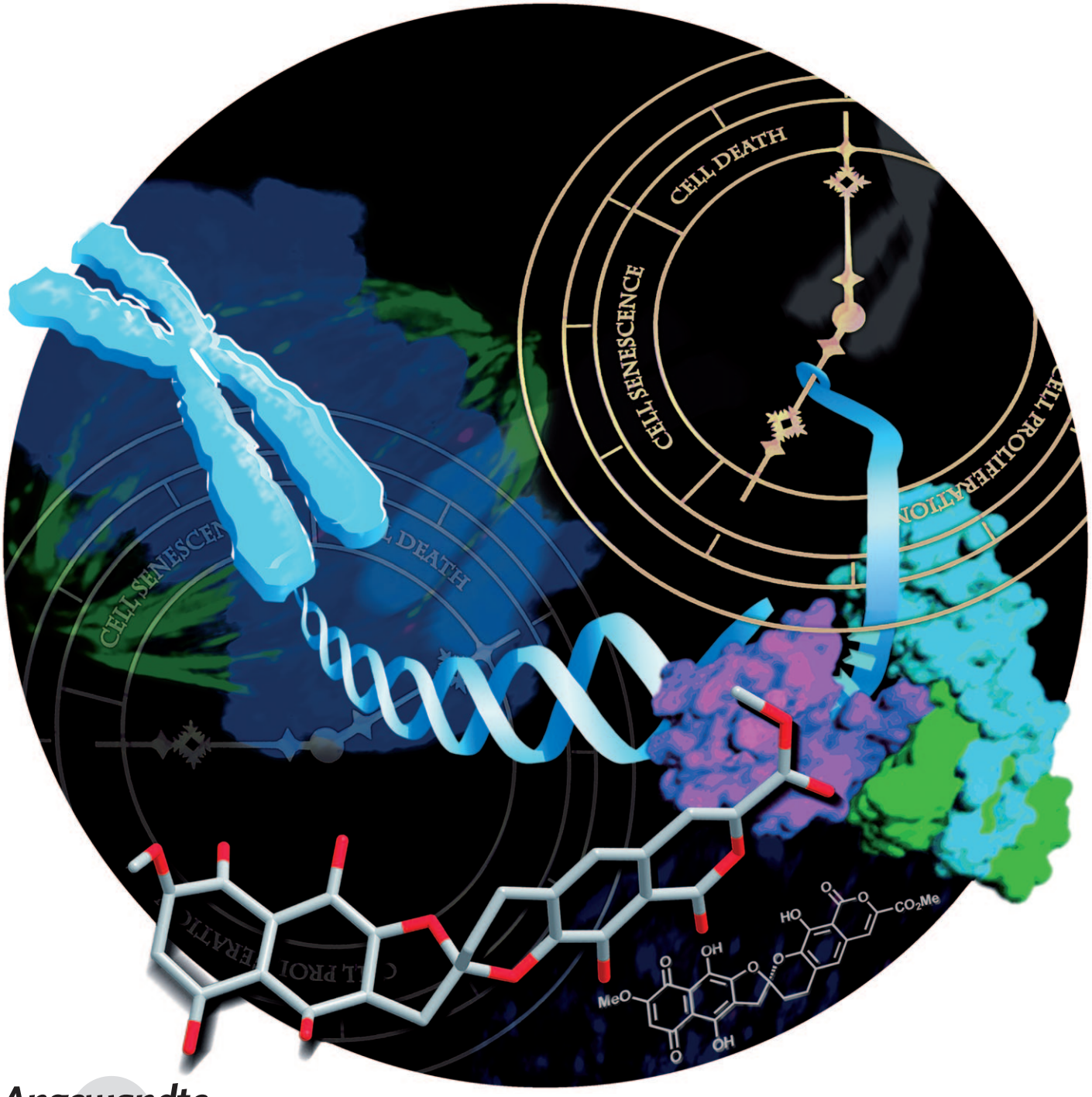
VIP Natural Product Synthesis

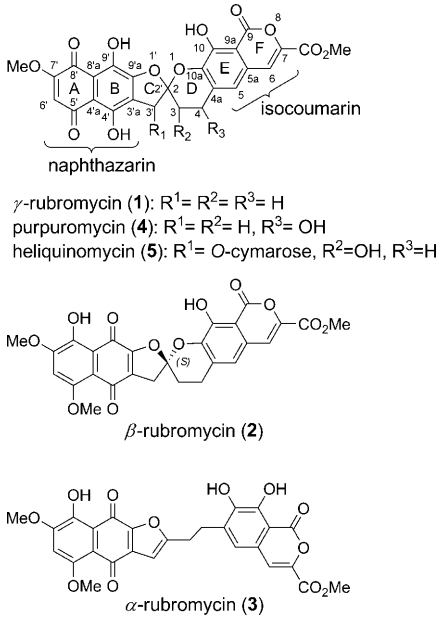
DOI: 10.1002/anie.200903316

# An Efficient Formal Synthesis of the Human Telomerase Inhibitor (±)-γ-Rubromycin**

*Dominea C. K. Rathwell, Sung-Hyun Yang, Kit Y. Tsang, and Margaret A. Brimble**



γ-Rubromycin[1] (**1**, Scheme 1), the prototypical member of a structurally related family of antibiotics known as the rubromycins, consists of a densely oxygenated naphthazarin ring and an isocoumarin moiety linked through a unique aromatic 5,6-spiroketal ring system (Scheme 1). The rubromycins exhibit a wide range of biological activity including




***Scheme 1.*** Selected natural products of the rubromycin class of antibiotics.

antimicrobial and anticancer properties.[1–6] β-Rubromycin (**2**) and γ-rubromycin display potent activity against human telomerase ($IC_{50} = 3\ \mu M$), the reverse transcriptase of HIV-1, and the moloney murine leukemia virus.[4–7] α-Rubromycin (**3**), the only member of the group to lack the aryl spiroketal moiety, exhibits substantially decreased inhibitory potency towards telomerase ($IC_{50} > 200\ \mu M$), which suggests that the spiroketal core is an essential pharmacophore for the inhibition of telomerase.[4] Purpuromycin (**4**),[8] a potential topical agent for vaginal infections,[2] and heliquinomycin (**5**), a selective inhibitor of DNA helicase are structurally related to the rubromycins.[6] Thus, 56 years since the isolation of **1**, the rubromycins still attract considerable synthetic efforts because of their unprecedented structures and intriguing pharmacological profile.[5] Numerous syntheses of the naphthalene[9–11] and isocoumarin[12] fragments together with model doubly benzannelated[13–16] and naphthyl-benzannelated[11,15] spiroketal systems have been reported. However, only two successful total syntheses have been achieved to date, namely, the aglycon of (±)-**5** by Danishefsky and co-workers,[17] in which spiroketalization of a hemiketal occurred under Mitsunobu conditions, and (±)-**1** by Kita et al.[18] who used a double aromatic Pummerer-type reaction to install the spiroketal.

In spite of these investigations, an efficient and flexible strategy for the total synthesis of these natural products has not been forthcoming; the most striking problem in the synthesis is the acid-mediated spiroketalization to construct the unique 5,6-bisbenzannelated spiroketal core. Difficulties in the application of this seemingly straightforward approach to the natural product have been reported by the research groups of Danishefsky,[17] Kozlowski,[11] and Reißig[5,16] in their respective synthetic efforts towards **5** and purpuromycin **4**. Through comprehensive modeling studies, Kozlowski, Reißig, and their respective co-workers concluded that the presence of electron-withdrawing groups in the isocoumarin unit markedly diminish the nucleophilicity of the phenolic hydroxyl group, a key factor that prohibits the critical spiroketalization step.[5,11,16]

In previous studies, our research group has demonstrated that steric hindrance associated with the presence of methoxy groups at C-9′ and C-10 (bis-*ortho* substituents) hamper the spirocyclization step to result in the undesired formation of the thermodynamically favored benzofuran.[19] We have also reported a mild, efficient deprotection and spiroketalization protocol that leads to the successful formation of the doubly benzannulated spiroketal.[19] Inspired by Reißig and co-workers,[5] who noted that with delicate electronic balancing of aromatic units the acid-promoted spiroketalization should be possible, we were convinced that use of a mild acid mediated spiroketalization step that pays due attention to the use of an appropriate isocoumarin ring precursor, should provide a practical method for the synthesis of the rubromycins and related analogues.

Herein, we report an efficient synthesis of Kita's pentacyclic spiroketal **6**[18] in which isocoumarin ring formation is delayed until after the key spiroketalization. This approach thus constitutes a formal synthesis of (±)-γ-rubromycin **1** (Scheme 2). Elimination of the electron-withdrawing mesomeric effect on the eastern fragment of the advanced spiroketal precursor **7** was expected to increase the nucleophilicity of the phenol, thus encouraging the key acid-mediated cyclization to form the D ring (see the insert in Scheme 2). Alkynol **8** was synthesized by the Sonogashira acetylide coupling strategy[14] that we developed to construct simpler aryl spiroketals, and was envisaged to be a flexible building block from which several members of the rubromycin family of antibiotics could be accessed.

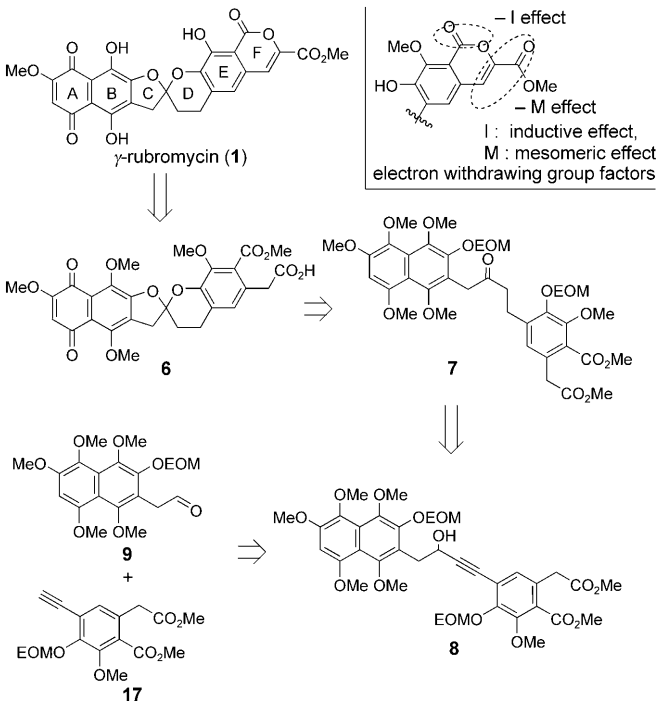
Our initial attention focused on the development of an efficient route to the naphthazarin fragment **9** that has already challenged several research groups.[5,9–11] In the process, a novel allyloxylation/Claisen rearrangement strategy that facilitated regioselective *ipso* and *ortho* functionalization of 1,4-naphthoquinones was developed.

[*] D. C. K. Rathwell, S.-H. Yang, K. Y. Tsang, Prof. Dr. M. A. Brimble
Department of Chemistry, The University of Auckland
23 Symonds Street, Auckland (New Zealand)
Fax: (+64) 9-373-7422
http://www.che.auckland.ac.nz/staffsites/BrimbleM/
E-mail: m.brimble@auckland.ac.nz

[**] We thank the New Zealand Foundation for Research, Science and Technology for the award of a Top Achiever Doctoral Scholarship to K.Y.T. and D.C.K.R. We are also grateful to Profs. Y. Kita and Fujioka for providing an authentic sample of **6**.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200903316.

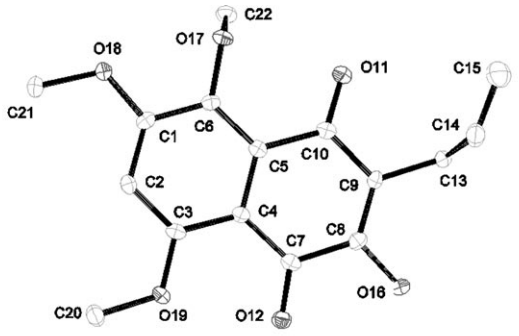



***Scheme 2.*** Retrosynthesis of **1**.

The synthesis of naphthalene fragment **9** hinged on the Claisen rearrangement of allyloxynaphthoquinone **11a** that started from the known bromoquinone **10**[10,20] (Scheme 3). Surprisingly, direct C-2 allyloxylation of bromoquinone **10** that would allow a subsequent Claisen rearrangement proved problematic. In preliminary studies, allyloxylation of bromoquinone **10** by formation of the sodium or potassium allyloxide was accompanied by degradation, and afforded **11a** in poor yields (20–30%). Eventually, the facile allyloxylation of bromoquinone **10** using excess allyl alcohol and cesium carbonate took place, but disappointingly afforded a mixture of regioisomers **11a** and **11b**.

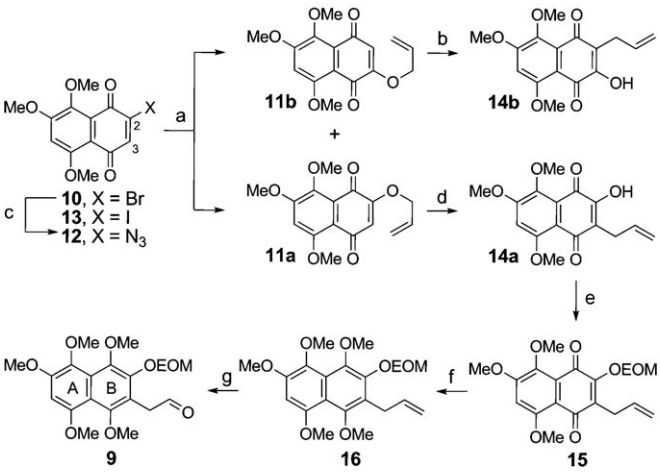
Normal substitution proceeds in an *ipso* fashion by a nucleophilic attack on the carbon bonded to the halogen (C-2) with subsequent expulsion of halide to give **11a**, whilst similar nucleophilic attack on the adjacent carbon (C-3) gives an intermediate adduct which upon dehydrohalogenation affords the *cine*-substitution product **11b**. The regiochemistry of **11b** was determined by its conversion into the crystalline Claisen product **14b**. A single-crystal X-ray structure was obtained for **14b**, which confirmed the regiochemistry of the *cine* product **11b** obtained from the allyloxylation.[21] After many unproductive attempts to improve the regiochemistry by activating the double bond of the quinone with iodine in the presence of transition metal salts ($CeCl_3$,[22] $Cu(OAc)_2$,[23] or HgO[24]), we decided to investigate the effect of the leaving group (X) on the regiochemical outcome of the key allyloxylation step.

To this end, bromoquinone **10** was converted to azidoquinone **12** by using sodium azide, whilst iodoquinone **13** was prepared in a similar manner to bromoquinone **10** (leaving-group effect, Scheme 3). Pleasingly, the nature of the leaving group dramatically affected the regiochemical outcome of



| | | X | Yield | (11a/11b) |
|---|---|---|---|---|
| leaving-group effect on regiochemical outcome of alloxylation (step a) | 13 | I | 86 % | 0:100 |
| | 10 | Br | 64 % | 60:40 |
| | 12 | $N_3$ | 80 %* | 100:0 |

* yield over 2 steps from bromoquinone **10**




***Scheme 3.*** Top: X-ray crystal structure of **14b**. Thermal ellipsoids are set at 50% probability. Center: Leaving-group effect on the outcome of step a. Bottom: Synthesis of naphthazarin **9**: a) $Cs_2CO_3$, allyl alcohol (excess), toluene, RT, 1 h, then filtration; b) microwave, 140°C, toluene, 1 h, 97%; c) $NaN_3$, MeCN, RT, 3 days, then filtration and evaporation; d) microwave, 140°C, toluene, 0.5 h, then evaporation; e) chloromethylethyl ether (EOMCl), $iPr_2NEt$, $CH_2Cl_2$, 0°C→RT, 70% from bromoquinone **10**; f) 1) $Na_2S_2O_4$, $nBu_4NBr$, THF/$H_2O$ (1:1), 0.5 h; 2) MeI, $Cs_2CO_3$, $Na_2S_2O_4$ (cat.), $H_2$, DMF, 60°C, 2 h, 92%; g) 2,6-lutidine, $OsO_4$ (cat.), $NaIO_4$, dioxane/water (3:1), 90%.

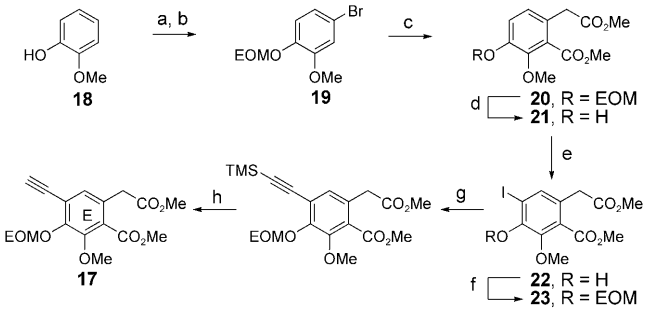
this reaction. Azidoquinone **12** underwent clean conversion to the desired allyl ether **11a** (normal substitution) as a single product, whilst iodoquinone **13** reacted cleanly to afford only the *cine* substitution product **11b**. The high regioselectivity observed can be attributed to a combination of steric and bond-dipole effects. Thus, decreasing the steric bulk at the C–X bond ($I > Br \gg N_3$) facilitates the desired *ipso* substitution at C-2. Also, if one considers the azide fragment as a pseudohalogen (absolute electronegativity: I 6.7, Br 7.5, $N_3$ 7.7 eV),[25] increased C–X bond polarization may also lead to *ipso* attack being favored over *cine* substitution. While a limited number of examples of the use of azide as a leaving group have been reported,[26] its application to the synthesis of complex molecules remains relatively unexplored. In the present work, the highly substituted nature of the target

naphthazarin necessitated extension of our synthetic repertoire to the use of an azide leaving group.

Subsequently, microwave irradiation of allyl ether **11a** was used to effect the desired Claisen rearrangement and delivered naphthol **14a**, which was subsequently protected as an ethoxymethyl (EOM) ether **15**. Significantly, the conversion of bromoquinone **10** to **15** only required one purification step and proceeded in 70% yield over four steps, thus facilitating an efficient large-scale synthesis. Reductive dimethylation of **15** required the careful use of a reductive environment (hydrogen atmosphere and sodium dithionite) using cesium carbonate as base to overcome the considerable steric constraints imposed when introducing additional alkoxy groups onto the heavily substituted naphthalene ring. One-pot oxidative cleavage[27] of the terminal olefin **16** subsequently provided aldehyde **9**.

The synthesis of isocoumarin precursor **17** commenced with commercially available guaiacol (**18**, Scheme 4). *Para*-selective bromination, followed by protection as an EOM

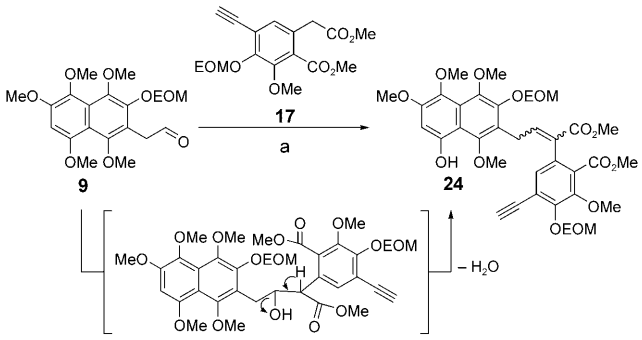


***Scheme 4.*** Syntheses of isocoumarin precursors, iodide **23**, and acetylene intermediate **17**: a) NBS, DMF, 0°C, 0.5 h, 83%; b) EOMCl, $iPr_2NEt$, $CH_2Cl_2$, 0°C→RT, 89%; c) LDA, THF, 0°C, 0.5 h, then dimethyl malonate, 0°C, 0.5 h, then **19**, THF, 0°C, 71%; d) $NaHSO_4/SiO_2$, $CH_2Cl_2$, RT, 16 h, 92%; e) $NaHCO_3$, tetramethylammonium dichloroiodate, $CH_2Cl_2$, RT, 2 h, 51%; f) EOMCl, $iPr_2NEt$, $CH_2Cl_2$, 0°C→RT, 93%; g) trimethylsilylacetylene, $Et_3N$, CuI (15 mol%), $PPh_3$ (25 mol%), $Pd(OAc)_2$ (10 mol%), DMF, RT, 16 h, 98%; h) $K_2CO_3$, THF/MeOH (1:1), RT, 1.5 h, 91%.

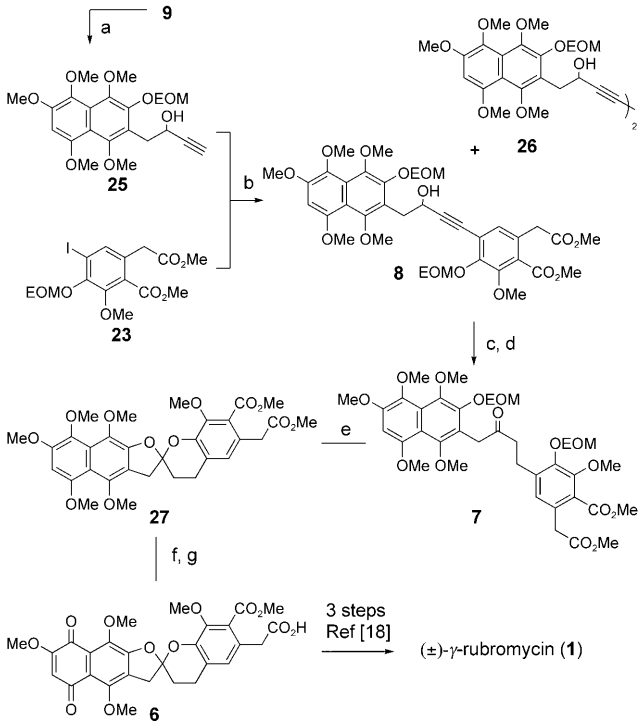
ether provided bromophenol **19**, which was then converted to diester **20** by slow dropwise addition of LDA to dimethyl malonate followed by addition of bromide **19**.[18,28] After removal of the EOM group, iodination of the resulting naphthol **21** with tetramethylammonium dichloroiodate afforded iodide **22**. After reprotection as an EOM ether (**23**), Sonogashira coupling with trimethylsilylacetylene followed by desilylation under mild basic conditions provided the key acetylene coupling partner **17**.

With the key naphthazarin **9** and isocoumarin precursor **17** fragments in hand, their union by acetylide addition was explored (Scheme 5). To our surprise, attempts to generate the lithiated acetylide of isocoumarin precursor **17** afforded adduct **24**, which resulted from the aldol condensation of aldehyde **9** with the ester enolate derived from **17**.

Accordingly, the coupling strategy for the naphthalene and isocoumarin fragments was revised (Scheme 6) so that iodide **23** (prepared as depicted in Scheme 4) was coupled to



***Scheme 5.*** Attempted lithiated acetylide coupling: a) LiHMDS, THF, −78°C, 1 h, 41%.



***Scheme 6.*** Completion of advanced spiroketal intermediate **6**: a) ethynyl magnesium bromide (0.5 M in THF), THF, 0°C, 1 h, 90%; b) $[Pd(PPh_3)_4]$ (10 mol%), CuI (15 mol%), $Cs_2CO_3$, DMF, 6 h, **8** (91%), **26** (trace); c) Pd/C, $H_2$, $NaHCO_3$, EtOAc, 3 h, 99%; d) IBX, DMSO, 60°C, 0.5 h, 95%; e) $NaHSO_4/SiO_2$, $CH_2Cl_2$, RT, 4 h, 80%; f) 10% KOH, MeOH, 0°C→RT, 16 h; g) cerium(IV) ammonium nitrate, $MeCN/H_2O$ (5:1), 0°C, 0.5 h (58% over two steps).

acetylene **25** using a Sonogashira reaction. Aldehyde **9** was converted to the key propargyl alcohol fragment **25** by Grignard addition with ethynyl magnesium bromide. Pleasingly, Sonogashira reaction of key alkyne **25** with iodide **23** proceeded in an excellent yield using a non-amine base (cesium carbonate) and degassed solvents, in order to minimize formation of the homocoupled product **26**. Hydrogenation of the resulting alkyne **8** in ethyl acetate buffered with sodium bicarbonate over 10% Pd/C then provided the saturated secondary alcohol in quantitative yield. Oxidation

of the alcohol with 2-iodoxybenzoic acid (IBX) in dimethylsulfoxide afforded ketone **7**.

The valuable spiroketalization precursor **7** was then subjected to the mild spiroketalization conditions previously developed by our group for the synthesis of model aryl 5,6-spiroketals.[19] Gratifyingly, treatment of ketone **7** with excess activated silica supported sodium hydrogen sulfate in dichloromethane at room temperature for 4 hours successfully afforded the desired spiroketal **27** in 80% yield. Finally, a selective hydrolysis of the aliphatic ester to the carboxylic acid and final adjustment of the oxidation states of the naphthalene unit furnished Kita's advanced intermediate **6**.[18] The spectroscopic data of the synthetic material were in full agreement with those recorded for an authentic sample kindly provided by Professor Kita (see the Supporting Information).

In conclusion, we have completed an efficient formal total synthesis of (±)-γ-rubromycin (**1**) in 17 steps from 1,2,4-trimethoxybenzene. The synthesis is significantly shorter than Kita's approach[18] and produces **1** in a much better overall yield. The present synthesis demonstrates the previously unrealized application of acid-mediated spiroketalization for the synthesis of the challenging rubromycin family of natural products. The synthetic route also makes the use of a novel regioselective allyloxylation of a 2-azido-1,4-naphthoquinone to facilitate a subsequent Claisen rearrangement. Further work will focus on extension of this synthetic strategy to the synthesis of purpuromycin (**4**) and naphthoquinone analogues thereof.

Received: June 19, 2009
Published online: July 30, 2009

**Keywords:** antibiotics · azides · natural product synthesis · spiroketals · total synthesis

[1] a) H. Brockmann, K. H. Renneberg, *Naturwissenschaften* **1953**, *40*, 59–60; b) H. Brockmann, K. H. Renneberg, *Naturwissenschaften* **1953**, *40*, 166–167; c) H. Brockmann, W. Lenk, G. Schwantje, A. Zeeck, *Tetrahedron Lett.* **1966**, *7*, 3525–3530.

[2] a) A. Trani, C. Dallanoce, G. Panzone, F. Ripamonti, B. P. Goldstein, R. Ciabatti, *J. Med. Chem.* **1997**, *40*, 967–971; b) B. P. Goldstein, A. King, F. Ripamonti, A. Trani, I. Phillips, *J. Antimicrob. Chemother.* **1995**, *36*, 1061–1065.

[3] a) C. Coronell, H. Pagani, M. R. Bardone, G. C. Lancini, *J. Antibiot.* **1974**, *27*, 161–168; b) C. Puder, S. Loya, A. Hizi, A. Zeeck, *Eur. J. Org. Chem.* **2000**, 729–735.

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

[5] M. Brasholz, S. Sorgel, C. Azap, H. U. Reißig, *Eur. J. Org. Chem.* **2007**, 3801–3814, and references therein.

[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) M. Chino, K. Nishikawa, M. Umekita, C. Hayashi, T. Yamazaki, T. Tsuchida, T. Sawa, M. Hamada, T. Takeuchi, *J. Antibiot.* **1996**, *49*, 752–757.

[7] M. E. Goldman, G. S. Salituro, J. A. Bowen, J. M. Williamson, D. L. Zink, W. A. Schleif, E. A. Emini, *Mol. Pharmacol.* **1990**, *38*, 20–25.

[8] M. R. Bardone, E. Martinelli, E. F. Zerilli, C. Coronelli, *Tetrahedron* **1974**, *30*, 2747–2754.

[9] a) X. Xie, M. C. Kozlowski, *Org. Lett.* **2001**, *3*, 2661–2663; b) J. L. Stevens, T. D. Welton, J. P. Deville, V. Behar, *Tetrahedron Lett.* **2003**, *44*, 8901–8903; c) A. N. Lowell, M. W. Fennie, M. C. Kozlowski, *J. Org. Chem.* **2008**, *73*, 1911–1918.

[10] S. Sorgel, C. Azap, H. U. Reißig, *Eur. J. Org. Chem.* **2006**, 4405–4418.

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

[12] a) S. P. Waters, M. C. Kozlowski, *Tetrahedron Lett.* **2001**, *42*, 3567–3570; b) M. Brasholz, H. U. Reißig, *Synlett* **2004**, 2736–2738; c) M. Brasholz, X. S. Luan, H. U. Reißig, *Synthesis* **2005**, 3571–3580.

[13] a) D. H. Qin, R. X. Ren, T. Siu, C. S. Zheng, S. J. Danishefsky, *Angew. Chem.* **2001**, *113*, 4845–4849; *Angew. Chem. Int. Ed.* **2001**, *40*, 4709–4713; b) T. Capecchi, C. B. de Koning, J. P. Michael, *Tetrahedron Lett.* **1998**, *39*, 5429–5432; c) G. L. Zhou, D. P. Zheng, S. J. Da, Z. X. Xie, Y. Li, *Tetrahedron Lett.* **2006**, *47*, 3349–3352; d) Y. Zhang, J. J. Xue, Z. J. Xin, Z. X. Xie, Y. Li, *Synlett* **2008**, 940–944; e) S. P. Waters, M. W. Fennie, M. C. Kozlowski, *Tetrahedron Lett.* **2006**, *47*, 5409–5413.

[14] K. Y. Tsang, M. A. Brimble, J. B. Bremner, *Org. Lett.* **2003**, *5*, 4425–4427.

[15] a) C. C. Lindsey, K. L. Wu, T. R. R. Pettus, *Org. Lett.* **2006**, *8*, 2365–2367; b) S. Sorgel, C. Azap, H. U. Reißig, *Org. Lett.* **2006**, *8*, 4875–4878.

[16] C. Venkatesh, H. U. Reißig, *Synthesis* **2008**, 3605–3614.

[17] T. Siu, D. H. Qin, S. J. Danishefsky, *Angew. Chem.* **2001**, *113*, 4849–4852; *Angew. Chem. Int. Ed.* **2001**, *40*, 4713–4716.

[18] 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.

[19] K. Y. Tsang, M. A. Brimble, *Tetrahedron* **2007**, *63*, 6015–6034.

[20] D. L. J. Clive, A. Khodabocus, P. G. Vernon, A. G. Angoh, L. Bordeleau, D. S. Middleton, C. Lowe, D. Kellner, *J. Chem. Soc. Perkin Trans. 1* **1991**, 1433–1444.

[21] D. C. K. Rathwell, K. Y. Tsang, K. W. Choi, P. D. W. Boyd, M. A. Brimble, *Acta Crystallogr.* **2008**, *E64*, o1929.

[22] M. Kidwai, P. Kumar, S. Kohli, *J. Chem. Res. Synop.* **1997**, 24–25.

[23] R. N. Khanna, K. P. Singh, S. K. Yadav, S. Srinivas, *Synth. Commun.* **1989**, *19*, 3151–3157.

[24] V. Bansal, J. Sharma, R. N. Khanna, *J. Chem. Res. Synop.* **1998**, 720–721.

[25] I. C. Tornieporth-Oetting, T. M. Klapötke, *Angew. Chem.* **1995**, *107*, 559–568; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 511–520.

[26] a) J. F. Burnett, R. E. Zahler, *Chem. Rev.* **1951**, *49*, 273–412; b) N. D. Pokhilo, A. Y. Yakubovskaya, V. A. Denisenko, V. P. Anufriev, *Tetrahedron Lett.* **2006**, *47*, 1385–1387.

[27] W. Yu, Y. Mei, Y. Kang, Z. Hua, Z. Jin, *Org. Lett.* **2004**, *6*, 3217–3219.

[28] M. D. Shair, T. Y. Yoon, K. K. Mosny, T. C. Chou, S. J. Danishefsky, *J. Am. Chem. Soc.* **1996**, *118*, 9509–9525.