Chemical Science

RSC Publishing

EDGE ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Chem. Sci.*, 2013, **4**, 1615

Symmetry-driven synthesis of 9-demethyl-10,15-dideoxyryanodol†

Daisuke Urabe, Masanori Nagatomo, Koji Hagiwara, Kengo Masuda and Masayuki Inoue*

Received 4th January 2013
Accepted 17th January 2013

DOI: 10.1039/c3sc00023k

www.rsc.org/chemicalscience

Ryanodine, a potent modulator of calcium release channels, possesses a highly oxygenated multicyclic structure. To develop a new unified strategy for the construction of ryanodine and its derivatives, we designed 9-demethyl-10,15-dideoxyryanodol (**1**) as a model compound. Here we report an efficient synthesis of **1** with seven contiguous tetrasubstituted carbons by taking advantage of the C_2-symmetric substructure embedded within its main structure.

Ryanodine (Fig. 1)[1] was isolated as a potent insecticide, and shown to target a membrane protein known as the ryanodine receptor.[2] The ryanodine receptors are responsible for the release of Ca^{2+} within cells and thus control many Ca^{2+}-dependent biological processes. Accordingly, altered functions of the receptors have been linked to many skeletal and cardiac diseases.[3] Since ryanodine modulates intrinsic channel gating, ryanodine and its natural and artificial derivatives have been expected to serve as specific biological probes and potential therapeutic agents.

The potent activity of ryanodine originates from its highly complex molecular architecture.[4] The pentacyclic ABCDE-ring system of ryanodine is fabricated with eight oxy (C2, 3, 4, 6, 10, 11, 12, 15), three methyl (C1, 5, 9), and one isopropyl (C2) groups. From a synthetic point of view, its densely functionalized structure is a daunting challenge for chemical synthesis. To date, the only total synthesis of this class of natural products was reported by Deslongchamps and co-workers: they successfully constructed ryanodol, a hydrolyzed analogue of ryanodine, in 1979.[5,6]

From our perspective, ryanodine and related molecules present an ideal platform to test new efficient strategies for building highly oxygenated multicyclic carboskeletons. In addition, the development of a flexible scheme would enable the generation of chemical derivatives with different functional properties toward the ryanodine receptors. These two aims prompted us to devise a new synthetic route to the ryanodine structures.[7] The challenge of ryanodine synthesis is significantly heightened by the seven contiguous tetrasubstituted stereocenters on the A- and B-rings (C1, 2, 4, 5, 6, 11, 12). To examine this issue, we designed 9-demethyl-10,15-dideoxyryanodol (**1**, Fig. 1) as a model compound, which possesses the entire ABCDE-ring structure of ryanodine. In this edge article, we report the efficient synthesis of the highly congested pentacyclic structure of **1** *via* judiciously controlled stereoselective introductions of the five adjacent oxygen-substituted carbon centers. The novel strategy described here would provide the basis for unified syntheses of ryanodine and their derivatives.

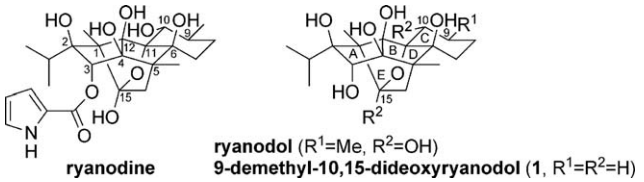
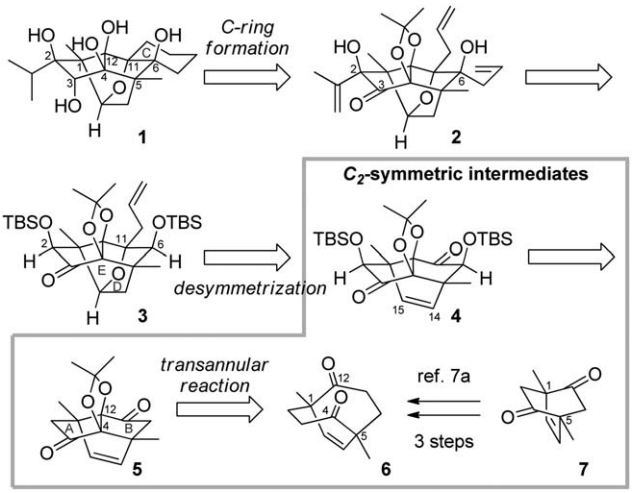


Fig. 1 Structures of ryanodine and its derivatives.

To minimize the total number of steps in the synthesis of **1**, our synthetic plan took advantage of its intrinsic C_2-symmetric substructure (Scheme 1).[8] Namely, the functionalized C_2-symmetric tricyclic compound **4** was envisioned as a subtarget for the construction of **1**. By doing so, a concise synthesis of **4** was planned to be attained by applying pairwise functionalizations to a series of C_2-symmetric intermediates. In the synthetic direction, the eight-membered ring **6**, previously prepared from racemic **7**,[7a] was to be oxidized and cyclized through a transannular reaction into the fused AB-ring system **5**, and further manipulations from **5** would give rise to **4**.[9] Then, desymmetrization of **4** and introduction of the C11-tetrasubstituted carbon would generate the oxygen-bridged DE-ring **3**, to which two different three-carbon units would be attached for the construction of the C2- and C6-tetrasubstituted carbons of **2**. Finally, **1** was envisioned to be synthesized from **2** through C-ring formation and C3-ketone reduction. Since the starting **6** contains C1- and C5-quaternary carbons, the most critical

Graduate School of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: inoue@mol.f.u-tokyo.ac.jp; Fax: +81-3-5841-0568

† Electronic supplementary information (ESI) available: Experimental protocols, characterization data, and NMR spectra of all new compounds. CCDC 914022 and 914023. For ESI and crystallographic data in CIF or other electronic format. See DOI: 10.1039/c3sc00023k



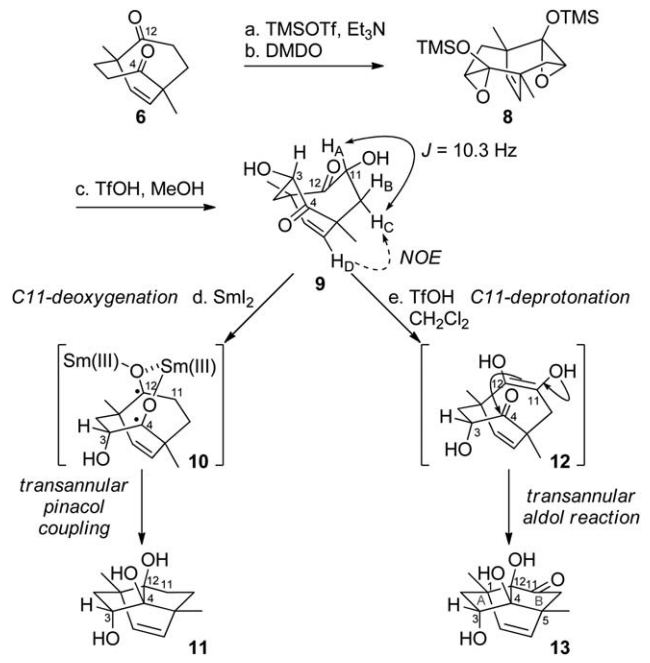
Scheme 1 Synthetic plan of **1**.

issues in this strategy to obtain **1** were the stereoselective installations of the six adjacent oxygen-substituted centers at C2, C3, C4, C6, C11 and C12.

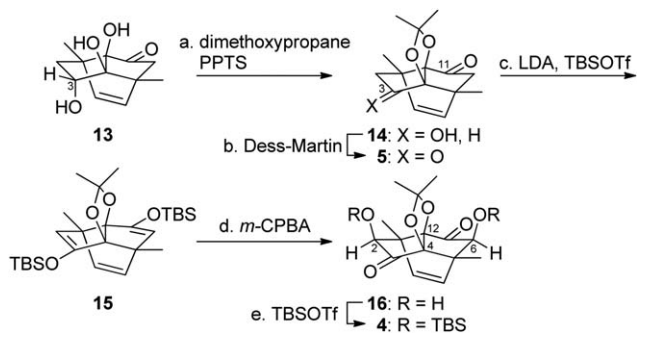
Construction of the AB-ring system was achieved from the racemic C_2-symmetric diketone **6** *via* only three pairwise transformations and one transannular cyclization (Scheme 2). Treatment of **6** with TMSOTf and Et_3N induced formation of the bis-TMS-enol ether, which was oxidized with DMDO from the bottom face to afford the unstable bis-epoxide **8** as the single isomer. Proto-desilylation of **8** using catalytic TfOH in MeOH generated diketone **9** with C3- and C11-hydroxy groups. Interestingly, the large coupling constant ($J_{HA,HC}$ = 10.3 Hz) and the NOE correlation (H_C and H_D) indicated that the eight-membered ring of **8** adopted the boat–boat conformation, which was presumably stabilized by the hydrogen bonds between the proximal carbonyl and hydroxy groups.

The transannular C–C bond formation reactions for cyclization of the AB-ring were then explored. Although SmI_2-promoted reductive coupling between the C4- and C12-ketones indeed transformed the eight-membered ring into the fused bicycle, the undesired reductive elimination of the C11-hydroxy group occurred prior to the ring closure of **10** to generate **11** in 47% yield.[10] Upon extensive screening of the reactions and substrates to suppress the undesired pathway, it was found that the mere presence of catalytic Brønsted acid effected the requisite cyclization from **9.** Treatment of **9** under the optimized conditions (3 mol% TfOH in CH_2Cl_2 at 65 °C) resulted in formation of **13**, the C11-oxo analogue of **11**, in 65% yield (4 steps). Consequently, simultaneous formation of the vicinal C4- and C12-tertiary hydroxy groups was realized without decreasing the oxidation state. This intriguing transformation involves two distinct steps: formation of enediol **12** by the C11-deprotonation and the subsequent transannular aldol reaction of **12** by attacking of the C12-carbon on the C4-ketone.[11] Release of the severe transannular interaction between the C11–H and C3–H of **9** is attributable for the facile α-hydroxyl ketone tautomerization of **9** to provide enediol **12**. The C12–C4 bond subsequently formed to produce the 5,5-ring system of **13** (the C11–C4 bond formation leads to the less stable 4,6-ring system).

The subtarget **4** was synthesized from the obtained **13** in five steps (Scheme 3). Protection of the 1,2-diol of **13** as an acetonide, followed by oxidation of the C3-hydroxy group of **14** with the Dess–Martin reagent[12] provided C_2-symmetric diketone **5**. Bis-α-hydroxylation of the C3- and C11-ketones of **5** was then performed *via* formation of bis-TBS-enol ether **15** with the reagent combination of LDA and TBSOTf and the following oxidation with *m*-CPBA, leading to **16** as a single isomer. The X-ray crystallographic analysis of **16** confirmed the newly introduced stereochemistries at C2, C4, C6, and C12 (Fig. 3).



Scheme 2 Transannular reactions of eight-membered ring **9**. *Reagents and conditions*: (a) TMSOTf, Et_3N, CH_2Cl_2, RT; (b) DMDO, CH_2Cl_2, 0 °C; (c) TfOH (1 mol%), MeOH, RT; (d) SmI_2, THF, 0 °C, 47% (from **9**); (e) TfOH (3 mol%), CH_2Cl_2, 65 °C, 65% (4 steps from **6**). DMDO = dimethyldioxirane; TfOH = trifluoromethanesulfonic acid; TMS = trimethylsilyl.

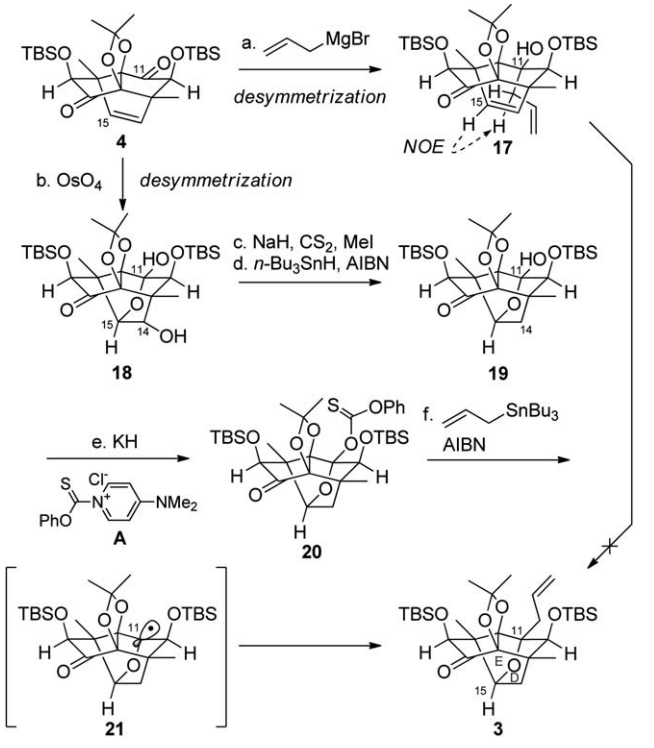


Scheme 3 Synthesis of C_2-symmetric diketone **4**. *Reagents and conditions*: (a) 2,2-dimethoxypropane, PPTS, benzene, reflux, 72%; (b) Dess–Martin reagent, $NaHCO_3$, CH_2Cl_2, RT, 81%; (c) LDA, THF, −78 °C, then TBSOTf, 85%; (d) *m*-CPBA, CH_2Cl_2, $NaHCO_3$, RT, 84%; (e) TBSOTf, Et_3N, $(CH_2Cl)_2$, 80 °C, 99%. LDA = lithium diisopropylamide; *m*-CPBA = *m*-chloroperbenzoic acid; PPTS = pyridinium *p*-toluenesulfonate; TBS = *tert*-butyldimethylsilyl.

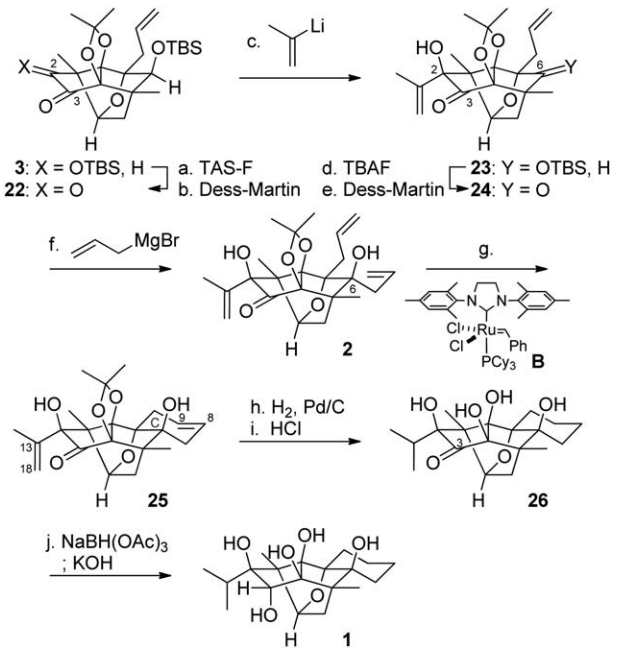
The resultant diol **16** was in turn converted to bis-TBS ether **4** using TBSOTf and Et_3N. Thus, the present symmetry-driven strategy enabled the preparation of the multiply oxidized C_2-symmetric tricarbocycle **4** in only nine steps from the simple diketone **6**.

With the subtarget **4** in hand, the next task was to synthesize the desymmetrized compound **3** *via* formation of the C10–C11 and C15–O bonds (Scheme 4). Nucleophilic monofunctionalization of diketone **4** using allylmagnesium bromide at −78 °C resulted in desymmetrization. However, monoketone **17** was found to possess the undesired C11-stereochemistry for the etherification (**17** → **3**). The acetonide in **4** appeared to kinetically protect the top face of the AB-ring from the nucleophile addition, as anticipated from the X-ray crystallographic structure of **16** (Fig. 3). To reverse the C11-stereochemical outcome, we opted for radical-based allylation of the configurationally predefined C11-α-alkoxy bridgehead radical **21**.[13] The radical precursor **20** was prepared in four steps from C_2-symmetric **4**. Osmylation of the C14–C15 double bond of **4** afforded the desymmetrized **18**, in which the C15-hydroxy group cyclized with the C11-ketone to form the DE-ring system. The C14-secondary alcohol of **18** was chemoselectively removed to produce **19** by the Barton–McCombie protocol,[14] which involves the methyl xanthate formation and subsequent *n*-Bu_3SnH reduction. Then, the remaining hindered C11-tertiary alcohol of **19** was treated with KH and the potently electrophilic pyridinium salt **A** to yield thiocarbonate **20**.[15] Thus obtained **20** underwent C11-allylation by the action of allyltributyltin and AIBN in refluxing benzene, deriving the requisite **3** in 66% yield. Therefore, the present powerful bridgehead radical reaction converted the C11–O bond into the C11–C10 bond within the sterically demanding polycyclic environment, and set the C11-tetrasubstitued carbon in a stereospecific fashion.

The target compound **1** was constructed from the core structure **3** *via* stepwise installations of the two carbon chains at C2 and C6 and the subsequent C-ring formation (Scheme 5). First, the C2-hydroxy group was selectively liberated from bis-TBS ether **3** using TAS-F[16] to generate the alcohol, which was oxidized with the Dess–Martin reagent to the C2-ketone of **22**.[17] Isopropenyl lithium reacted with C2,3-diketone **22** in the presence of TMEDA, leading to the desired C2-stereoisomer **23** as the sole product. Then, the TBAF deprotection and subsequent Dess–Martin oxidation transformed the C6-OTBS group of **23** to the C6-ketone of **24**. Addition of allylmagnesium bromide to C3,6-diketone **24** afforded the desired C6-stereoisomer **2** again as the single product, and hence introductions of the seven contiguous tetrasubstituted carbons were successfully completed at this stage. Importantly, the isopropenyl and allyl anions both attacked from the bottom face of the AB-ring, indicating the acetonide effectively functioned as the stereochemical controlling element. It is also worthy of note that the C3-ketone remained intact under these nucleophilic conditions



Scheme 4 Synthesis of the desymmetrized intermediate **3**. *Reagents and conditions*: (a) $CH_2{=}CHCH_2MgBr$, THF, −78 °C, 82%; (b) OsO_4, pyridine; aq. $NaHSO_3$, RT, 90%; (c) NaH, CS_2; MeI, THF, RT, 85%; (d) *n*-Bu_3SnH, AIBN, benzene, 100 °C, 82%; (e) KH, **A**, THF; −20 °C, 81%; (f) $CH_2{=}CHCH_2SnBu_3$, AIBN, benzene, reflux, 66%. AIBN = 2,2′-azobisisobutyronitrile.



Scheme 5 Synthesis of 9-demethyl-10,15-dideoxyryanodol **1**. *Reagents and conditions*: (a) TAS-F, DMF, RT; (b) Dess–Martin reagent, $NaHCO_3$, CH_2Cl_2, 0 °C; (c) *t*-BuLi, 2-bromopropene, TMEDA, THF, −78 °C, 48% (3 steps); (d) TBAF, CH_3CN, 60 °C, 80%; (e) Dess–Martin reagent, $NaHCO_3$, CH_2Cl_2, RT, 91%; (f) $CH_2{=}CHCH_2MgBr$, THF, −30 °C, 95%; (g) **B** (60 mol%), CH_2Cl_2, RT, 68%; (h) H_2, Pd/C, EtOAc, RT; (i) 1 M HCl in EtOAc, MeOH, 50 °C; (j) $NaBH(OAc)_3$, benzene–AcOH, 70 °C (2 cycles); KOH, MeOH, 50 °C, (3 cycles), 65% (3 steps). TAS-F = tris(dimethylamino)sulfonium difluorotrimethylsilicate; TBAF = tetra-*n*-butylammonium fluoride; TMEDA = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.

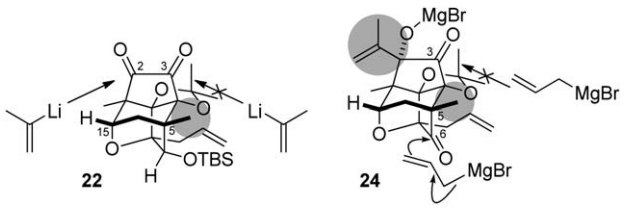


Fig. 2 Possible explanation for the diastereo- and regioselectivity of the two nucleophilic additions.

(Fig. 2), presumably because not only the acetonide groups but also the bulky groups highlighted in gray obstructed the reagent approach from the top and bottom faces of the C3-ketones of **22** and **24**, respectively.

The introduced two allyl groups of **2** then participated in the ring-closing metathesis reaction upon treatment with Grubbs' 2nd generation catalyst **B**, resulting in formation of the pentacycle **25**.[18] The simultaneous hydrogenation of the C8–C9 and C13–C18 double bonds of **25**, followed by acid-promoted deprotection, provided tetraol **26**. After detachment of the sterically cumbersome acetonide from **25**, the hydride attack on the C3-ketone from the same face with the four hydroxy groups became possible; the hydroxy-directed reduction of **26** with $NaBH(OAc)_3$ and subsequent hydrolysis of the resultant borate delivered 9-demethyl-10,15-dideoxyryanodol **1** as a single stereoisomer.[19,20,21] As shown in Fig. 3, the stereostructure of racemic pentacycle **1** was unambiguously determined by X-ray crystallographic analysis.

In summary, 9-demethyl-10,15-dideoxyryanodol **1** was synthesized from the simple C_2-symmetric bicycle **6** in 24 steps by taking advantage of the intrinsic symmetric element in the ryanodine structure. The key features of this synthesis are (1) transannular aldol reaction of the eight-membered ring **9** to build the tricyclic structure **13**, (2) minimization of the synthetic steps up to the C_2-symmetric **4** by the eight pairwise functionalizations, (3) stereospecific C11-allylation of **20** by employing the bridgehead radical reaction, (4) diastereo- and regioselective installations of the two different carbon chains at C2 and C6 of **22** and **24**, respectively, by utilizing the acetonide as the stereocontrolling element, (5) C-ring construction by the ring-closing metathesis of **2**, and (6) hydroxy-directed reduction of the C3-ketone of **26**. Further synthetic studies of ryanodine and its derivatives based on the newly developed strategy are underway in our laboratory.

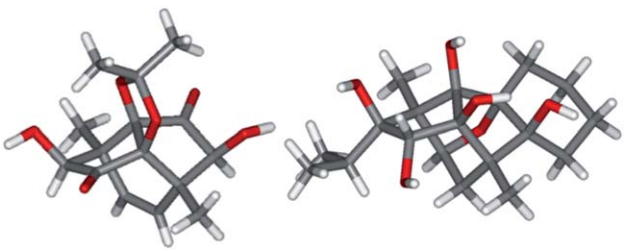


Fig. 3 X-Ray crystal structures of **16** (left) and **1** (right). CCDC 914022 and 914023 (**16** and **1**, respectively).†

Acknowledgements

This research was financially supported by the Funding Program for Next Generation World-Leading Researchers (JSPS) to M.I. and a Grant-in-Aid for Young Scientists (B) (JSPS) to D.U. Fellowships from JSPS to M.N. and K.H. are gratefully acknowledged. We thank Dr Eunsang Kwon (Tohoku University) and Dr Kenji Yoza (Bruker AXS) for X-ray crystallographic analyses, and the Health & Crop Sciences Research Laboratory, Sumitomo Chemical Co., Ltd. for bioactivity evaluation.

Notes and references

1 (*a*) E. F. Rogers, F. R. Koniuszy, J. Shavel Jr and K. Folkers, *J. Am. Chem. Soc.*, 1948, **70**, 3086–3088; (*b*) K. Wiesner, Z. Valenta and J. A. Findlay, *Tetrahedron Lett.*, 1967, **8**, 221–225; (*c*) S. N. Srivastava and M. Przybylska, *Can. J. Chem.*, 1968, **46**, 795–797.

2 For reviews on ryanodine receptors, see: (*a*) R. Zucchi and S. Ronca-Testoni, *Pharmacol. Rev.*, 1997, **49**, 1–51; (*b*) *Ryanodine Receptors: Structure, Function and Dysfunction in Clinical Disease*, ed. X. H. T. Wehrens and A. R. Marks, Springer, 2005.

3 For reviews, see: (*a*) M. J. Betzenhauser and A. R. Marks, *Eur. J. Physiol.*, 2010, **460**, 467–480; (*b*) J. J. Mackrill, *Biochem. Pharmacol.*, 2010, **79**, 1535–1543. See also ref. 2*b*.

4 For a review on the structure–activity relationship studies of ryanodine, see: J. L. Sutko, J. A. Airey, W. Welch and L. Ruest, *Pharmacol. Rev.*, 1997, **49**, 53–98.

5 For synthetic studies of ryanodine and related compounds, see: (*a*) S. Mc, N. Sieburth and E. D. Santos, *Tetrahedron Lett.*, 1994, **35**, 8127–8130; (*b*) J. L. Wood, J. K. Graeber and J. T. Njardarson, *Tetrahedron*, 2003, **59**, 8855–8858.

6 (*a*) A. Bélanger, D. J. F. Berney, H.-J. Borschberg, R. Brousseau, A. Doutheau, R. Durand, H. Katayama, R. Lapalme, D. M. Leturc, C.-C. Liao, F. N. MacLachlan, J.-P. Maffrand, F. Marazza, R. Martino, C. Moreau, L. Saint-Laurent, R. Saintonge, P. Soucy, L. Ruest and P. Deslongchamps, *Can. J. Chem.*, 1979, **57**, 3348–3354; (*b*) P. Deslongchamps, A. Bélanger, D. J. F. Berney, H.-J. Borschberg, R. Brousseau, A. Doutheau, R. Durand, H. Katayama, R. Lapalme, D. M. Leturc, C.-C. Liao, F. N. MacLachlan, J.-P. Maffrand, F. Marazza, R. Martino, C. Moreau, L. Ruest, L. Saint-Laurent, R. Saintonge and P. Soucy, *Can. J. Chem.*, 1990, **68**, 186–192, and references cited therein.

7 (*a*) K. Hagiwara, M. Himuro, M. Hirama and M. Inoue, *Tetrahedron Lett.*, 2009, **50**, 1035–1037; (*b*) M. Iwatsu, D. Urabe and M. Inoue, *Heterocycles*, 2010, **82**, 491–504.

8 We previously reported the symmetry-driven total synthesis of merrilactone A: (*a*) M. Inoue, T. Sato and M. Hirama, *J. Am. Chem. Soc.*, 2003, **125**, 10772–10773; (*b*) M. Inoue, T. Sato and M. Hirama, *Angew. Chem., Int. Ed.*, 2006, **45**, 4843–4848; (*c*) M. Inoue, N. Lee, S. Kasuya, T. Sato, M. Hirama, M. Moriyama and Y. Fukuyama, *J. Org. Chem.*, 2007, **72**, 3065–3075.

9 For reviews on pairwise functionalization, see: (*a*) S. R. Magnuson, *Tetrahedron*, 1995, **51**, 2167–2213; (*b*) C. S. Poss and S. L. Schreiber, *Acc. Chem. Res.*, 1994, **27**, 9–17; (*c*) R. W. Hoffman, *Angew. Chem., Int. Ed.*, 2003, **42**, 1096–1109.

10 (*a*) G. A. Molander and G. Hahn, *J. Org. Chem.*, 1986, **51**, 1135–1138. For reviews, see: (*b*) G. A. Molander and C. R. Harris, *Chem. Rev.*, 1996, **96**, 307–338; (*c*) H. B. Kagan, *Tetrahedron*, 2003, **59**, 10351–10372; (*d*) J. M. Concellón and H. Rodríguez-Solla, *Chem. Soc. Rev.*, 2004, **33**, 599–609.

11 For examples on bicyclo[3.3.0]octane formation by transannular aldol reaction of cyclooctane derivatives, see: (*a*) J. T. Negri, T. Morwick, J. Doyon, P. D. Wilson, E. R. Hickey and L. A. Paquette, *J. Am. Chem. Soc.*, 1993, **115**, 12189–12190; (*b*) L. A. Paquette and F. Geng, *J. Am. Chem. Soc.*, 2002, **124**, 9199–9203. For a review of extensive studies in this field by Paquette, see: (*c*) L. A. Paquette, *Eur. J. Org. Chem.*, 1998, 1709–1728; (*d*) P. A. Wender, G. G. Gamber, R. D. Hubbard and L. Zhang, *J. Am. Chem. Soc.*, 2002, **124**, 2876–2877; (*e*) M. Zora, I. Koyuncu and B. Yucel, *Tetrahedron Lett.*, 2000, **41**, 7111–7114; (*f*) S. K. Verma, E. B. Fleischer and H. W. Moore, *J. Org. Chem.*, 2000, **65**, 8564–8573; (*g*) K. G. Dongol, R. Wartchow and H. Butenschön, *Eur. J. Org. Chem.*, 2002, 1972–1983; (*h*) T. Hamura, S. Tsuji, T. Matsumoto and K. Suzuki, *Chem. Lett.*, 2002, **31**, 280–281; (*i*) C. L. Chandler and B. List, *J. Am. Chem. Soc.*, 2008, **130**, 6737–6739. See also ref. 8.

12 D. B. Dess and J. C. Martin, *J. Org. Chem.*, 1983, **48**, 4155–4156.

13 D. Urabe, H. Yamaguchi and M. Inoue, *Org. Lett.*, 2011, **13**, 4778–4781.

14 (*a*) D. H. R. Barton and S. W. McCombie, *J. Chem. Soc., Perkin Trans. 1*, 1975, 1574–1585. For reviews, see: (*b*) D. H. R. Barton and W. B. Motherwell, *Pure Appl. Chem.*, 1981, **53**, 15–31; (*c*) D. Crich and L. Quintero, *Chem. Rev.*, 1989, **89**, 1413–1432.

15 (*a*) M. J. Robins and J. S. Wilson, *J. Am. Chem. Soc.*, 1981, **103**, 932–933; (*b*) T. R. R. Pettus, M. Inoue, X.-T. Chen and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2000, **122**, 6160–6168.

16 (*a*) R. Noyori, I. Nishida, J. Sakata and M. Nishizawa, *J. Am. Chem. Soc.*, 1980, **102**, 1223–1225; (*b*) K. A. Scheidt, H. Chen, B. C. Follows, S. R. Chemler, D. S. Coffey and W. R. Roush, *J. Org. Chem.*, 1998, **63**, 6436–6437.

17 The electron-withdrawing effect of the proximal C3-ketone would increase the reactivity of the C2-OTBS group toward the nucleophile, TAS-F, in comparison to that of the C6-OTBS group, which is close to the electron-donating tertiary C11-ether.

18 M. Scholl, S. Ding, C. W. Lee and R. H. Grubbs, *Org. Lett.*, 1999, **1**, 953–956.

19 (*a*) G. W. Gribble and D. C. Ferguson, *J. Chem. Soc., Chem. Commun.*, 1975, 535–536; (*b*) C. F. Nutaitis and G. W. Gribble, *Tetrahedron Lett.*, 1983, **24**, 4287–4290; (*c*) D. A. Evans, K. T. Chapman and E. M. Carreira, *J. Am. Chem. Soc.*, 1988, **110**, 3560–3578.

20 Compound **1** has a propensity to form the corresponding borate with B_2O_3 leached from borosilicate glassware (Pyrex glass). Therefore, soda-lime or quartz glassware was used for the purification of **1**. For a related example, see: A. Kawamura, J. Guo, Y. Itagaki, C. Bell, Y. Wang, G. T. Haupert Jr, S. Magil, R. T. Gallagher, N. Berova and K. Nakanishi, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 6654–6659.

21 A preliminary toxicity study of ryanodine, ryanodol and racemic **1** was carried out in house flies. While natural ryanodine displayed low LD_{100} values (15.6 ppm), ryanodol showed only weak toxicity (200 ppm), and **1** did not exhibit detectable activity (>200 ppm).