Total Synthesis

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A Divergent Enantioselective Strategy for the Synthesis of Griseusins

*Yinan Zhang, Qing Ye, Xiachang Wang, Qing-Bai She, and Jon S. Thorson**

Abstract: *The first enantioselective total synthesis of griseusin A, griseusin C, 4′-deacetyl-griseusin A, and two non-native counterparts in 11–14 steps is reported. This strategy highlights a key hydroxy-directed C–H olefination of 1-methylene isochroman with an α,β-unsaturated ketone followed by subsequent stereoselective epoxidation and regioselective cyclization to afford the signature tetrahydro-spiropyran ring. Colorectal cancer cell cytotoxicities of the final products highlight the impact of the griseusin tetrahydro-spiropyran ring on bioactivity. As the first divergent enantioselective synthesis, the strategy put forth sets the stage for further griseusin mechanism-of-action and SAR studies.*

The griseusins produced by *Streptomyces griseus* and *Nocardiopsis* sp. are pyranonaphthoquinone metabolites that contain a fused spiro-ring C/E system (Figure 1).[1] Ring E of this signature structural motif is further elaborated through oxidation, acetylation, and/or glycosylation and, similar to members of the simpler frenolicin-type pyranonaphthoquinones,[2] some griseusin members also contain an open D-ring. Additional distinguishing features among members include stereo-inversion at C_3, C_4, $C_{3'}$, $C_{4'}$, $C_{6'}$, and/or the ring C/E spiro-ring junction C_1. Although griseusins have been noted for their potent antibiotic, antifungal, and anticancer activities, their fundamental mechanism of action remains unclear. For example, the recent identification of a representative naturally occurring griseusin as COMPARE-negative implicates a potentially novel anticancer mechanism.[1g] Griseusin synthetic development has been inspired by these cumulative properties, beginning with the first total synthesis of an enantiomer of griseusin A by Kometani et al. in 1983.[3] Yet, although notably elegant strategies to construct the griseusin core scaffold have since been developed,[4] the total syntheses of naturally occurring griseusins have not been reported, a major challenge of which stems from C_1 epimerization within the context of spiropyran construction. Toward this end, herein we describe an efficient divergent enantioselective strategy for griseusin synthesis and the corresponding total synthesis of griseusin A, 4′-deacetyl griseusin A, griseusin C, and two unnatural analogues. Highlights of the fundamental strategy include the rapid assembly of core chiral fragments, a novel C–H activation to facilitate early-stage fragment coupling assembly, and an enabling diastereoselective epoxidation–cyclization cascade approach to tetrahydro-spiropyran formation that avoids the C_1 epimerization. The subsequent comparison of the anticancer cytotoxicities of this griseusin series for the first time reveals that E-ring substitution modulates potency. This enabling synthetic method sets the stage for future in-depth SAR and mechanistic studies of this intriguing natural product family.

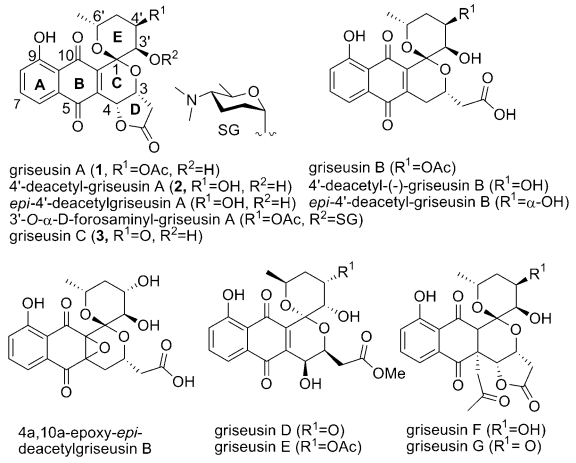

Figure 1. Naturally occurring griseusins.

An initial intent was to leverage our recently reported diastereoselective oxa-Pictet–Spengler-based strategy developed for the construction of frenolicin-type pyranonaphthoquinones.[5] However, all attempts to do so proved to be incompatible with the requisite griseusin tetrahydro-spiropyran ring. Thus, an alternative approach was designed based on the division of the griseusins into four main subclasses that diverge synthetically from griseusin C (**3**, Scheme 1). In this strategy, the key griseusin α-hydroxy tetrahydro-spiropyran moiety was obtained from a diastereoselective epoxidation–cyclization cascade of the key intermediate 1-methylene isochroman **4**, a precursor prepared by direct C–H activation and conjugation of **5** and **6**. Sharpless dihydroxylation[6] was thereby conceived to set the key stereocenters en route to **5** from commercially available naphthalene **7**. Importantly, the modular design of this strategy was anticipated to enable access to a range of divergent griseusin-based analogues.

The synthesis commenced with the asymmetric preparation of intermediate **5** (Scheme 2) through preparation of benzoquinone **8** from commercially available 1,5-dihydroxynaphthalene **7** (55% overall yield, 20 gram scale).[5] Deacetylation under acid reflux gave 5-hydroxy benzoquinone **9**, which was converted to naphthalene bromide **10** through sequential reduction, acetonide protection, and methylation (three steps, 65% overall yield). Heck coupling of **10** with

[*] Dr. Y. Zhang, Dr. X. Wang, Prof. J. S. Thorson
Center for Pharmaceutical Research and Innovation
College of Pharmacy, University of Kentucky
789 South Limestone Street, Lexington, KY 40536 (USA)
E-mail: jsthorson@uky.edu
Dr. Q. Ye, Prof. Q.-B. She
Markey Cancer Center, University of Kentucky
741 South Limestone Street, Lexington, KY 40536 (USA)

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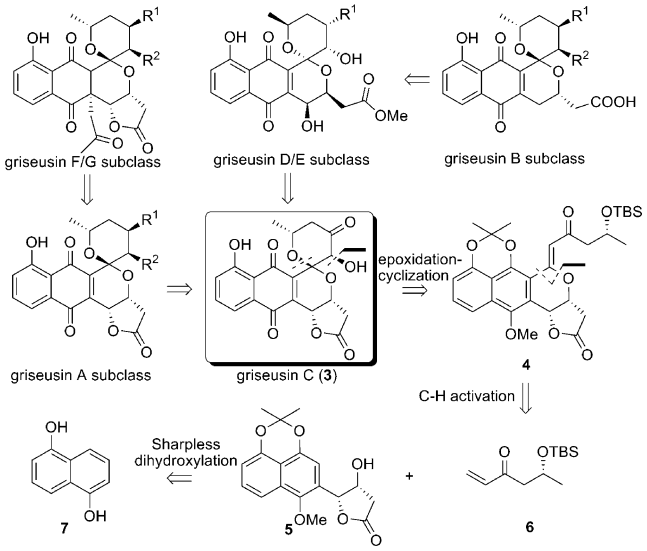
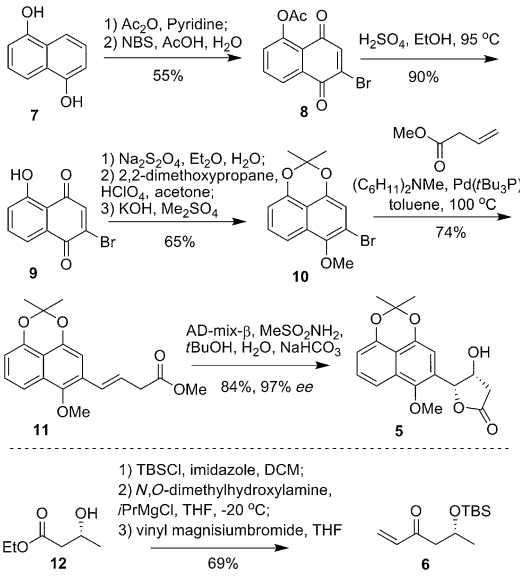


Scheme 1. Retrosynthetic analysis of griseusins. TBS = *tert*-butyldimethyl silyl.

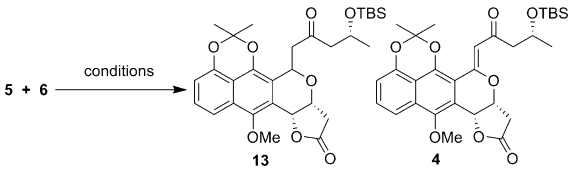


Scheme 2. Preparation of intermediates **5** and **6**. NBS = *N*-bromosuccinimide.

methyl but-3-enoate afforded the desired ester **11** in 74% yield. Whereas the ester **11** was unstable under standard Sharpless dihydroxylation conditions (strong base), alternative use of $NaHCO_3$ led to intermediate **5** in excellent yield (84%, 97% *ee*).[7] The corresponding synthesis of building block **6** was achieved in three steps from ethyl (*R*)-3-hydroxybutyrate (**12**)[8] to set the stage for the key hydroxy-directed C–H olefination reaction.

Although palladium catalyzed C–H activation has been extensively studied, its use in total synthesis remains limited.[9] Following the precedent set by Yu and co-workers for hydroxy-directed C–H olefination[10] in the context of isochromanone synthesis,[11] the desired methylene isochroman **4** was obtained in poor yield (10%, entry 1, Table 1). This may

Table 1: Optimization of the C–H olefination reaction to set the stage for Scheme 3.[a]

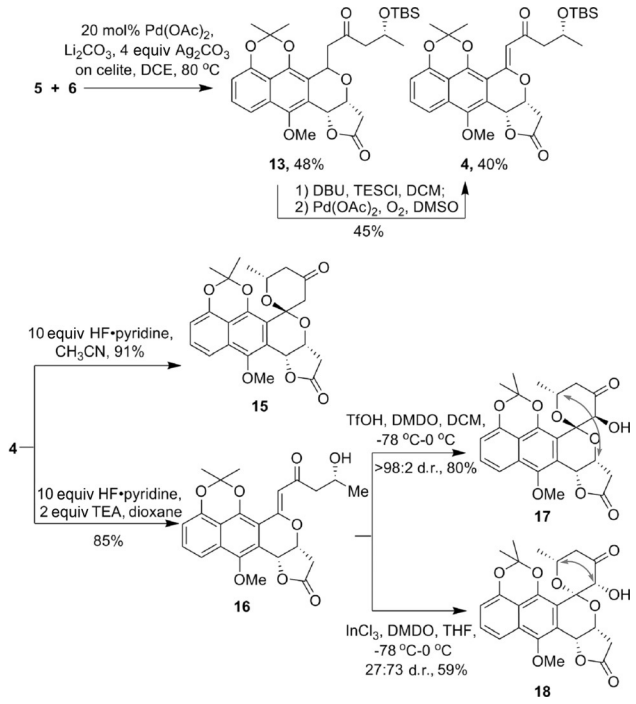


Entry	Pd catalyst	Oxidant	*T* [°C]	Yields of **13/4/5** [%][b]
1[c]	$Pd(OAc)_2$	AgOAc	80	47/10/40[d]
2	$Pd(OAc)_2$	Ag_2CO_3	80	60/32/6[d]
3	$Pd(OAc)_2$	Ag_2CO_3/celite	80	53/37/< 5[d]
4	$Pd(OAc)_2$	AgOPiv	80	64/10/22
5[e]	$Pd(OAc)_2$	Ag_2CO_3	80	55/32/10
6[f]	$Pd(OAc)_2$	–	80	40/35/10
7	$Pd(OPiv)_2$	Ag_2CO_3	80	22/0/71
8[g]	$Pd(OAc)_2$	Ag_2CO_3	40	25/19/50
9[h]	$Pd(OAc)_2$	Ag_2CO_3	60	48/30/20
10[i]	$Pd(OAc)_2$	Ag_2CO_3	100	60/32/3
11[j]	$Pd(OAc)_2$	Ag_2CO_3/celite	80	48/40/< 5[d]
12[k]	$Pd(OAc)_2$	Ag_2CO_3	80	85/0/9[d]

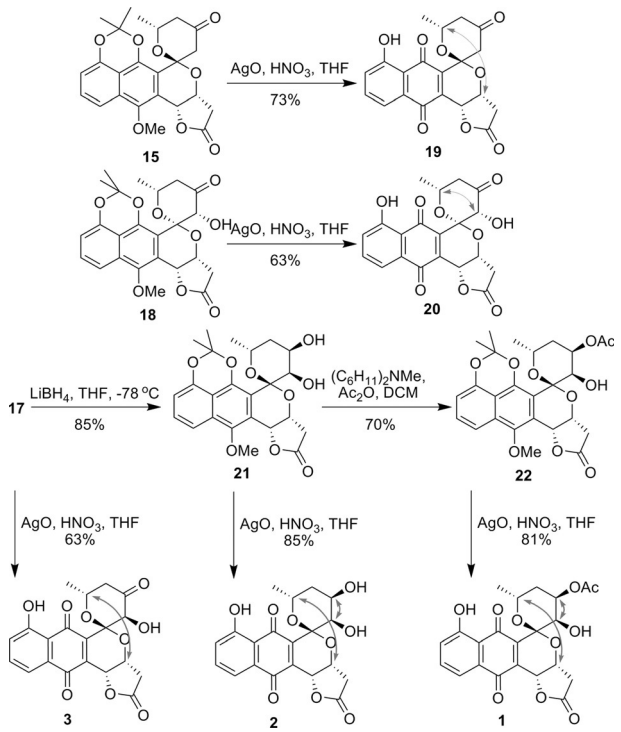
[a] Reactant **5** (0.05 mmol), Pd catalyst (0.01 mmol), oxidant (0.2 mmol), Li_2CO_3 (0.05 mmol), DCE (0.3 mL), and reactant **6** (0.06 mmol) was stirred at 80 °C for 16–24 h. [b] Yield was determined by ^{1}H NMR analysis of the crude reaction mixture using 1-bromo-3,5-dichlorobenzene as an internal standard. [c] Previously described method.[10] [d] Yield of isolated product. [e] 8 Equiv Ag_2CO_3 was added. [f] 200% $Pd(OAc)_2$ was used. [g] 80 h reaction time. [h] 40 h reaction time. [i] 8 h reaction time. [j] 10 mmol scale. [k] 20 mol% $(BnO)_2P(O)OH$ as ligand and no base.

be due to steric inhibition of palladium coordination in the secondary oxidative cyclization and competition by the corresponding hetero-Michael addition,[10] consistent with the observed major product **13** (Figure S1). Extensive screening of alternative solvents, ligands, and auxiliary oxidants led to a ca. threefold improvement in yield of the desired **4** (entry 2, Table 1 and Tables S1–S4). While palladium pivalate, silver pivalate,[12] or excess oxidant/catalyst failed to provide further improvements, silver carbonate on celite gave **4** in 37% yield (entries 3–7, Table 1). Lower temperature reduced the yield even with extended reaction time whereas elevated temperature increased reaction rates but with no improvement in desired product (entries 8–10, Table 1). The yield of **13** and **4** on a larger scale were comparable (entry 11, Table 1) and they were easily isolated by standard silica gel chromatography. It is also important to note that the use of $(BnO)_2P(O)OH$ as the ligand[13] dramatically improved the yield of **13** (entry 12, Table 1) as an alternative route to **4** through α,β-dehydrogenation.[14] Within this context, enol silylation and subsequent Saegusa–Ito oxidation[15] were found to be the best conditions to achieve the desired product (**4**, two steps, 45% yield under un-optimized conditions, 62% yield in total from **5** to **4**).

With the key intermediate **4** in hand, our attention shifted to deprotection and tetrahydro-spiropyran formation (Scheme 3). The TBS group could be easily removed by hydrogen fluoride in CH_3CN to give cyclized 3′-dehydroxy griseusin precursor (**15**, 91%) as the predominant product. Subsequent optimization of potential neutralizing bases in this reaction revealed the addition of two equivalents of



Scheme 3. Tetrahydro-spiropyran formation. Key NOESY correlations in final products **17** and **18** are highlighted in gray. DCE = 1,2-dichloroethane, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, TEA = triethylamine, Tf = trifluoromethanesulfonyl.



Scheme 4. Completion of the **1**–**3**, **19**, and **20** total syntheses. Key NOESY correlations in final products **1**–**3**, **19**, and **20** are highlighted in gray.

triethylamine to afford desired **16** in 85% yield (Table S5). While few, if any, examples exist of sequential unsaturated ketone epoxidation and cyclization, the seminal work of Tan and co-workers on the stereoselectivity of allyl silyl ether-based spiroketal formation served as a basis for this strategy.[16] Subsequent screening of a range of oxidants, solvents, and Lewis or Brønsted acids revealed that the addition of dimethyldioxirane (DMDO)[17] to a 1:1 mixture of **16** and TfOH in DCM afforded the desired griseusin C-type precursor **17** (80% yield, >98:2 d.r., Tables S6 and S7). In contrast, the same reaction in the presence of $InCl_3$ in THF led to the corresponding 1,3′-*epi*-griseusin C precursor **18** (59% yield, 73:27 d.r.). Distinct from previous examples,[16c,d] diastereoselectivity may derive from facial selectivity of **16** epoxidation (TfOH predominantly *si*-face; $InCl_3$ *re*-face bias), in which the sequential cyclization follows a C_1-inversion mechanism in both cases. For all products, the relative configuration at newly generated stereocenters was assigned by cross peaks in NOESY and other 2D NMR spectra (Scheme 3).

From **17**, the remaining steps proceeded as planned (Scheme 4). Specifically, lithium borohydride reduction gave the natural 4′-axial diastereomer **21** exclusively. Selective 4′-acetylation was accomplished using a sterically hindered base (dicyclohexylmethyl amine) to yield **22**. Final global deprotection of the pyranonaphthoquinone core afforded the desired natural products griseusin A (**1**), 4′-deacetyl griseusin (**2**), griseusin C[1f] (**3**, also known as 4′-dehydro-deacetyl griseusin A[1h]), and two griseusin analogues, 3′-dehydroxy griseusin C (**19**), and 1,3′-*epi*-griseusin C (**20**). Optical rotations of the synthetically derived griseusins agreed well with values previously reported for the corresponding natural products (**1**[1b] $[\alpha]_D^{23}=-148$, synthetic $[\alpha]_D^{20}=-153$; **2**[1e] $[\alpha]_D^{24}=-198$, synthetic $[\alpha]_D^{20}=-202$; **3**[1h] $[\alpha]_D^{15}=-114$, synthetic $[\alpha]_D^{20}=-100$).

Table 2: Cytotoxicity of **1**–**3**, **19**, and **20**.[a]

Compounds	IC_{50} [nM] HCT116	DLD-1	SW620
1	201 ± 3	170 ± 9	138 ± 8
2	305 ± 5	258 ± 9	210 ± 8
3	127 ± 2	94 ± 3	67 ± 2
19	133 ± 2	57 ± 2	63 ± 1
20	345 ± 5	290 ± 6	242 ± 6

[a] Data are presented as IC_{50} ± SD values. Experiments were performed in triplicate.

The cancer cell line cytotoxicity of this set was subsequently evaluated against three colon cancer cell lines (HC116, DLD-1, and SW620; Table 2). All griseusins were found to be potent cancer cell line cytotoxins to which the stereochemistry and substituent pattern of ring E contributed to potency modulation. Specifically, although all members were found to display notable potency ($IC_{50} < 350$ nM), griseusin C (**3**) and its corresponding 3′-dehydroxy analogue **19** were found to be most active. C4′-reduction and/or modification led to reductions in potency, whereas analogues with variation at C3′ (hydroxy versus deoxy) were roughly equipotent.

In conclusion, this work highlights the first concise, asymmetric divergent strategy for the synthesis of naturally

occurring and non-native griseusins in 11–14 steps from commercially available materials. Key highlights include the first applications of hydroxy-directed C–H olefination in a total synthesis and the key regioselective and diastereoselective cyclization to efficiently access the signature tetrahydro-spiropyran ring E while avoiding C_1 epimerization, an issue that has plagued griseusin total synthesis efforts to date. Subsequent bioactivity assays reveal that the stereochemistry and functionalization of ring E modulates anticancer potency with 3′-dehydroxy griseusin C as the most potent member (57–133 nM). Work is underway to further probe griseusin SAR and fundamental mechanism of action, the outcome of which will be reported in due course.

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[1] a) N. Tsuji, M. Kobayashi, Y. Wakisaka, K. Kawamura, M. Mayama, K. Matsumoto, *J. Antibiot.* **1976**, *29*, 7; b) N. Tsuji, M. Kobayashi, Y. Terui, K. Tori, *Tetrahedron* **1976**, *32*, 2207; c) M. Maruyama, C. Nishida, Y. Takahashi, H. Naganawa, M. Hamada, T. Takeuchi, *J. Antibiot.* **1994**, *47*, 952; d) T. W. Yu, M. J. Bibb, W. P. Revill, D. A. Hopwood, *J. Bacteriol.* **1994**, *176*, 2627; e) M. Igarashi, W. Chen, T. Tsuchida, M. Umekita, T. Sawa, H. Naganawa, M. Hamada, T. Takeuchi, *J. Antibiot.* **1995**, *48*, 1502; f) X. Li, Y. Zheng, I. Sattler, W. Lin, *Arch. Pharmacal Res.* **2006**, *29*, 942; g) Y. Q. Li, M. G. Li, W. Li, J. Y. Zhao, Z. G. Ding, X. L. Cui, M. L. Wen, *J. Antibiot.* **2007**, *60*, 757; h) J. He, E. Roemer, C. Lange, X. Huang, A. Maier, G. Kelter, Y. Jiang, L. H. Xu, K. D. Menzel, S. Grabley, H. H. Fiebig, C. L. Jiang, I. Sattler, *J. Med. Chem.* **2007**, *50*, 5168; i) M. S. Abdelfattah, T. Kazufumi, M. Ishibashi, *J. Antibiot.* **2011**, *64*, 729; j) Z. G. Ding, J. Y. Zhao, M. G. Li, R. Huang, Q. M. Li, X. L. Cui, H. J. Zhu, M. L. Wen, *J. Nat. Prod.* **2012**, *75*, 1994.

[2] a) G. A. Ellestad, H. A. Whaley, E. L. Patterson, *J. Am. Chem. Soc.* **1966**, *88*, 4109; b) Y. Iwai, A. Kora, Y. Takahashi, T. Hayashi, J. Awaya, R. Masuma, R. Oiwa, S. Ōmura, *J. Antibiot.* **1978**, *31*, 959; c) M. A. Brimble, L. J. Duncalf, M. R. Nairn, *Nat. Prod. Rep.* **1999**, *16*, 267; d) X. Wang, K. A. Shaaban, S. I. Elshahawi, L. V. Ponomareva, M. Sunkara, Y. Zhang, G. C. Copley, J. C. Hower, A. J. Morris, M. K. Kharel, J. S. Thorson, *J. Nat. Prod.* **2013**, *76*, 1441; e) X. Wang, S. I. Elshahawi, K. A. Shaaban, L. Fang, L. V. Ponomareva, Y. Zhang, G. C. Copley, J. C. Hower, C.-G. Zhan, M. K. Kharel, J. S. Thorson, *Org. Lett.* **2014**, *16*, 456; f) S. I. Elshahawi, K. A. Shaaban, M. K. Kharel, J. S. Thorson, *Chem. Soc. Rev.* **2015**, DOI: 10.1039/c4cs00426d.

[3] a) T. Kometani, Y. Takeuchi, E. Yoshii, *J. Org. Chem.* **1982**, *47*, 4725; b) T. Kometani, Y. Takeuchil, E. Yoshii, *J. Org. Chem.* **1983**, *48*, 2311.

[4] a) M. A. Brimble, M. R. Nairn, J. Park, *Org. Lett.* **1999**, *1*, 1459; b) M. A. Brimble, M. R. Nairn, J. Park, *J. Chem. Soc. Perkin Trans. 2* **2000**, 697; c) K. A. Parker, T. L. Mindt, Y. H. Koh, *Org. Lett.* **2006**, *8*, 1759; d) B. J. Naysmith, M. A. Brimble, *Org. Lett.* **2013**, *15*, 2006; e) B. J. Naysmith, D. Furkert, M. A. Brimble, *Tetrahedron* **2014**, *70*, 1199.

[5] Y. Zhang, X. Wang, M. Sunkara, Q. Ye, L. V. Ponomereva, Q. B. She, A. J. Morris, J. S. Thorson, *Org. Lett.* **2013**, *15*, 5566.

[6] H. C. Kolb, M. S. Van Nieuwenhze, K. B. Sharpless, *Chem. Rev.* **1994**, *94*, 2483.

[7] Enantioselectivity was determined by the NMR analysis of (*R*)-Mosher esters **5-MTPA** and **5′-MTPA** obtained from intermediate **5** (Scheme 2) and its enantiomer **5′** (derived from AD-mix-α) following standard protocols (see T. M. Hoye, C. S. Jeffrey, F. Shao, *Nat. Protoc.* **2007**, *2*, 2451, and references therein).

[8] K. R. Anderson, S. L. G. Akinson, T. Fujiwara, M. E. Giles, T. Matsumoto, E. Merifield, J. T. Singleton, T. Saito, T. Sotoguchi, J. A. Tornos, E. L. Way, *Org. Process Res. Dev.* **2010**, *14*, 58.

[9] a) D. Y.-K. Chen, S. W. Youn, *Chem. Eur. J.* **2012**, *18*, 9452; b) K. M. Engle, T.-S. Mei, M. Wasa, J.-Q. Yu, *Acc. Chem. Res.* **2012**, *45*, 788; c) G. Li, D. Leow, L. Wan, J.-Q. Yu, *Angew. Chem. Int. Ed.* **2013**, *52*, 1245; *Angew. Chem.* **2013**, *125*, 1283.

[10] Y. Lu, D.-H. Wang, K. M. Engle, J.-Q. Yu, *J. Am. Chem. Soc.* **2010**, *132*, 5916.

[11] Y. Lu, D. Leow, X. Wang, E. M. Engle, J.-Q. Yu, *Chem. Sci.* **2011**, *2*, 967.

[12] a) D. Leow, G. Li, T.-S. Mei, J.-Q. Yu, *Nature* **2012**, *486*, 518; b) R.-Y. Zhu, J. He, X.-C. Wang, J.-Q. Yu, *J. Am. Chem. Soc.* **2014**, *136*, 13194.

[13] Q. Wang, J. Han, C. Wang, J. Zhang, Z. Huang, D. Shi, Y. Zhao, *Chem. Sci.* **2014**, *5*, 4962.

[14] a) Y. Izawa, D. Pun, S. S. Stahl, *Science* **2011**, *333*, 209; b) T. Diao, S. S. Stahl, *J. Am. Chem. Soc.* **2011**, *133*, 14566; c) W. Gao, Z. He, Y. Qian, J. Zhao, Y. Huang, *Chem. Sci.* **2012**, *3*, 883; d) T. Diao, T. J. Wadzinski, S. S. Stahl, *Chem. Sci.* **2012**, *3*, 887; e) Y. Shang, X. Jie, J. Zhou, P. Hu, S. Huang, W. Su, *Angew. Chem. Int. Ed.* **2013**, *52*, 1299; *Angew. Chem.* **2013**, *125*, 1337.

[15] a) Y. Ito, T. Hirao, T. Saegusa, *J. Org. Chem.* **1978**, *43*, 1011; b) R. C. Larock, T. R. Hightower, G. A. Kraus, P. Hahn, D. Zheng, *Tetrahedron Lett.* **1995**, *36*, 2423.

[16] a) S. B. Moilanen, J. S. Potuzak, D. S. Tan, *J. Am. Chem. Soc.* **2006**, *128*, 1792; b) G. Liu, J. M. Wurst, D. S. Tan, *Org. Lett.* **2009**, *11*, 3670; c) J. M. Wurst, G. Liu, D. S. Tan, *J. Am. Chem. Soc.* **2011**, *133*, 7916; d) I. Sharma, J. M. Wurst, D. S. Tan, *Org. Lett.* **2014**, *16*, 2474.

[17] D. F. Taber, P. W. DeMatteo, R. A. Hassan, *Org. Synth.* **2013**, *90*, 350.

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