Full Paper

Israel Journal of Chemistry

DOI: 10.1002/ijch.201600110

Synthesis of the Right-Side Structure of Type B Physalins

Masaki Morita,[a, b] Shuntaro Kojima,[a] Megumi Ohkubo,[a] Hiroyuki Koshino,[a, c] Daisuke Hashizume,[d] Go Hirai,*[a, b, c] Keiji Maruoka,[e] and Mikiko Sodeoka*[a, b, c]

This article is dedicated to Professors K. C. Nicolaou and Stuart L. Schreiber in celebration of their 2016 Wolf Prize.

Abstract: We present a full account of our synthetic studies on the racemic DEFGH-ring moiety of physalins, featuring domino ring transformation of a tricyclic key intermediate. We also report the results of a detailed mechanistic examination of the domino ring transformation, as well as a reoptimization of the 2,3-Wittig rearrangement and methylation steps. Furthermore, we have newly established a method for the preparation of an optically active synthetic intermediate by enzymatic kinetic resolution. Our work provides access to both natural and nonnatural right-side physalin structures.

Keywords: domino reactions · natural products · optical resolution · physalins · steroids

1. Introduction

Physalins are the bitter components of *Physalis* plants, and were first isolated from winter cherry in 1852.[1] A century later, Matsuura and coworkers determined the structures of two bitter substances isolated from the leaves of *Physalis alkekengi* var. *franchetii* and named them physalin A[2] (**1**) and physalin B[3] (**2**) (Figure 1). Since then, more than 30 physalins have been identified.[4,5] Physalins share a unique 13,14-*seco*-16,24-cycloergostane skeleton, with a highly oxygenated, complex structure. Type B physalins, such as physalin B, have an H-ring with a *C*14–O–*C*27 bond and a cage-shaped structure, while Type A physalins do not. Prior to the work of our group, no synthetic study of physalins had been reported, other than derivatization of natural products.

In addition to the intriguing structure, there is considerable interest in the biological activities of physalins, which include antitumor activity,[6,7] anti-inflammatory activity,[8] and inhibitory activity on NF-κB signaling.[9] Recently, inhibition of the hedgehog signaling pathway[10] and the ubiquitin proteasome pathway[11] have also been reported. Thus, physalins have the potential to regulate a broad range of biological events. However, the mechanisms of these biological activities at the molecular level remain unknown. The AB-ring of physalins, which is commonly found in plant steroids, has been suggested to be involved in these biological activities. For instance, Ma and coworkers suggested that the A-ring of physalin A could form a covalent bond with cysteine residues of IKKβ.[12] In contrast, little attention has been paid to the contribution of the cage-shaped right-side structure of Type B physalins. We hypothesized that this unique partial structure would play an important role in the biological activity.

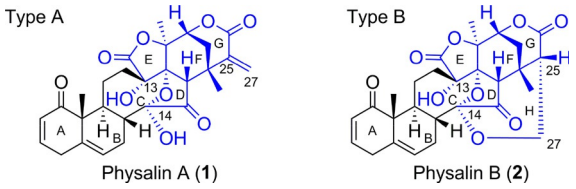


Figure 1. Structure of physalins **1** and **2**.

[a] M. Morita, S. Kojima, M. Ohkubo, H. Koshino, G. Hirai, M. Sodeoka
Synthetic Organic Chemistry Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198 (Japan)
Tel.: (+81) 48-467-9373, Fax: (+81) 48-462-4666
e-mail: gohirai@riken.jp
sodeoka@riken.jp

[b] M. Morita, G. Hirai, M. Sodeoka
AMED-CREST, 2-1 Hirosawa, Wako, Saitama 351-0198 (Japan)

[c] H. Koshino, G. Hirai, M. Sodeoka
RIKEN Center for Sustainable Resource Science, 2-1 Hirosawa, Wako, Saitama 351-0198 (Japan)

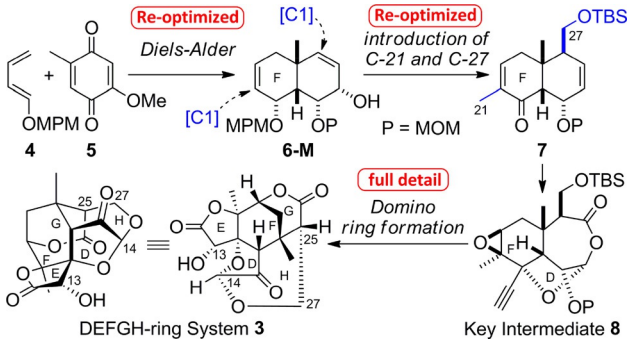
[d] D. Hashizume
RIKEN Center for Emergent Matter Science, 2-1 Hirosawa, Wako, Saitama 351-0198 (Japan)

[e] K. Maruoka
Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502 (Japan)

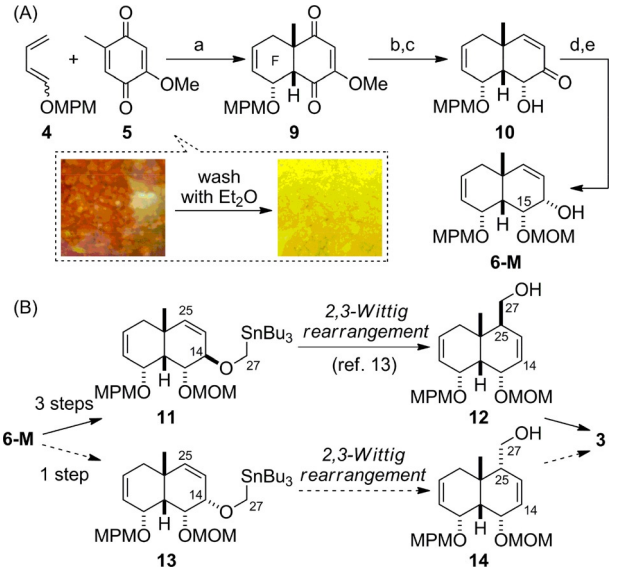
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ijch.201600110.

Israel Journal of Chemistry

We have already succeeded in synthesizing the cage-shaped molecule **3** (Scheme 1),[13,14] and we confirmed that the right-side structure of physalins indeed contributes to NF-κB-inhibitory activity.[5] The synthesis of **3** featured precise construction of tricyclic key intermediate **8** through a Diels–Alder reaction and the installation of two C1 units, and its "domino ring transformation", leading to DEFGH-ring compound **3**. In this full paper, we present full details of the domino ring transformation to **3**, together with the results of reoptimization of the synthesis of **8**, and a newly established method to prepare optically pure intermediate **6-M** by enzymatic resolution.



Scheme 1. Synthesis of DEFGH-ring compound **3**. MOM = methoxymethyl, MPM = *para*-methoxyphenylmethyl, P = protecting group, TBS = *tert*-butyldimethylsilyl.



Scheme 2. (A) Construction of *cis*-decalin framework; (B) plan for introduction of the *C*27 moiety by 2,3-Wittig rearrangement. Reaction conditions: a) toluene, 100 °C, 2 days (77%); b) DIBAL-H, CH_2Cl_2, 0 °C; c) 10% H_2SO_4 aq., CH_2Cl_2, RT (74%, over 2 steps); d) MOMCl, iPr_2NEt, CH_2Cl_2, reflux (98%); e) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, RT (98%). aq. = aqueous solution; DIBAL-H = diisobutylaluminium hydride; RT = room temperature.

2. Results and Discussion

2.1 Synthesis of Tricyclic Intermediate

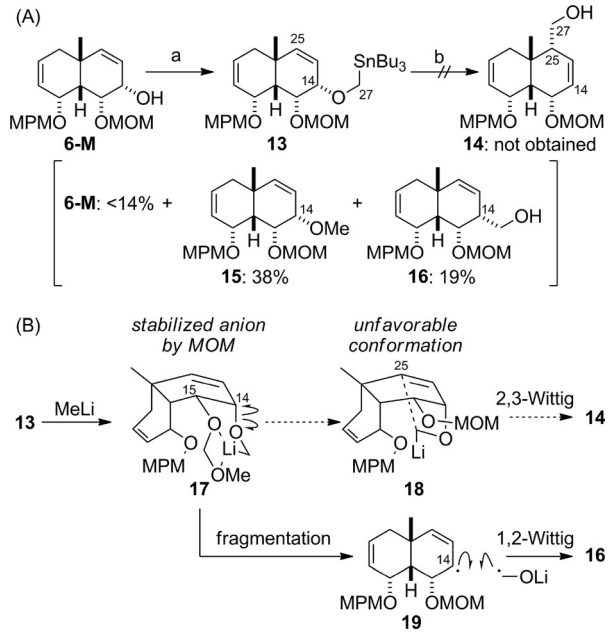
The preparation of **8** was commenced with the Diels–Alder reaction of **4**[15] with **5**[16] (Scheme 2A) to afford bicyclic **9** in moderate yield. However, we encountered poor reproducibility (25–65% yield) in this reaction, especially at a gram scale. We speculated that impurities in quinone **5** might be responsible for this, and found that **5** could be obtained as a pale yellow solid by washing the crude brown solid with ether. When purified **5** was subjected to a Diels–Alder reaction, **9** was obtained in 77% yield in a reproducible manner. The two carbonyl groups were reduced with DIBAL-H, and the resulting diol was converted to enone **10** by acid treatment. Protection of *OH*15 with MOM followed by Luche reduction gave α-allylic alcohol **6-M**.

The next key step is 2,3-Wittig rearrangement to introduce a hydroxymethyl group at *C*25. In our original report,[13] β-allylic alcohol derivative **11** was utilized as a substrate (Scheme 2B). The stereochemistry of *C*25 generated by 2,3-Wittig rearrangement should not be critical, because it is lost during the domino ring transformation. If homoallylic alcohol **14**, which would be obtained by 2,3-Wittig rearrangement of **13**, is applicable to the synthesis of **3**, it would be possible to reduce the number of synthetic steps.

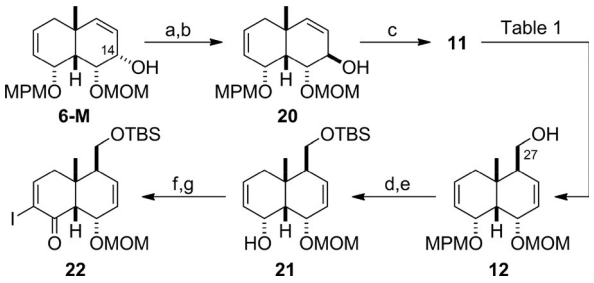
The precursor **13** was prepared by Williamson ether synthesis with ICH_2SnBu_3 (Scheme 3A).[17] Unfortunately, treatment of **13** with MeLi[18] in THF at −5 °C did not give 2,3-Wittig rearrangement product **14** at all. Instead, allylic alcohol **6-M**, methyl ether **15**, and primary alcohol **16** were obtained, probably owing to stabilization of lithiated intermediate **17** by interaction with the MOM group, as well as the unfavorable transition state **18** (Scheme 3B). α-Elimination or protonation of **17** leads to the formation of **6-M** or **15**, respectively, and 1,2-Wittig rearrangement of **19** yielded **16**. Although we examined changing the alkyl lithium reagent, solvent, and temperature, **14** was not produced at all, suggesting that introduction of the hydroxymethyl group should be performed from the original precursor **11**.

For 2,3-Wittig rearrangement of **11**, *OH*14 in **6-M** was inverted to afford the corresponding β-alcohol **20** by a Mitsunobu reaction, followed by solvolysis.[19] Preparation of **11** was accomplished in a similar manner to that described for **13** (Scheme 4). In sharp contrast to the reaction of **13**, a 2,3-Wittig rearrangement of **11** proceeded well. When **11** was treated with *n*-BuLi in THF, the desired primary alcohol **12** was obtained in 61% yield, along with **20** in 7% yield and **24** in 15% yield (Table 1, entry 1). Further optimization using *n*-BuLi turned out to be ineffective. We then switched to MeLi,[18] and found that the yield of **12** gradually increased as the reaction

Israel Journal of Chemistry



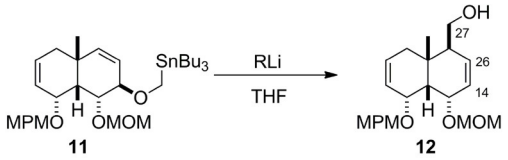
Scheme 3. (A) A trial for 2,3-Wittig rearrangement from **13**; (B) intermediates for 2,3-Wittig rearrangement, and a plausible mechanism for formation of **16**. Reaction conditions: a) Bu_3SnCH_2I, NaH, DMF-THF (3:2), RT (88%); b) MeLi (5 equiv.), THF, −5 °C (**6-M**: <14%, **15**: 38%, **16**: 19%). DMF = *N*,*N*-dimethylformamide; THF = tetrahydrofuran.



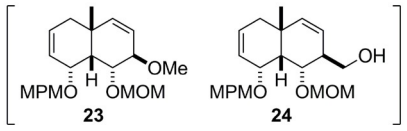
Scheme 4. Synthesis of **22**. Reaction conditions: a) DIAD, PPh_3, pNO_2-$C_6H_4CO_2H$, THF, RT; b) K_2CO_3, MeOH-Et_2O (3:1), RT (86%, over 2 steps); c) Bu_3SnCH_2I, NaH, DMF-THF (1:1), RT (76%); d) TBSCl, Et_3N, DMAP, CH_2Cl_2, RT; e) DDQ, CH_2Cl_2, pH 7 buffer, RT (68%, over 3 steps from **11**); f) MnO_2, CH_2Cl_2, RT (93%); g) $TMSN_3$; then I_2, Py, CH_2Cl_2, 0 °C to RT (97%). DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DIAD = diisopropylazodicarboxylate; DMAP = 4-dimethylaminopyridine; Py = pyridine, TMS = trimethylsilyl.

temperature was raised, and the formation of **20** was suppressed. Finally, **12** was obtained in 87% yield as a result of careful control of the reaction temperature at −5 °C; this is a better result than in our original report.[13,20] The higher temperature probably favors the appropriate conformation for the rearrangement. These results clearly indicate that the stereochemistry of the precursor is critical in this case. Since it was difficult to separate the mixture of **12**, **20**, and **24** by normal silica gel chromatography, the mixture was subjected to protection of *OH*27 with TBS,

Table 1. Optimization of the 2,3-Wittig rearrangement from **11**.



entry	RLi (equiv)	temperature	**12**[a]	**20**[a]	**23**	**24**[a]
1	^{n}BuLi (9)	0 °C to RT	61%	7%	-	15%
2	MeLi (5)	-78 °C	15%	85%	-	-
3	MeLi (5)	-40 °C	63%	11%	24%	1%
4	MeLi (5)	-5 °C	87%	4%	-	9%

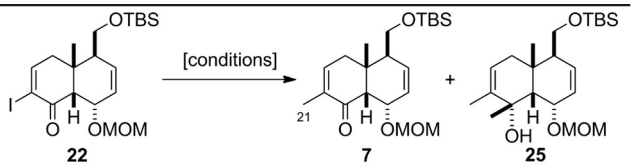


[a] The yields of **12**, **20**, and **24** were calculated based on the product ratio determined by ^{1}H NMR of the mixture (see Supporting Information).

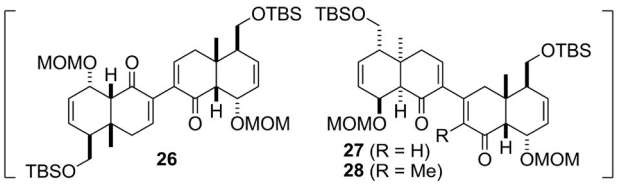
and deprotection of MPM, to give allylic alcohol **21** in pure form. MnO_2 oxidation and iodination[21] of the α-position of the resulting enone afforded **22**.

The introduction of the *C*21 methyl group was originally achieved by iron-mediated coupling,[22] and α-methylenone **7** was obtained in 75% yield. However, the reproducibility of this reaction was also found to be problematic. Namely, predominant formation of tertiary alcohol **25** was occasionally observed (Table 2, entry 1). Although the formation of **7** is likely to depend on the addition speed of MeMgBr (slow addition of MeMgBr led to significant formation of **25**), we investigated other transition metal-catalyzed methods for methylation of **22** to obtain

Table 2. Optimization of methylation to introduce *C*21.



entry	conditions	yield of **7**	byproducts
1	MeMgBr, $Fe(acac)_3$, NMP, THF	8–75%	**25**: 91–8%
2	Me_4Sn, Pd_2dba_3, Ph_3As, CuI, NMP	29%	**26**: 15%
3	$(MeOB)_3$, $PdCl_2(dppf)$, Cs_2CO_3, dioxane	51%	**27**: 19%, **28**: 15%
4	Me_2Zn, $PdCl_2(PPh_3)_2$, DMF-THF (1:1)	75%	**26**: 23%

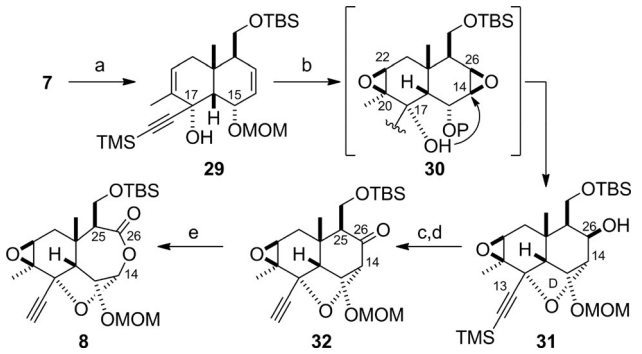


acac = acetylacetonate; dba = dibenzylideneacetone, dppf = 1,1′-bis(diphenylphosphino)ferrocene; NMP = *N*-methylpyrrolidone.

Israel Journal of Chemistry

the desired **7** more consistently. Although Stille coupling using tetramethyltin[23] or Suzuki–Miyaura coupling using trimethylboroxine[24] gave **7** in moderate yield, the concomitant formation of dimers **26** (entry 2) or **27** and **28** (entry 3) was observed. Although Negishi coupling with dimethylzinc[25] also generated dimer **26**, the desired **7** was obtained in 75% yield, comparable with the best result (entry 1), but in a more reproducible manner (entry 4).

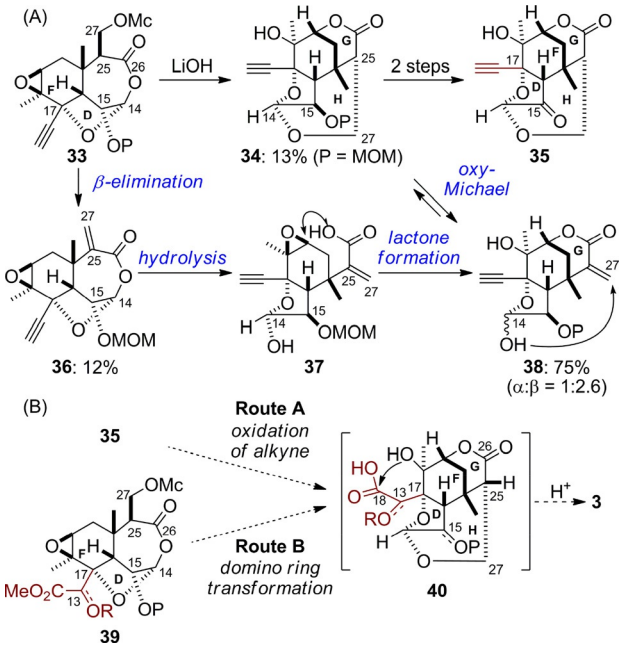
The diastereoselective addition of an alkyne to **7** was achieved with lithium acetylide in the presence of anhydrous $CeCl_3$, without β-elimination of the oxygen functionality at *C*15 (Scheme 5). Epoxidation of diene **29** from the convex face with *m*CPBA was accompanied by tetrahydrofuran formation through attack of *OH*17 on the right-side epoxide to give **31**. Desilylation and Dess–Martin oxidation of the secondary alcohol afforded ketone **32**. Baeyer–Villiger oxidation of **32** with *m*CPBA favored rearrangement of the more electron-rich *C*14 carbon, and **8** was obtained.



Scheme 5. Synthesis of key intermediate **8**. Reaction conditions: a) TMS-acetylene, *n*BuLi, $CeCl_3$, THF, −78 °C (96%); b) *m*CPBA (10 equiv.), CH_2Cl_2, 0 °C to RT (73%); c) K_2CO_3, MeOH, RT; d) Dess–Martin periodinane, CH_2Cl_2, RT (92%, over 2 steps); e) *m*CPBA, $NaHCO_3$, CH_2Cl_2, RT (92%). *m*CPBA = *m*-chloroperbenzoic acid.

2.2 Domino Ring Transformation

We had earlier established a synthetic methodology for the DFGH-ring system **35**,[13] lacking the E-ring. The DFGH-ring core **34** was constructed from a tricyclic framework via domino ring transformation (Scheme 6A). By simple LiOH treatment of **33**, which was prepared from **8** in 2 steps,[13] the DFGH-ring compound **34** was obtained in 13% yield, along with hemiacetal **38** in 75% yield and *exo*-olefin **36** in 12% yield. Based on the formation of these by-products, we propose the following consecutive mechanism. Treatment with LiOH would lead to β-elimination (**33**→**36**), and hydrolysis of the seven-membered lactone would occur (**36**→**37**), forming hemiacetal and carboxylate. The resulting carboxylate would intramolecularly attack the epoxide to form the G-ring (**37**→**38**), and then the hemiacetal would attack the α,β-unsaturated lactone via an oxy-Michael reaction to form the H-ring (**38**→**34**). We also demonstrated that **34** and **38** are in equilibrium (**34**:**38** = ~1:6) under the reaction conditions, indicating that **38** is more stable than **34**. This thermodynamically controlled process prevented further optimization to improve the yield of **34**. Deprotection and oxidation of *OH*15 afforded **35**.

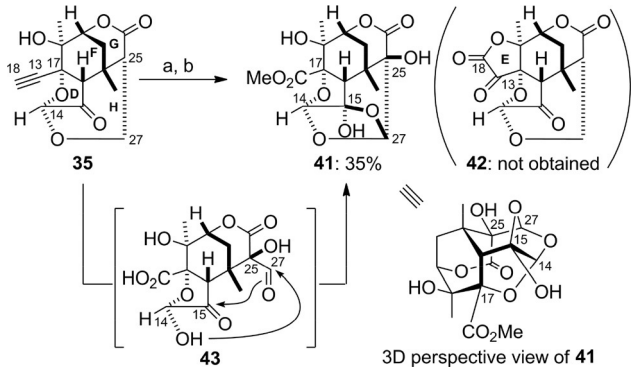


Scheme 6. (A) Synthesis of DFGH-ring compound **35** via domino ring transformation from **33** to **34**; (B) two plans for construction of the E-ring, leading to DEFGH-ring system **3**. Mc = monochloromethanesulfonyl.

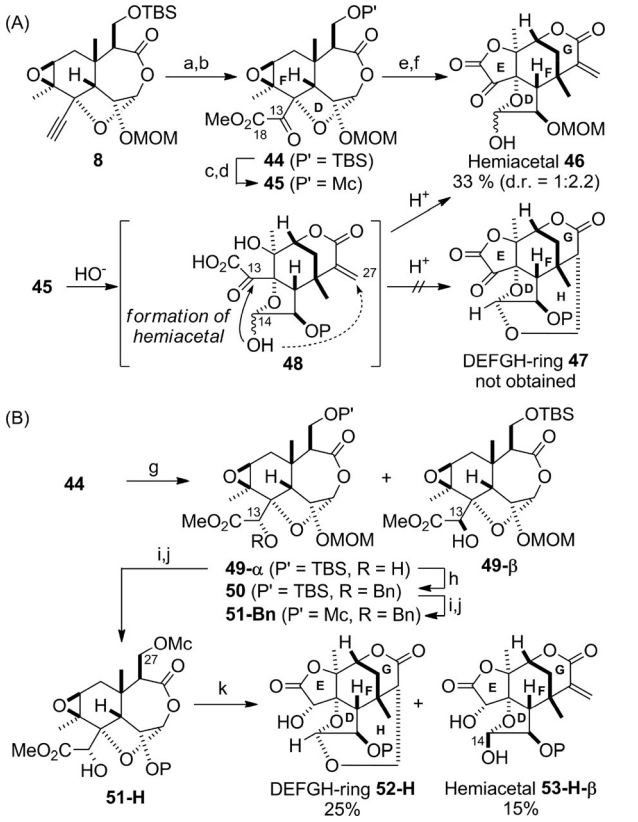
To obtain the DEFGH-ring system **3**, we designed two synthetic routes (Scheme 6B). In Route A, the alkyne moiety of **35** would be directly oxidized to α-ketocarboxylic acid **40**, which would be transformed to **3** by acid treatment and reduction of the ketone at *C*13. In this route, the stability of the DFGH-ring system under oxidative conditions is critical. In Route B, the α-keto or α-hydroxyester moiety should be installed in advance. Domino ring transformation of **39** would form the GH-ring to furnish carboxylic acid **40**, as in the case of **33**. Subsequent acid treatment would afford **3** after deprotection and oxidation of *OH*15. We expected that the stability advantage of the DEFGH-ring (like Type B physalins) over the corresponding hemiacetal DEFG-ring (like Type A physalins) would be enhanced, compared with that observed in **34** and **38**, because most natural physalins possess Type B structure.

We first examined Route A. Unfortunately, treatment of **35** with $KMnO_4$ and $NaIO_4$[26] in ^tBuOH-H_2O, followed by acidification, did not provide **42** (Scheme 7). Instead, the generation of polar intermediates (probably carboxylic acid) was observed by TLC analysis. Thus, the resulting reaction mixture was treated with $TMSCHN_2$. Unexpect-

Israel Journal of Chemistry



Scheme 7. Trial for construction of the E-ring via Route A. Reaction conditions: a) $KMnO_4$, $NaIO_4$, $NaHCO_3$, *t*BuOH-H_2O (2:1), 50 °C, 0.1 N HCl aq.; b) $TMSCHN_2$, Et_2O-MeOH (1:1), RT (35%, over 2 steps).



Scheme 8. Trial for construction of the E-ring via Route B. (A) Synthesis of precursor **45** and its domino ring transformation; (B) synthesis of precursors **51-H** and **51-Bn** and domino ring transformation of **51-H**. Reaction conditions: a) $KMnO_4$, $NaIO_4$, $NaHCO_3$, *t*BuOH-H_2O (2:1), 50 °C, 0.1 N HCl aq.; b) $TMSCHN_2$, Et_2O-MeOH (1:1), RT (88%, over 2 steps); c) HF·Py, CH_2Cl_2-Py (1:1), 0 °C (96%); d) McCl, Py, 0 °C (87%); e) LiOH·H_2O (4 equiv.), THF-H_2O (1:1), RT, 1.5 h; 1 N HCl aq.; f) 1 N HCl aq., Et_2O-H_2O (1:1), RT (33%, *d.r.* = 1:2.2, over 2 steps); g) $NaBH_4$, $CeCl_3$·$7H_2O$, MeOH, −78 °C (99%, $\alpha:\beta = 9:1$); h) NaH, BnBr, $n$$Bu_4NI$, THF-DMF (1:1), 0 °C (63%); i) HF·Py, CH_2Cl_2-Py (1:1), 0 °C; j) McCl, Py, −30 °C (for **51-H**: 98%, over 2 steps; for **51-Bn**: 77%, over 2 steps); k) LiOH·H_2O (4 equiv.), THF-H_2O (1:1), RT, 1.5 h; 1 N HCl aq., RT, 4 h (**52-H**: 25%, **53-H-*β***: 15%). Bn = benzyl; *d.r.* = diastereomeric ratio.

edly, the only isolated product was *C*15–O–*C*27 bridged hemiacetal **41**, which does not have a *C*18 carbon. Although the precise mechanism of formation of **41** remains unclear, we considered that intermediate **43** would be generated by opening of the H-ring and oxidation of *C*25 and *C*27. Sequential formation of hemiacetal from *OH*14, *CHO*27, and ketone at *C*15 might give **41**. These results indicate that the oxidation of alkyne in the presence of H-ring acetal and/or ketone in Route A is not a promising strategy for the synthesis of **3**.

Then, we examined Route B (Scheme 6B). Oxidation of alkyne **8** with $KMnO_4$ and $NaIO_4$ proceeded without loss of *C*18, and the resulting carboxylic acid was esterified with $TMSCHN_2$, affording the desired α-ketoester **44** in 88% yield. Removal of the TBS group and installation of a monochloromethanesulfonyl (Mc) group furnished **45** (Scheme 8A).

As in the case of **33**, **45** was treated with LiOH in THF-H_2O. A new spot, probably due to the α-ketoacid species derived from hydrolysis of the α-ketoester, was observed on TLC. After 1.5 h, the reaction mixture was acidified with 1 N HCl aq., and a new, less-polar spot appeared on TLC. Unfortunately, the product was hemiacetal **46**, and the target molecule **47** was not detected at all. This result indicates that although the E-ring was successfully formed by acid treatment, the H-ring was not formed in the presence of α-ketolactone moiety. This might be due to transient formation of a hemiacetal between *OH*14 and the ketone at *C*13, which would block the desired H-ring-forming oxy-Michael reaction. Another possibility is instability of the putative product **47**, because it has an sp^2 carbon at *C*13, whereas physalins have an sp^3 carbon at this position.

Then we prepared **51** bearing an α-hydroxyester group by reduction of the α-ketoester. Although the ketone at *C*13 is sterically hindered, it is expected to be reduced preferentially, due to its higher electrophilicity, compared with lactone, and ester functionality. Indeed, Luche reduction of **44** afforded the hydroxyester **49** in 99% yield ($\alpha:\beta = 9:1$, Scheme 8B). Removal of the protecting group at *OH*27 (P′) and introduction of an Mc group provided precursor **51-H**. Benzyl-protected precursor **51-Bn** was also prepared as follows. Treatment of **49-α** with BnBr and NaH in the presence of $^nBu_4NI$ gave **50**, which was then converted to **51-Bn** in two steps.

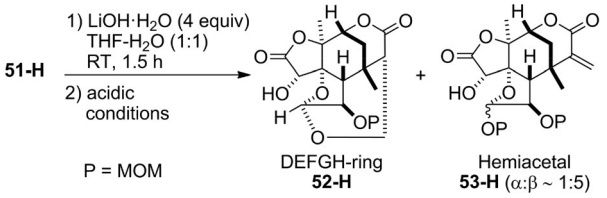
With the precursors in hand, we examined the domino ring transformation of **51-H**. When **51-H** was treated with 4 equiv. of LiOH for 1.5 h, almost complete conversion of the substrate, with generation of a carboxylic acid species, was observed on TLC. Pleasingly, subsequent acidification with 1 N HCl aq. predominantly afforded the target DEFGH-ring compound **52-H** in 25% yield, along with hemiacetal **53-H-β** in 15% yield. This result clearly indi-

Israel Journal of Chemistry

cates that the keto functionality in **45** disturbed H-ring formation.

We optimized the acidic conditions, because the low mass balance should be attributable to inefficiency of lactone formation (Table 3). Use of concentrated HCl resulted in cleavage of the MOM group, and no desired product was obtained (entry 2). Employment of an AcOH-H_2O (20:1) system[27] at higher temperature improved the outcome (entries 3–6). When the acid treatment was conducted at 100 °C for 1 h, a mixture of **52-H** and **53-H** was obtained in 70% total yield (entry 5). Treatment for a shorter or longer time decreased the yield (entries 7 and 8). While the content of water did not affect the reaction efficiency (entries 9 and 10), replacement of acetic acid with dichloroacetic acid resulted in deprotection of the MOM group (entry 11).

Table 3. Optimization of acidic conditions for domino ring transformation of **51-H**.



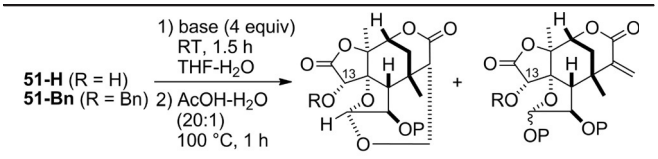
entry	acidic conditions	temperature	time	**52-H**	**53-H**
1	1 N HCl aq.	RT	4 h	25%	15%
2	conc. HCl aq.	RT	1 h	0%	0%
3	AcOH-H_2O (20:1)	RT	1 h	13%	16%
4	AcOH-H_2O (20:1)	80 °C	1 h	23%	20%
5	**AcOH-H_2O (20:1)**	**100 °C**	**1 h**	33%	37%
6	AcOH-H_2O (20:1)	reflux	1 h	14%	26%
7	AcOH-H_2O (20:1)	100 °C	20 min	24%	18%
8	AcOH-H_2O (20:1)	100 °C	3 h	15%	16%
9	AcOH-H_2O (2:1)	100 °C	1 h	29%	27%
10	AcOH	100 °C	1 h	29%	32%
11	DCA-H_2O (20:1)	100 °C	1 h	0%	0%

Ac = acetyl; conc. = concentrated; DCA = dichloroacetic acid.

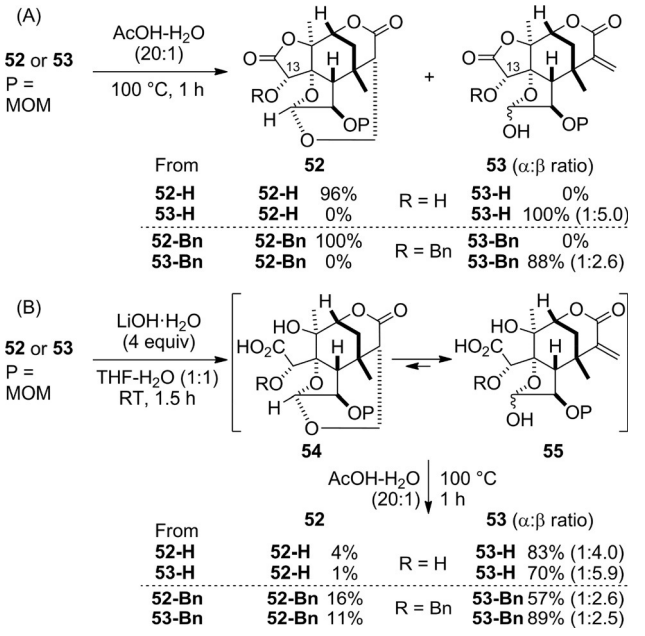
We next examined various basic conditions to improve the ratio of **52**/**53**. As shown in Table 4, the use of 4 equiv. of base at room temperature for 1.5 h was found to be the best, and shorter or longer reaction times resulted in decreased yield of **52**.[14] Reduction of the water ratio decreased the formation of **52-H** (entry 2). The counter cation also had a significant influence, and LiOH gave the best result (entries 3 and 4). When we applied the optimal conditions (entry 1) to the Bn-protected **51-Bn**, the desired **52-Bn** was obtained in a better yield (50%, entry 5), along with hemiacetal **53-Bn** (44%).

In contrast to the domino ring transformation of **33**, the product ratio of **52** and **53** varied, depending upon the reaction conditions, indicating that the product ratio is probably not determined by the relative stability of **52** and **53**. Treatment of **52** or **53** under acidic conditions did not induce isomerization, indicating that H-ring formation or breakage did not proceed upon AcOH treatment (Scheme 9A). On the other hand, treatment of DEFGH-ring compound **52** under basic conditions generated a polar intermediate (like **54** and **55**) and subsequent acid treatment gave **53** as a major product, regardless of the protecting group on *OH*13. When the same experiment was conducted starting from hemiacetal **53**, DEFGH-ring compound **52** was produced only in up to 11% yield. These results clearly indicate that formation of the H-ring from **55** to **54** by oxy-Michael reaction under the basic conditions is an unfavorable process. In other words, the formation of the H-ring occurred via a different process. Indeed, when we treated **51-H** or **51-Bn** with 2 equiv. of

Table 4. Optimization of basic conditions for domino ring transformation of **51**.



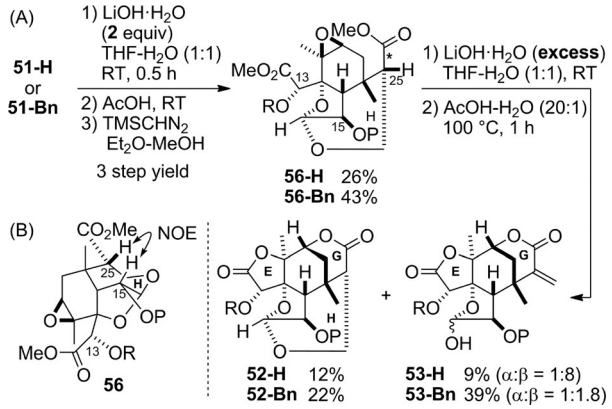
entry	precursor	base	solvent ratio	**52**	**53** (α:β ~ 1:5)
1	**51-H**	LiOH·H_2O	1:1	**52-H**: 33%	**53-H**: 37%
2	**51-H**	LiOH·H_2O	3:1	**52-H**: 19%	**53-H**: 30%
3	**51-H**	NaOH	1:1	**52-H**: 25%	**53-H**: 39%
4	**51-H**	KOH	1:1	**52-H**: 28%	**53-H**: 40%
5	**51-Bn**	LiOH·H_2O	1:1	**52-Bn**: 50%	**53-Bn**: 44%



Scheme 9. (A) Retreatment of products under acidic conditions; (B) retreatment of products under basic conditions, followed by acidic conditions.

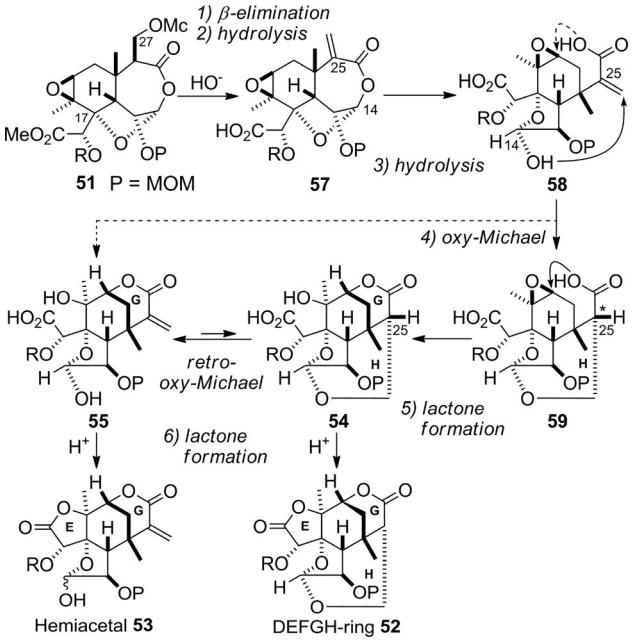
Israel Journal of Chemistry

LiOH for 30 min, followed by acidification and esterification, diester **56-H** or **56-Bn** bearing the H-ring without the G-ring, was isolated as a single isomer in 26% and 43% yield, respectively, along with **52** and **53** (Scheme 10).[28] Furthermore, treatment of **56** with an excess amount of LiOH, followed by acid treatment, provided **52** and **53**,[28,29] indicating that the corresponding dicarboxylic acid was probably formed as an intermediate during domino ring transformation.



Scheme 10. (A) Identification and retreatment of intermediates; (B) NOE correlation of **56**.

A plausible mechanism of the domino ring transformation from **51** is shown in Scheme 11. As in the reaction from **33**, β-elimination of $^-$OMc and hydrolysis of methyl-ester and seven-membered lactone would give α,β-unsaturated carboxylic acid **58**. Then, 7-*endo* oxy-Michael addition[30] would occur to form the H-ring, followed by diastereoselective protonation at *C*25 to generate **59**. Subsequent formation of δ-lactone through ring opening of the epoxide by the carboxylic acid would give **54**. Finally, acid treatment would form γ-lactone to furnish **52**. This proposal is supported by the observation that the process from **55** to **54** is unfavorable, together with the formation of **56. 54** should be kinetically formed and gradually converted to **55** by retro-oxy-Michael reaction, leading to hemiacetal **53**. The substituent at *C*17 favors domino ring transformation, and thus efficient formation of the cage-shaped DEFGH-ring compound was achieved.



Scheme 11. Plausible mechanism of the domino ring transformation.

2.3 Transformation to 3

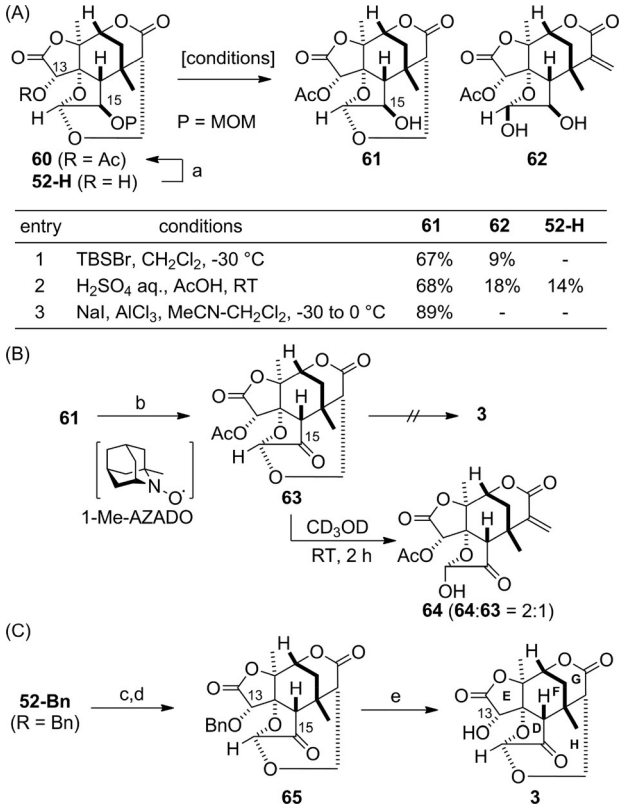
Remaining tasks to complete the synthesis of **3** were removal of the MOM group and oxidation of *OH*15. For selective oxidation of *OH*15 over *OH*13, *OH*13 was first protected as the acetate to give **60**. Treatment of **60** with TMSBr, which had been used for the synthesis of DFGH-ring **35**, gave the desired **61** in 67% yield, along with retro-oxy-Michael reaction product **62** (Scheme 12A, entry 1). The use of catalytic H_2SO_4 in AcOH gave a similar result to entry 1, with concomitant removal of the acetyl group (entry 2). After further investigations, we found that treatment with $AlCl_3$ and NaI[31] effectively provided **61** in 89% yield (entry 3). Oxidation of **61** to ketone was then examined (Scheme 12B). Dess–Martin oxidation, as used in the synthesis of **35**, unexpectedly did not proceed, presumably because of the steric hindrance around *OH*15 and ring strain of **61**. On the other hand, 1-Me-AZADO[32] was found to be an effective oxidant, affording the desired ketone **63** quantitatively.

We then examined removal of the acetyl group. Unfortunately, basic, acidic, or enzymatic conditions did not give **3** at all. The main product was hemiacetal **64**, which was formed via retro-oxy-Michael reaction. Surprisingly, even when **63** was dissolved in CD_3OD and left at room temperature for 2 days, formation of **64** was observed by ^{1}H NMR. This result suggests that the H-ring is susceptible to even a protic solvent. Therefore, we selected benzyl ether as a protecting group at *OH*13 instead of acetate. Benzyl-protected **65** was obtained by means of the same procedure established above, without opening of the H-ring (Scheme 12C). As we had hoped, removal of the benzyl group by hydrogenolysis was accomplished with $Pd(OH)_2$/C (Pearlman's catalyst) in AcOEt, and DEFGH-ring compound **3** was obtained quantitatively.

2.4 Optical Resolution of Synthetic Intermediate

We performed the synthetic studies using racemic compounds, but it would be better to have optically active compounds for future structure–activity relationship studies. Furthermore, for target identification, comparison of

Israel Journal of Chemistry



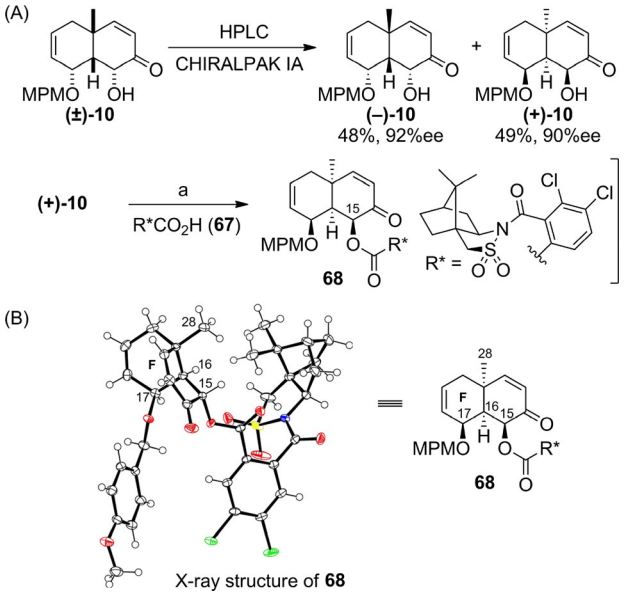
entry	conditions	61	62	52-H
1	TBSBr, CH_2Cl_2, -30 °C	67%	9%	-
2	H_2SO_4 aq., AcOH, RT	68%	18%	14%
3	NaI, $AlCl_3$, MeCN-CH_2Cl_2, -30 to 0 °C	89%	-	-

Scheme 12. (A) Optimization of removal of the MOM group; (B) a trial for the synthesis of **3** from **61** by deprotection of the acetyl group; (C) synthesis of **3**. Reaction conditions: a) Ac_2O, DMAP, Py, 0 °C (87%); b) 1-Me-AZADO, NaOCl, KBr, nBu_4NBr, CH_2Cl_2-$^{sat.}NaHCO_3$ aq., 0 °C (quant); c) NaI, $AlCl_3$, CH_3CN-CH_2Cl_2 (2.5:1), RT; d) 1-Me-AZADO, NaOCl, KBr, nBu_4NBr, CH_2Cl_2-$^{sat.}NaHCO_3$ aq., 0 °C (72%, over 2 steps); e) H_2, $Pd(OH)_2$/C, AcOEt, RT (quant). AZADO = 2-azaadamantane-*N*-oxyl; sat. = saturated.

the natural and unnatural enantiomers is valuable.[33] Therefore, we set out to establish a method to obtain an optically active synthetic intermediate.

First, we tried optical resolution of **(±)-10**. The enantiomers were separated by preparative HPLC using CHRALPAK IA to give optically active **(−)-10** and **(+)-10** (Scheme 13A). The absolute stereochemistry of **(+)-10** was determined by X-ray crystallography analysis of **68**, which was prepared by condensation of **(+)-10** with chiral carboxylic acid **67**.[28] As a result, **(+)-10** was found to have the absolute stereochemistry leading to the unnatural enantiomer of physalins (Scheme 13B). Hence, **(−)-10** is the enantiomer required for the natural product synthesis. Since HPLC resolution is not suitable for large-scale preparation of the optically active synthetic intermediate, we next investigated the enzymatic kinetic resolution of **(±)-10**.

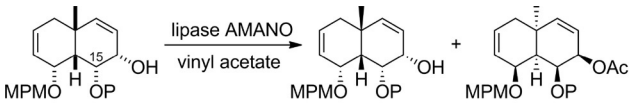
The secondary alcohol **(±)-10** was treated with lipase in vinyl acetate. We screened various types of lipases, but unfortunately, no reaction occurred. We then tried other synthetic intermediates bearing a secondary alcohol, such



Scheme 13. (A) Separation of **(+)-10** and **(−)-10** by HPLC and synthesis of **68**. (B) ORTEP figure of **68**. Reaction conditions: a) **67**, EDC·HCl, DMAP, CH_2Cl_2, RT (37%). EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

as **(±)-20**, **(±)-21** and **(±)-31**, but again almost no conversion of substrates was observed. During the development of the synthetic route to key intermediate **8**, we also prepared 15-*OBOM*-protected compounds **6-B**. When **(±)-6-B** was used as a substrate, the corresponding acetate **66** was finally detected. Acetylation with lipase SL gave the desired **(+)-6-B** in 61% yield and 51% *ee* (Table 5, entry 1). After the screening of various enzymes to improve the efficiency of the optical resolution, we eventually found that treatment of **(±)-6-B** with lipase AK for 21 h gave **(−)-66-B** in 34% yield as an almost optically pure form (entry 2), although the enantiomeric excess of **(+)-6-B** remained at only 46%. A longer reaction time

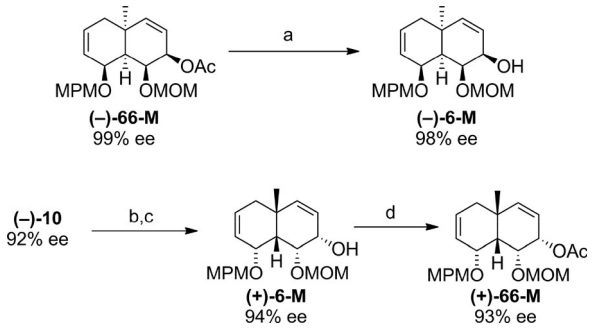
Table 5. Enzymatic optical resolution of 6-B and 6-M.



entry	6	liapse	temperature	time	yield of (+)-6	yield of (−)-66
1	**6-B**	SL	70 °C	128 h	61%, 51% ee	41%, 79% ee
2	**6-B**	AK	55 °C	21 h	66%, 46% ee	34%, 99% ee
3	**6-B**	AK	55 °C	72 h	53%, 64% ee	35%, 95% ee
4	**6-B**	AK	55 °C	154 h	50%, 87% ee	50%, 96% ee
5[a]	**6-B**	AK	55 °C	170 h	66%, 41% ee	25%, 98% ee
6	**6-M**	AK	55 °C	132 h	50%, 99% ee	50%, 99% ee

[a] Isopropenyl acetate was used as a solvent. BOM = benzyloxymethyl group.

improved both yield and *ee*, and **(+)-6-B** was obtained in 50% yield and 87% *ee* after 154 h (entry 4). As the reaction proceeded, the generated acetaldehyde might decrease the enzymatic activity due to Schiff base formation.[34] To avoid the formation of acetaldehyde, the reaction in isopropenyl acetate was also examined, but this resulted in low conversion (entry 5). Finally treatment of MOM-protected **(±)-6-M** with lipase AK in vinyl acetate at 55 °C afforded the desired **(+)-6-M** in 50% yield and 99% *ee* (entry 6). Importantly, the corresponding acetate **(−)-66-M** was also formed in 50% yield and 99% *ee*, and this was converted to **(−)-6-M** (Scheme 14). The absolute stereochemistry of **(+)-6-M** was determined to be the one leading to the natural enantiomer of physalin by conversion from **(−)-10** using the same procedure as for the racemate synthesis (Scheme 14).[28]



Scheme 14. Conversion of (−)-**6-M** to (−)-**6-M** and (−)-**10** to (+)-**6-M** and (+)-**66-M**. Reaction conditions: a) K_2CO_3, MeOH-Et_2O (3 : 1), RT, (97%, 98% *ee*); b) MOMCl, *i*Pr_2NEt, CH_2Cl_2, reflux (58%); c) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, RT (91%, 94% *ee*); d) Ac_2O, DMAP, Py, RT (90%, 93% *ee*). EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

Selectivity of optical resolution can be evaluated in terms of *E* value.[35] The *E* value of optical resolution of **6-B** with lipase SL (entry 1) was $E = \sim 15$, while with lipase AK (entry 4), the value was $E = \sim 150$, clearly indicating that lipase AK was a superior catalyst for this system. The *E* value of the reaction of **6-M** with lipase AK (entry 6) was $E > 1000$, so that both enantiomers were obtained in almost optically pure forms. Thus, the methodology to obtain both the natural and unnatural enantiomers of the right-side structure of physalins was established.

3. Conclusion

We have further developed and optimized our synthetic methodologies for the complex cage-shaped right-side structure of physalins. We propose a mechanism for the key domino ring transformation employed to construct the DEFGH-ring system on the basis of experimental evidence, including isolation of an intermediate. We have also established an efficient kinetic resolution of a synthetic intermediate. This should enable us to obtain optically active DEFGH-ring compounds. Further work, aimed at the total synthesis of physalins, is underway.

Acknowledgements

We would like to thank Prof. Yoshiharu Iwabuchi (Tohoku University, Japan) for providing 1-Me-AZADO, and Amano Enzyme Inc. for providing lipases. We also would like to thank C. J. Henderson for checking part of this manuscript. This work was partially supported by RIKEN project funding and JSPS KAKENHI, Grant Number 16H01167 for the Middle Molecular Strategy and 15K12760.

References

[1] V. Dessaignes, J. Chautard, *J. Prakt. Chem.* **1852**, *55*, 323–325.
[2] T. Matsuura, M. Kawai, R. Nakashima, Y. Butsugan, *Tetrahedron Lett.* **1969**, *10*, 1083–1086.
[3] T. Matsuura, M. Kawai, *Tetrahedron Lett.* **1969**, *10*, 1765–1766.
[4] For selected references, see: a) M. Kawai, T. Matsuura, *Tetrahedron* **1970**, *26*, 1743–1745; b) L. R. Row, N. S. Sarma, K. S. Reddy, T. Matsuura, R. Nakashima, *Phytochemistry* **1978**, *17*, 1647–1650; c) L. R. Row, K. S. Reddy, N. S. Sarma, T. Matsuura, R. Nakashima, *Phytochemistry* **1980**, *19*, 1175–1181; d) M. Kawai, T. Matsuura, S. Kyuno, H. Matsuki, M. Takenaka, T. Katsuoka, Y. Butsugan, K. Saito, *Phytochemistry* **1987**, *26*, 3313–3317; e) M. Kawai, T. Ogura, M. Nakanishi, T. Matsuura, Y. Butsugan, Y. Mori, K. Harada, M. Suzuki, *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2696–2698; f) M. Kawai, T. Ogura, B. Makino, A. Matsumoto, H. Yamamura, Y. Butsugan, M. Hayashi, *Phytochemistry* **1992**, *31*, 4299–4302; g) M. Kawai, A. Matsumoto, B. Makino, H. Mori, T. Ogura, Y. Butsugan, K. Ogawa, M. Hayashi, *Bull. Chem. Soc. Jpn.* **1993**, *66*, 1299–1300; h) B. Makino, M. Kawai, Y. Iwata, H. Yamamura, Y. Butsugan, K. Ogawa, M. Hayashi, *Bull. Chem. Soc. Jpn.* **1995**, *68*, 219–226; i) B. Makino, M. Kawai, T. Ogura, M. Nakanishi, H. Yamamura, Y. Butsugan, *J. Nat. Prod.* **1995**, *58*, 1668–1674; j) B. Makino, M. Kawai, K. Kito, H. Yamamura, Y. Butsugan, *Tetrahedron* **1995**, *51*, 12529–12538; k) M. Kawai, T. Yamamoto, B. Makino, H. Yamamura, S. Araki, Y. Butsugan, K. Saito, *J. Asian Nat. Prod. Res.* **2001**, *3*, 199–205; l) P.-C. Kuo, T.-H. Kuo, A. G. Damu, C.-R. Su, E.-J. Lee, T.-S. Wu, R. Shu, C.-M. Chen, K. F. Bastow, T.-H. Chen, K.-H. Lee, *Org. Lett.* **2006**, *8*, 2953–2956; m) R. Chen, J. Liang, R. Liu, *Helv. Chim. Acta* **2007**, *90*, 963–966; n) L. Qiu, F. Zhao, Z.-H. Jiang, L.-X. Chen, Q. Zhao, H.-X. Liu, X.-S. Yao, F. Qiu, *J. Nat. Prod.* **2008**, *71*, 642–646.
[5] M. Ozawa, M. Morita, G. Hirai, S. Tamura, M. Kawai, A. Tsuchiya, K. Oonuma, K. Maruoka, M. Sodeoka, *ACS Med. Chem. Lett.* **2013**, *4*, 730–735.
[6] M. D. Antoun, D. Abramson, R. L. Tyson, C. Chang, J. L. McLaughlin, G. Peck, J. M. Cassady, *J. Nat. Prod.* **1981**, *44*, 579–585.

[7] A. G. Damu, P.-C. Kuo, C.-R. Su, T.-H. Kuo, T.-H. Chen, K. F. Bastow, K.-H. Lee, T.-S. Wu, *J. Nat. Prod.* **2007**, *70*, 1146–1152.
[8] A. T. Vieira, V. Pinho, L. B. Lepsch, C. Scavone, I. M. Ribeiro, T. Tomassini, R. Ribeiro-dos-Santos, M. B. P. Soares, M. M. Teixeira, D. G. Souza, *Br. J. Pharmacol.* **2005**, *146*, 244–251.
[9] N. J. Jacobo-Herrera, P. Bremner, N. Márquez, M. P. Gupta, S. Gibbons, E. Muñoz, M. Heinrich, *J. Nat. Prod.* **2006**, *69*, 328–331.
[10] T. Hosoya, M. A. Arai, T. Koyano, T. Kowithayakorn, M. Ishibashi, *ChemBioChem* **2008**, *9*, 1082–1092.
[11] I. Vandenberghe, L. Créancier, S. Vispé, J.-P. P. Annereau, J.-M. M. Barret, I. Pouny, A. Samson, Y. Aussagues, G. Massiot, F. Ausseil, C. Bailly, A. Kruczynski, *Biochem. Pharmacol.* **2008**, *76*, 453–462.
[12] L. Ji, Y. Yuan, L. Luo, Z. Chen, X. Ma, Z. Ma, L. Cheng, *Steroids* **2012**, *77*, 441–447.
[13] M. Ohkubo, G. Hirai, M. Sodeoka, *Angew. Chem. Int. Ed.* **2009**, *48*, 3862–3866.
[14] M. Morita, G. Hirai, M. Ohkubo, H. Koshino, D. Hashizume, K. Maruoka, M. Sodeoka, *Org. Lett.* **2012**, *14*, 3434–3437.
[15] S. D. S. Burke, J. E. Cobb, K. Takeuchi, *J. Org. Chem.* **1990**, *55*, 2138–2151.
[16] P. A. Wender, D. B. Rawlins, *Tetrahedron* **1992**, *48*, 7033–7048.
[17] J. Åhman, P. Somfai, *Synth. Commun.* **1994**, *24*, 1117–1120.
[18] P. A. Caruana, A. J. Frontier, *Tetrahedron* **2004**, *60*, 10921–10926.
[19] L. Gentric, I. Hanna, L. Ricard, *Org. Lett.* **2003**, *5*, 1139–1142.
[20] The improved yield is attributed to accurate temperature control, using an isothermal bath. Reactions conducted with ice or dry ice baths resulted in a decreased yield of **12**.
[21] C.-K. Sha, S.-J. Huang, *Tetrahedron Lett.* **1995**, *36*, 6927–6928.
[22] G. Cahiez, H. Avedissian, *Synthesis* **1998**, 1199–1205.
[23] H. Yokoe, C. Mitsuhashi, Y. Matsuoka, T. Yoshimura, M. Yoshida, K. Shishido, *J. Am. Chem. Soc.* **2011**, *133*, 8854–8857.
[24] T. Taniguchi, G. Tanabe, O. Muraoka, H. Ishibashi, *Org. Lett.* **2008**, *10*, 197–199.
[25] S. I. Ngi, K. Cherry, V. Héran, L. Commeiras, J. L. Parrain, A. Duchêne, M. Abarbri, J. Thibonnet, *Chem. Eur. J.* **2011**, *17*, 13692–13696.
[26] a) J. H. Tatlock, *J. Org. Chem.* **1995**, *60*, 6221–6223; b) D. Urabe, T. Nishikawa, M. Isobe, *Chem. Asian. J.* **2006**, *1*, 125–135.
[27] T. Nakajima, D. Yamashita, K. Suzuki, A. Nakazaki, T. Suzuki, S. Kobayashi, *Org. Lett.* **2011**, *13*, 2980–2983.
[28] See Supporting Information.
[29] Since hydrolysis was so sluggish, an excess amount of LiOH was required for complete consumption of **56**.
[30] As far as we know, this is the first example of a simple intramolecular 7-*endo* oxy-Michael reaction. In most cases, simultaneous 5-*exo* cyclization occurs. For example, see: S. J. Danishefsky, W. H. Pearson, D. F. Harvey, C. J. Maring, J. P. Springer, *J. Am. Chem. Soc.* **1985**, *107*, 1256–1268.
[31] a) M. Node, K. Nishide, M. Sai, E. Fujita, S. Acids, *Tetrahedron Lett.* **1978**, *19*, 5211–5214; b) E. D. Moher, J. L. Collins, P. A. Grieco, *J. Am. Chem. Soc.* **1992**, *114*, 2764–2765.
[32] M. Shibuya, M. Tomizawa, I. Suzuki, Y. Iwabuchi, *J. Am. Chem. Soc.* **2006**, *128*, 8412–8413.
[33] Y. Nakamura, R. Miyatake, M. Ueda, *Angew. Chem. Int. Ed.* **2008**, *47*, 7289–7292.
[34] B. Berger, K. Faber, *J. Chem. Soc. Chem. Commun.* **1991**, 1198–1200.
[35] C. S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.

Received: September 3, 2016
Published online: November 9, 2016

