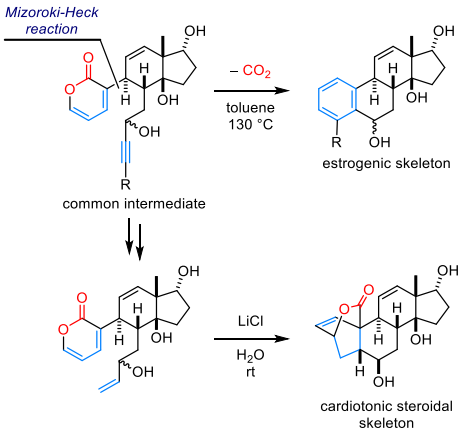
A Synthesis of Oxy-Functionalized Steroidal Skeletons via Mizoroki-Heck and Intramolecular Diels-Alder Reactions

Shogo Watanabe, Toshio Nishikawa, Atsuo Nakazaki*

Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8601, Japan

Supporting Information Placeholder



ABSTRACT: Estrogenic and cardiotonic steroidal skeletons were concisely constructed via Mizoroki-Heck and intramolecular Diels-Alder (IMDA) reactions. Simple modification of the dienophile unsaturation of the IMDA precursor enabled representative AB-ring systems of both steroid classes to be accessed from the same intermediate. The diastereoselectivity of the IMDA reaction used to access the cardiotonic steroidal skeleton was found to be significantly enhanced by performing the reaction in water.

Highly oxy-functionalized steroidal skeletons are privileged structures[1] in the context of drug discovery (Figure 1).[2] Cardiotonic steroids, such as digoxin and ouabain, and their derivatives are of particular interest,[3] having proven utility in the suppression of Th17 cell differentiation by antagonizing RORγt transcriptional activity[4] and in the inhibition of the α4-isoform of Na^+/K^+-ATPase.[5] Hence, a variety of approaches towards their synthesis have been disclosed.[6,7] However, these approaches are somewhat limited in that the target molecules obtained do not bear pendant alkene and hydroxy groups from which the oxy-functionalities necessary for the optimization of drug candidates could be derived. We hypothesized that more potent and/or isoform-selective drug candidates could be efficiently obtained if the synthesis was redesigned to proceed through an intermediate allowing facile oxy-functionalization of the steroidal skeleton. Herein, we disclose a new synthesis of the core skeletons of both estrogenic and cardiotonic steroids, both of which could serve as versatile synthetic intermediates for a wide variety of functionalized steroids.

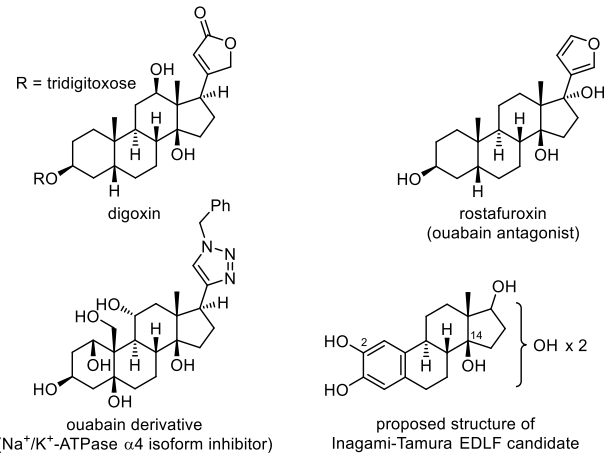
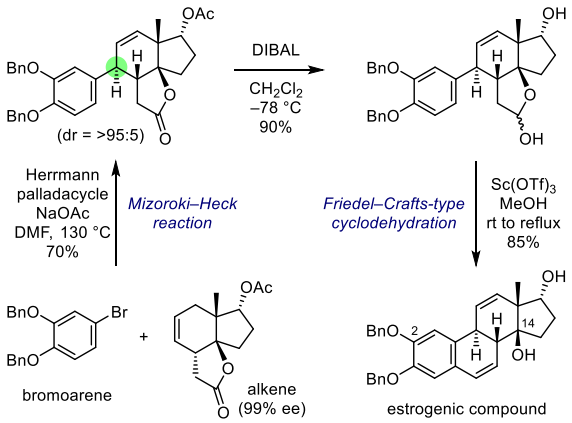


Figure 1. Structures of highly oxy-functionalized steroids and derivatives.

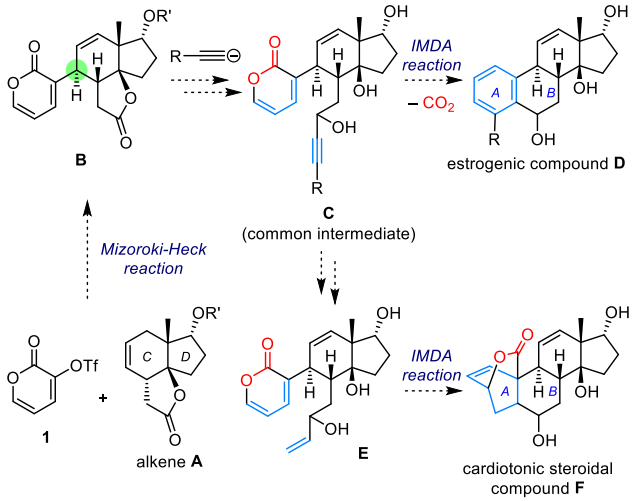
Recently, we reported the synthesis of estradiol analogues which we used to validate the proposed structures of a candidate of endogenous digitalis-like factor (EDLF)[8] (Figure 1).[9] In our synthetic approach as shown in Scheme 1, the Mizoroki-Heck reaction of bromoarene and alkene followed by a

Friedel-Crafts-type cyclodehydration were used to construct an estrogenic skeleton, which bore an alkene on the B- and C-rings for subsequent installation of the oxy-functionalities necessary to access seven regio- and stereoisomers of the 2,14β-dihydroxyestradiols. We envisaged that this synthetic approach would also be applicable to the construction of the cardiotonic steroidal skeleton; and further anticipated access of both estrogenic and cardiotonic targets via a common intermediate. For example, Mizoroki-Heck reaction of 2-pyrone triflate **1**[10] and alkene **A** followed by intramolecular Diels-Alder (IMDA) reaction of 2-pyrone[11,12] was anticipated to yield estrogenic and cardiotonic steroidal compounds **D** and **F** via common intermediate **C** (Scheme 2). IMDA reaction of **C** with concomitant decarboxylation would lead to estrogenic compound **D**; whereas partial reduction of the alkyne followed by a different IMDA reaction would produce cardiotonic steroidal compound **F** bearing a bridged lactone, which could be transformed into a series of cardiotonic steroids.

Scheme 1. Previous approach to estrogenic compound.



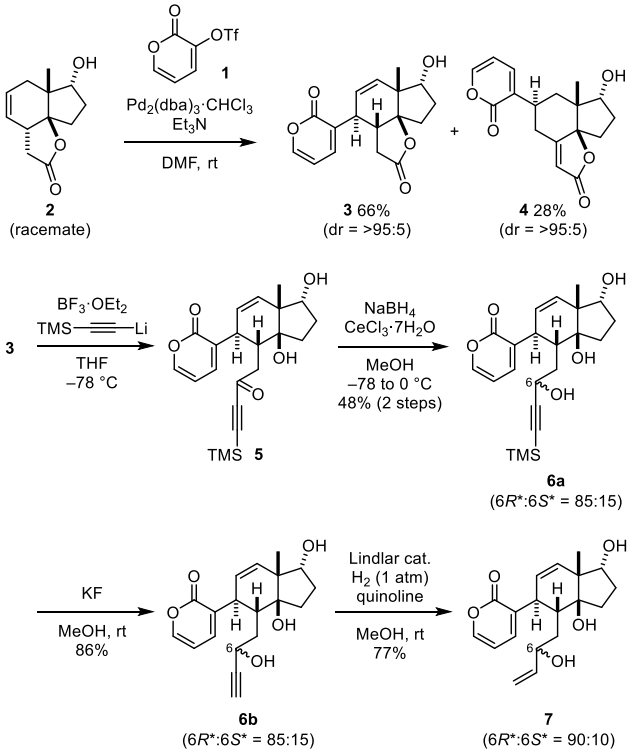
Scheme 2. Synthetic plan.



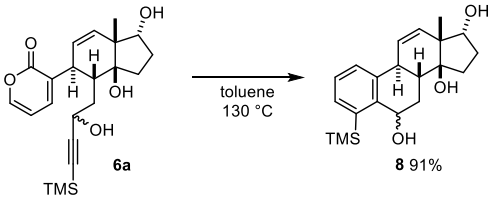
Synthesis of precursors **6a** and **7** for the IMDA reaction commenced with the Mizoroki-Heck reaction of 2-pyrone triflate **1** and alkene **2** (Scheme 3). By stirring **2**[13] and **1**, with a catalytic amount of $Pd_2(dba)_3{\cdot}CHCl_3$ and Et_3N in DMF, **3** was obtained in 66% yield as a single diastereomer, along with its regioisomer **4** in 28% yield. To obtain alkyne **6a**, lactone **3** was treated with lithium trimethylsilylacetylide in the presence of $BF_3{\cdot}OEt_2$ in THF to afford ynone **5**. Reduction of the resulting ketone of **5** using Luche conditions provided the desired alkyne **6a** in 48% yield from **3** as an 85:15 mixture of epimers. Alkene **7** was obtained in good yield by a two-step sequence involving removal of the TMS group in **6a** and subsequent half reduction of the alkyne in the resulting **6b**.

With the precursors **6a** and **7** in hand, we surveyed their reactivities in the IMDA reaction (Scheme 4 and Table 1). Upon heating alkyne **6a** in toluene at 130 °C in a sealed-tube, estrogenic compound **8** was exclusively obtained (Scheme 4).

Scheme 3. Synthesis of precursors 6a and 7 for the IMDA reaction.



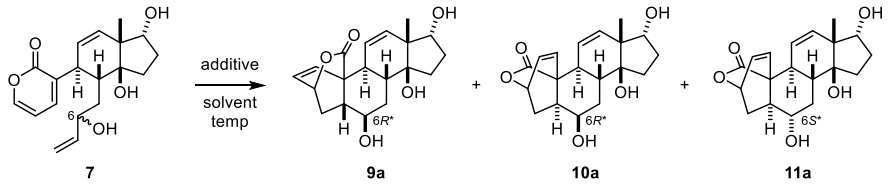
Scheme 4. IMDA reaction of alkyne 6a.



Construction of the cardiotonic steroidal skeleton was next examined (Table 1). First, alkene **7** (a 90:10 mixture of C6-epimers) was heated at 90 °C in THF in a sealed tube. The undesired cycloadducts **10a** and **11a** as major diastereomers for each starting epimer and only a trace amount of desired **9a** were obtained (entry 1);[14] the structures of **9a** and **10a** were unambiguously confirmed by single crystal X-ray diffraction analysis; the former after derivatization to the corresponding *p*-bromobenzoate **9b**.[15] The relative stereochemistry of **11a** was determined by NOESY analysis of the corresponding *p*-bromobenzoate. A variety of conventional organic solvents

were screened (THF, CH_2Cl_2, DMSO, HFIP, and MeOH) in an attempt to reverse the diastereoselectivity of this reaction; however, only the undesired diastereomers along with a substantial amount of the recovered **7** were consistently obtained (entries 2-6). In contrast, use of H_2O as the solvent dramatically increased both the reaction rate and diastereoselectivity (entry 7).[16,17] This result was not improved by conducting the reaction in THF-H_2O (entry 8) or under the Grieco conditions (ethereal $LiClO_4$, entry 9).[18] The highest diastereoselectivity was observed upon conducting the reaction in an aqueous solution of LiCl, an additive expected to increase the hydrophobic effect[19] [83% yield (**9a**:**10a**:**11a** = 59:33:8), entry 10].

Table 1. IMDA reaction of alkene **7**.



7 **9a** **10a** **11a**

entry	**7** (6*R**:6*S**)[a]	solvent	additive	temp	time (h)	cycloadducts % yield[b]	cycloadducts (**9a**:**10a**:**11a**)[a]	recovered **7** % yiled[b]	recovered **7** (6*R**:6*S**)[a]
1[c]	90:10	THF	–	90 °C	10	quant	(5:86:9)	0	–
2	92:8	THF	–	rt	96	19	(5:69:26)	81	(>95:5)
3	92:8	CH_2Cl_2	–	rt	96	10	(0:47:53)	90	(>95:5)
4	90:10	DMSO	–	rt	96	37	(10:78:12)	39	(>95:5)
5	92:8	HFIP	–	rt	96	21	(16:60:24)	79	(>95:5)
6	92:8	MeOH	–	rt	96	38	(12:71:17)	61	(>95:5)
7	92:8	H_2O	–	rt	96	85	(48:44:8)	15	(>95:5)
8	92:8	THF-H_2O	–	rt	96	64	(13:79:8)	22	(>95:5)
9[d]	90:10	Et_2O	$LiClO_4$	rt	218	67	(23:65:12)	32	(>95:5)
10[e]	90:10	H_2O	LiCl	rt	174	83	(59:33:8)	0	–

[a]Determined by ^{1}H NMR analysis. [b]Isolated yield. [c]Sealed tube was used. [d]5.0 M solution of $LiClO_4$ in Et_2O was used. [e]4.86 M aqueous solution of LiCl was used. HFIP = 1,1,1,3,3,3-hexafluoro-2-propanol.

Based on these results, stereochemical outcomes of the IMDA reaction using major epimer (6*R**)-**7** could be rationalized (Figures 2 to 4). Cycloadducts **12** and **13** were not observed, presumably because severe steric repulsions around the carbonyl moiety in disfavored *exo* **TS-3** and **TS-4**. However, *endo* **TS-1** and **TS-2** do not suffer from such steric repulsions; of these two, *endo*-chair **TS-2** is expected to be favored over *endo*-boat **TS-1** due to the chair-like conformation of the B-ring, leading to **10a** as a major diastereomer. DFT calculations also support this account for the observed diastereoselectivity (Figure 3), with a decreased free energy of activation ($\Delta\Delta G^{\ddagger}$) for **TS-1** in H_2O (+1.08 kcal/mol in H_2O for **TS-1a** vs +2.05 kcal/mol in THF for **TS-1b**).[20] Additionally, the α-face of the *endo*-boat **TS-1a** is expected to be hydrophobic owing to the orientation of the oxy-functionalities of the 2-pyrone to the β-face (Figure 4). Therefore, based on the hydrophobic nature of the α-face, the enhanced diastereoselectivity of **9a** in water (Table 1, entries 7 and 10) might also be rationalized by reference to the aggregation of substrates by hydrophobic interactions through the α-face,[21] stabilizing the favorable conformation leading to the *endo*-boat **TS-1**.

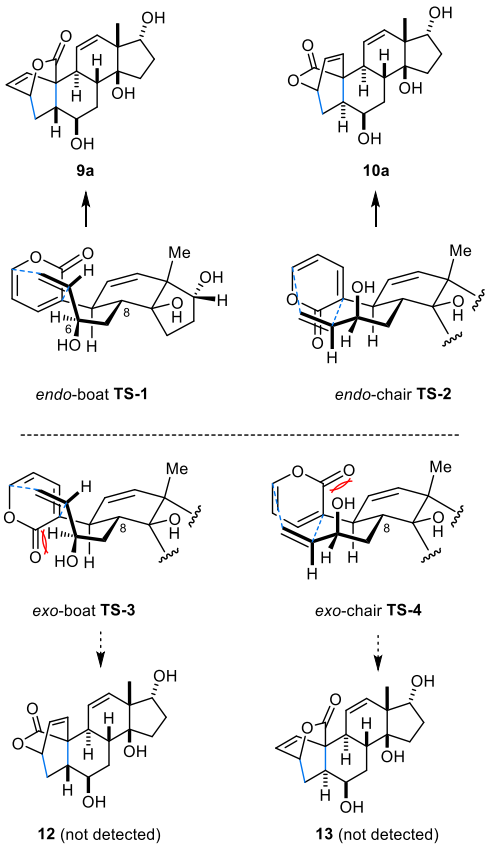


Figure 2. Possible transition state structures **TS-1** to **TS-4** of IMDA reaction using major epimer (6*R**)-**7**.

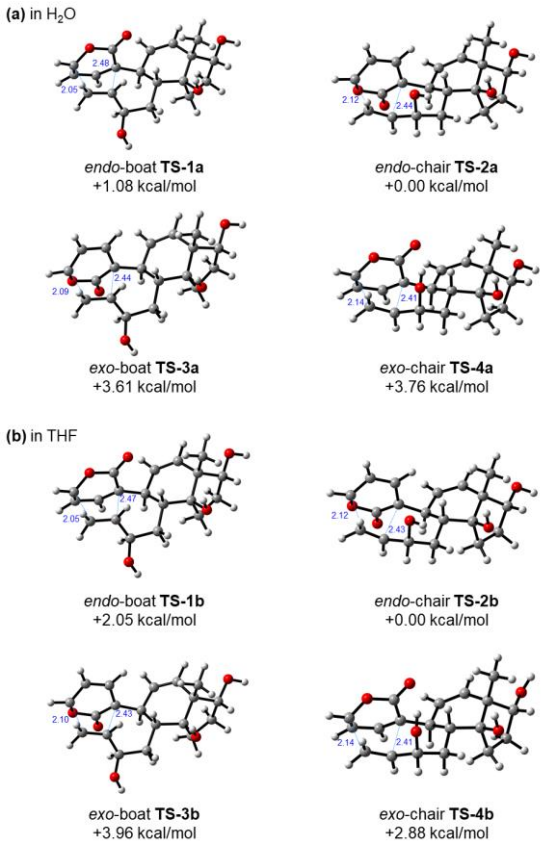


Figure 3. DFT calculated transition structures (a) **TS-1a** to **TS-4a** in H_2O and (b) **TS-1b** to **TS-4b** in THF, and their calculated free energies of activation ($\Delta\Delta G^{\ddagger}$) at the B3PW91/6-311+G(2d,2p)/SMD(solvent)//B3PW91/6-311+G(d,p)/CPCM(solvent) level of theory.

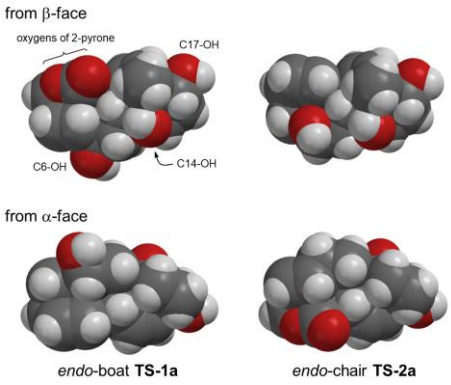


Figure 4. Space-filling model of the DFT calculated transition state structures **TS-1a** and **TS-2a** in H_2O.

In conclusion, we have successfully developed a new and concise synthesis of the highly functionalized core skeletons of the estrogenic and cardiotonic steroids, which are otherwise difficult to obtain. The key steps in our synthesis are Mizoroki-Heck and IMDA reactions, which is particularly efficient as it proceeds through a common intermediate. Because the obtained compounds bear pendant alkene and hydroxy groups,[9] they are anticipated to be useful precursors for both natural and unnatural steroids. We also made the serendipitous and unusual discovery that the diastereoselectivity of the IMDA reaction could be significantly enhanced by polar oxyfunctionalities (such as unprotected hydroxy and carbonyl groups) present in the substrate when the reaction was undertaken in water, and rationalized our findings using DFT calculations. This discovery is expected to constitute a general method to exert diastereocontrol over substrates bearing free hydroxy groups.[17,22] Its further study and application to the synthesis of biologically interesting steroids is ongoing and will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures and spectral data for all new compounds and X-ray crystallographic data for compounds **9b** and **10a** (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: nakazaki@agr.nagoya-u.ac.jp

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was financially supported by Grants-in-Aid for Scientific Research on Innovative Areas ‘‘Frontier Research on Chemical Communication’’ (No.17H06406) and “Middle Molecular Strategy” (No.18H04400), and the Program for Leading Graduate Schools: IGER program in Green Natural Sciences from MEXT. We thank Dr. Hiroyasu Sato (Rigaku Corporation) and Dr. Yasuhiro Ohki (Nagoya University) for advice on the X-ray analysis. We also appreciate to Dr. Kazunobu Igawa (Kyushu University) for fruitful suggestions of DFT calculations.

REFERENCES

(1) For a review, see: Schneider, P.; Schneider, G. Privileged Structures Revisited. *Angew. Chem. Int. Ed.* **2017**, *56*, 7971-7974.

(2) For reviews, see: (a) Salvador, J. A. R.; Carvalho, J. F. S.; Neves, M. A. C.; Silvestre, S. M.; Leitão, A. J.; Silva, M. M. C.; Luisa Sá e Melo, M. Anticancer steroids: linking natural and semi-synthetic compounds. *Nat. Prod. Rep.* **2013**, *30*, 324-374. (b) Blanco, M.-J.; La, D.; Coughlin, Q.; Newman, C. A.; Griffin, A. M.; Harrison, B. L.; Salituro, F. G. Breakthroughs in neuroactive steroid drug discovery. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 61-70.

(3) For a review, see: (a) Prassas, I.; Diamandis, E. P. Novel therapeutic applications of cardiac glycosides. *Nat. Rev. Drug Discov.* **2008**, *7*, 926-935. A recent example: (b) T. Fujii, T. Shimizu, S. Yamamoto, K. Funayama, K. Fujita, Y. Tabuchi, A. Ikari, H. Takeshima, H. Sakai, Fujii, T.; Shimizu, T.; Yamamoto, S.; Funayama, K.; Fujita, K.; Tabuchi, Y.; Ikari, A.; Takeshima, H.; Sakai, H. Crosstalk between Na^+,K^+-ATPase and a volume-regulated anion channel in membrane microdomains of human cancer cells. *BBA Mol. Basis Disease* **2018**, *1864*, 3792-3804.

(4) Huh, J. R.; Leung, M. W. L.; Huang, P.; Ryan, D. A.; Krout, M. R.; Malapaka, R. R. V.; Chow, J.; Manel, N.; Ciofani, M.; Kim, S. V.; Cuesta, A.; Santori, F. R.; Lafaille, J. J.; Xu, H. E.; Gin, D. Y.; Rastinejad, F.; Littman, D. R. Digoxin and its derivatives suppress

T_H17 cell differentiation by antagonizing RORγt activity. *Nature* **2011**, *472*, 486-490.
(5) S. S. Syeda, G. Sánchez, K. H. Hong, J. E. Hawkinson, G. I. Georg, G. Blanco, Syeda, S. S.; Sánchez, G.; Hong, K. H.; Hawkinson, J. E.; Georg, G. I.; Blanco, G. Design, Synthesis, and in Vitro and in Vivo Evaluation of Ouabain Analogues as Potent and Selective Na,K-ATPase α4 Isoform Inhibitors for Male Contraception. *J. Med. Chem.* **2018**, *61*, 1800-1820.
(6) For reviews of the synthesis of steroids, see: (a) R. Skoda-Földes, L. Kollár, Skoda-Földes, R. Kollár, L. Transition-Metal-Catalyzed Reactions in Steroid Synthesis. *Chem. Rev.* **2003**, *103*, 4095-4129. (b) Chapelon, A.-S.; Moraléda, D.; Rodriguez, R.; Ollivier, C. Santelli, M. Enantioselective synthesis of steroids. *Tetrahedron* **2007**, *63*, 11511-11616. (c) Gupta, P.; Panda, G. Asymmetric Assembly of Steroidal Tetracyclic Skeletons. *Eur. J. Org. Chem.* **2014**, 8004-8019.
(7) For reviews of the synthesis of cardiotonic steroids, see: (a) Heasley, B. Chemical Synthesis of the Cardiotonic Steroid Glycosides and Related Natural Products. *Chem. Eur. J.* **2012**, *18*, 3092-3120. (b) Michalak, M.; Michalak, K.; Wicha, J. The synthesis of cardenolide and bufadienolide aglycones, and related steroids bearing a heterocyclic subunit. *Nat. Prod. Rep.* **2017**, *34*, 361- 410. Selected examples: (c) Yoshii, E.; Oribe, T.; Tumura, K.; Koizumi, T. Studies on the Synthesis of Cardiotonic Steroids. 4. Synthesis of Strophanthidin. *J. Org. Chem.* **1978**, *43*, 3946-3950. (d) Wiesner, K.; Tsai, T. Y. R. Some recent progress in the synthetic and medicinal chemistry of cardioactive steroid glycosides. *Pure Appl. Chem.* **1986**, *58*, 799-810. (e) Kočovský, P.; Stieborová, I. Synthesis of strophanthidin. *Tetrahedron Lett.* **1989**, *30*, 4295-4298. (f) Stork, G.; West, F.; Lee, H. Y.; Issacs, R. C. A.; Manabe, S. The Total Synthesis of a Natural Cardenolide: (+)-Digitoxigenin. *J. Am. Chem. Soc.* **1996**, *118*, 10660-10661. (g) Overman, L. E.; Rucker, P. V. Enantioselective Synthesis of Cardenolide Precursors Using an Intramolecular Heck Reaction. *Tetrahedron Lett.* **1998**, *39*, 4643-4646. (h) Honma, M.; Nakada, M. Enantioselective total synthesis of (+)-digitoxigenin. *Tetrahedron Lett.* **2007**, *48*, 1541-1544. (i) Reddy, M. S.; Zhang, H.; Phonix, S.; Deslongchamps, P. Total Synthesis of Ouabagenin and Ouabain. *Chme. Asian, J.* **2009**, *4*, 725-741. (j) Jung, M. E.; Guzaev, M. Studies Toward the Enantiospecific Total Synthesis of Rhodexin A. *J. Org. Chem.* **2013**, *78*, 7518-7526. (k) Renata, H.; Zhou, Q.; Dünstl, G.; Felding, J.; Merchant, R. R.; Yeh, C.-H.; Baran, P. S. Development of a Concise Synthesis of Ouabagenin and Hydroxylated Corticosteroid Analogues. *J. Am. Chem. Soc.* **2015**, *137*, 1330-1340. (l) Urabe, D.; Nakagawa, Y.; Mukai, K.; Fukushima, K.; Aoki, N.; Itoh, H.; Nagatomo, M.; Inoue, M. Total Synthesis and Biological Evaluation of 19-Hydroxysarmentogenin-3-*O*-α-L-rhamnoside, Trewianin, and Their Aglycons. *J. Org. Chem.* **2018**, *83*, 13888-13910. (m) Khatri, H. R.; Bhattarai, B.; Kaplan, W.; Li, Z.; Long, M. J. C.; Aye, Y.; Nagorny, P. Modular Total Synthesis and Cell-Based Anticancer Activity Evaluation of Ouabagenin and Other Cardiotonic Steroids with Varying Degrees of Oxygenation. *J. Am. Chem. Soc.* **2019**, *141*, 4849-4860. (n) Peter, C.; Geoffroy, P.; Murata, T.; Tonoi, T.; Shiina, I.; Miesch, M. Diastereo-/enantioselective Diels-Alder Synthesis of 14β-hydroxysteroid scaffolds: A combined experimental and DFT study. *Heterocycles* **2019**, *99*, 1251-1275.
(8) For reviews of EDLF, see: (a) Nesher, M.; Shpolansky, U.; Rosen, H.; Lichtstein, D. The digitalis-like steroid hormones: New mechanisms of action and biological significance. *Life Sci.* **2007**, *80*, 2093-2107. (b) Schoner, W.; Scheiner-Bobis, G. Endogenous and exogenous cardiac glycosides: their roles in hypertension, salt metabolism, and cell growth. *Am. J. Physiol. Cell Physiol.* **2007**, *293*, C509-C536. (c) Bagrov, A. Y.; Shapiro, J. I. Endogenous digitalis: pathophysiologic roles and therapeutic applications. *Nature Rev. Nephrology* **2008**, *4*, 378-392. (d) Hamlyn, J. M.; Manunta, P. Endogenous Cardiotonic Steroids in Kidney Failure: A Review and an Hypothesis. *Adv. Chronic Kidney Dis.* **2015**, *22*, 232-244. (e) Blaustein, M. P. The pump, the exchanger, and the holy spirit: origins and 40-year evolution of ideas about the ouabain-Na^+ pump endocrine system. *Am. J. Physiol. Cell Physiol.* **2018**, *314*, C3-C26.
(9) Nakazaki, A.; Hashimoto, K.; Ikeda, A.; Shibata, T.; Nishikawa, T. De Novo Synthesis of Possible Candidates for the Inagami−Tamura Endogenous Digitalis-like Factor. *J. Org. Chem.* **2017**, *82*, 9097-9111.
(10) (a) Frébault, F.; Teresa Oliveira, M.; Wöstefeld, E.; Maulide, N. A Concise Access to 3-Substituted 2-Pyrones. *J. Org. Chem.* **2010**, *75*, 7962-7965. (b) Suzuki, T.; Watanabe, S.; Kobayashi, S.; Tanino, K. Enantioselective Total Synthesis of (+)-Iso-A82775C, a Proposed Biosynthetic Precursor of Chloropupukeananin. *Org. Lett.* **2017**, *19*, 922-925.
(11) For reviews of Diels-Alder reaction using 2-pyrones, see: (a) Afarinkia, K.; Vinader, V.; Nelson, T. D.; Posner, G. H. Diels-Alder Cycloadditions of 2-Pyrones and 2-Pyridones. *Tetrahedron* **1992**, *48*, 9111-9171. (b) Goel, A.; Ram, V. J. Natural and synthetic 2H-pyran-2-ones and their versatility in organic synthesis. *Tetrahedron* **2009**, *65*, 7865-7913. (c) Lee, J. S. Recent Advances in the Synthesis of 2-Pyrones. *Mar. Drugs* **2015**, *13*, 1581-1620.
(12) For reviews of approaches to steroid synthesis using Diels-Alder reaction, see: (a) Ibrahim-Ouali, M. Diels-Alder route to steroids and associated structures. *Steroids* **2009**, *74*, 133-162. (b) Mackay, E. G.; Sherburn, M. S. The Diels–Alder Reaction in Steroid Synthesis. *Synthesis* **2015**, *47*, 1-21. A recent example: (c) Fan, J.-H.; Hu, Y.-J.; Guo, Q.; Li, S.; Zhao, J.; Li, C.-C. Asymmetric synthesis of the tetracyclic core of bufogargarizin C by an intramolecular [5 + 2] cycloaddition. *Org. Chem. Front.* **2019**, *6*, 22-26.
(13) In this study, racemic **2** was used, although it is available as an enantiomerically pure form by our previous report (ref. 9).
(14) Obtained cycloadducts **9a**-**11a** were easily separable after TBS-protection of the C6 and/or C17-hydroxy group(s). See the Supporting Information for details.
(15) CCDC 1937513 (**9b**) and 1937514 (**10a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_ request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
(16) Acceleration and improvement of the diastereoselectivity could not be observed using $Sc(OTf)_3$ in MeOH. The thermal reactions at higher temperature (>50 °C) provided the ring-opened product of cycloadduct.
(17) For reviews of organic reactions in H_2O, see: (a) Li, C. J. Organic Reactions in Aqueous Media with a Focus on Carbon-Carbon Bond Formations: A Decade Update. *Chem. Rev.* **2005**, *105*, 3095-3165. (b) Lindström, U. M. *Organic Reactions in Water: Principles, Strategies and Applications*, Blackwell Publishing, Oxford, 1st edn, 2007. (c) Butler, R. N.; Coyne, A. G. Water: Nature's Reaction Enforcers–Comparative Effects for Organic Synthesis "In-Water" and "On-Water". *Chem. Rev.* **2010**, *110*, 6302-6337.
(18) Grieco, P. A.; Nunes, J. J.; Gaul, M. D. Dramatic rate accelerations of Diels-Alder reactions in 5 M lithium perchlorate-diethyl ether: the cantharidin problem reexamined. *J. Am. Chem. Soc.* **1990**, *112*, 4595-4596.
(19) Rideout, D. C.; Breslow, R. Hydrophobic Acceleration of Diels-Alder reactions. *J. Am. Chem. Soc.* **1980**, *102*, 7816-7817.
(20) DFT calculations also support rationalizing the observed stereochemical outcome of the reaction using minor epimer (6*S**)-**7**. See the Supporting Information for details.
(21) For a review of hydrophobic interactions, see: Otto, S.; Engberts, J. B. F. N. Hydrophobic interactions and chemical reactivity. *Org. Biomol. Chem.* **2003**, *1*, 2809-2820.
(22) For a review of stereoselective organic reactions in water, see: Lindström, U. M. Stereoselective Organic Reactions in Water. *Chem. Rev.* **2002**, *102*, 2751-2772.