Synthesis of the Right-Side Structure of Type B Physalins

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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-cycloerostane skeleton, with a highly oxygenated, complex structure. Type B physalins, such as physalin B, have an H-ring with a C14–O–C27 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.
Full Paper

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.\[9\] 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.

2. Results and Discussion

2.1 Synthesis of Tricyclic Intermediate

The preparation of 8 was commenced with the Diels–Alder reaction of 4 with 5 (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 OH15 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 C25. In our original report,\[19\] β-allylic alcohol derivative 11 was utilized as a substrate (Scheme 2B). The stereochemistry of C25 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$_2$SnBu$_3$ (Scheme 3A).\[15\] Unfortunately, treatment of 13 with McLi\[18\] 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, OH14 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,\[15\] and found that the yield of 12 gradually increased as the reaction progressed.
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 58°C; this is a better result than in our original report. 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 OH27 with TBS, and deprotection of MPM, to give allylic alcohol 21 in pure form. MnO2 oxidation and iodination[21] of the α-position of the resulting enone afforded 22.

The introduction of the C21 methyl group was originally achieved by iron-mediated coupling,[22] and α-methylene-ene 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 26.

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

| entry | RLi (equiv) | temperature | 12 | 20 | 23 | 24 |
|-------|-------------|-------------|----|----|----|----|
| 1     | 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 1H NMR of the mixture (see Supporting Information).

Table 2. Optimization of methylation to introduce C21.

| entry | conditions | yield of 7 | byproducts |
|-------|------------|------------|------------|
| 1     | MeMgBr, Fe(acac)3, NMP, THF | 8-75% | 25: 91-8% |
| 2     | Me2Sn, Pd2dba3, Ph3As, Cul, NMP | 29%  | 26: 15%  |
| 3     | (MeOB)3, PdCl2(dppe), Cs2CO3, dioxygen | 51%  | 27: 19%, 28: 15% |
| 4     | Me2Zn, PdCl2(dppe), DMF-THF (1:1) | 75%  | 26: 23%  |

acac = acetylacetone; dba = dibenzylideneacetone, dppe = 1,1’-bis(diphenylphosphino)ferrocene; NMP = N-methylpyrrolidone.
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 CeCl₃ without β-elimination of the oxygen functionality at C15 (Scheme 5). Epoxidation of diene 29 from the convex face with mCPBA was accompanied by tetrahydrofuran formation through attack of OH₁₇ 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 mCPBA favored rearrangement of the more electron-rich C14 carbon, and 8 was obtained.

2.2 Domino Ring Transformation

We had earlier established a synthetic methodology for the DFGH-ring system 38[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 OH₁₅ afforded 35.

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 C13. 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 OH₁₅. We expected that the stability advantage of the DFGH-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 KMnO₄ and NaIO₄ in BuOH-H₂O, 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 TMSCN₂. Unexpect-
edly, the only isolated product was C15–O–C27 bridged hemiacetal 41, which does not have a C18 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 C25 and C27. Sequential formation of hemiacetal from OH14, CHO27, and ketone at C15 might give 41. These results indicate that the oxidation of alkene 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 alkene 8 with KMnO4 and NaIO4 proceeded without loss of C18, and the resulting carboxylic acid was esterified with TMSCHN2, affording the desired α-ketoester 44 in 88% yield. Removal of the TBS group and installation of a monocloromethanesulfonyl (Mc) group furnished 45 (Scheme 8A).

As in the case of 33, 45 was treated with LiOH in THF-H2O. 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 OH14 and the ketone at C13, which would block the desired H-ring-forming oxy-Michael reaction. Another possibility is instability of the putative product 47, because it has an sp2 carbon at C13, whereas physalins have an sp3 carbon at this position.

Then we prepared 51 bearing an α-hydroxyester group by reduction of the α-ketoester. Although the ketone at C13 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 (α:β = 9:1, Scheme 8B). Removal of the protecting group at OH27 (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 Bu3NF 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 indicates...
icates 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 prod-

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

| entry | conditions | temperature | time | 52-H | 53-H (α:β - 1:5) |
|-------|------------|-------------|------|------|-----------------|
| 1     | 1 N HCl aq. | RT          | 4 h  | 25%  | 15%             |
| 2     | conc. HCl aq.| RT          | 1 h  | 0%   | 0%              |
| 3     | AcOH-H$_2$O (20:1) | 80 °C     | 1 h  | 13%  | 16%             |
| 4     | AcOH-H$_2$O (20:1) | 100 °C    | 1 h  | 23%  | 20%             |
| 5     | AcOH-H$_2$O (20:1) | reflux     | 1 h  | 33%  | 37%             |
| 6     | AcOH-H$_2$O (20:1) | 100 °C    | 20 min | 24% | 18%             |
| 7     | AcOH-H$_2$O (20:1) | 100 °C    | 1 h  | 15%  | 16%             |
| 8     | AcOH-H$_2$O (2:1) | 100 °C    | 1 h  | 29%  | 27%             |
| 9     | AcOH-H$_2$O (2:1) | 100 °C    | 1 h  | 29%  | 32%             |
| 10    | AcOH          | 100 °C    | 1 h  | 0%   | 0%              |

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

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 OH13. 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 | conditions | base | solvent ratio | 52-H | 53-H |
|-------|------------|------|---------------|------|------|
| 1     | 51-H       | LiOH-H$_2$O | 1:1 | 52-H: 33% | 53-H: 37% |
| 2     | 51-H       | LiOH-H$_2$O | 3:1 | 52-H: 19% | 53-H: 30% |
| 3     | 51-H       | NaOH  | 1:1 | 52-H: 25% | 53-H: 36% |
| 4     | 51-H       | KOH   | 1:1 | 52-H: 28% | 53-H: 40% |
| 5     | 51-Bn      | LiOH-H$_2$O | 1:1 | 52-Bn: 56% | 53-Bn: 44% |

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

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
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). Furthermore, treatment of 56 with an excess amount of LiOH, followed by acid treatment, provided 52 and 53, indicating that the corresponding dicarboxylic acid was probably formed as an intermediate during domino ring transformation.

Furthermore, treatment of 56 with an excess amount of LiOH, followed by acid treatment, provided 52 and 53, indicating that the corresponding dicarboxylic acid was probably formed as an intermediate during domino ring transformation.

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 would occur to form the H-ring, followed by diastereoselective protonation at C25 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 C17 favors domino ring transformation, and thus efficient formation of the cage-shaped DEFGH-ring compound was achieved.

2.3 Transformation to 3

Remaining tasks to complete the synthesis of 3 were removal of the MOM group and oxidation of OH15. For selective oxidation of OH15 over OH13, OH13 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 H2SO4 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 AlCl3 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 OH15 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 CD3OD and left at room temperature for 2 days, formation of 64 was observed by 1H 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 OH13 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
the natural and unnatural enantiomers is valuable.\textsuperscript{[33]}
Therefore, we set out to establish a method to obtain an optically active synthetic intermediate.

First, we tried optical resolution of \((\pm)-10\). The enantiomers were separated by preparative HPLC using CHIRALPAK 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 \((\pm)-10\) with chiral carboxylic acid 67.\textsuperscript{[28]} As a result, \((+)-10\) was found to have the absolute stereochemistry leading to the unnatural enantiomer of physalin (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 \((\pm)-10\).

The secondary alcohol \((\pm)-10\) 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 as \((\pm)-20\), \((\pm)-21\) and \((\pm)-31\), 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 \((\pm)-6-B\) was used as a substrate, the corresponding acetate 66 was finally detected. Acetylation with lipase SL gave the desired \((\pm)-6-B\) 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 \((\pm)-6-B\) 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

### 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\(_2\)O, DMAP, Py, 0 °C (87%); b) 1-Me-AZADO, NaOCl, KBr, nbuNBr, CH\(_2\)Cl\(_2\), NaHCO\(_3\)aq., 0 °C (quant); c) NaI, AlCl\(_3\), MeCN-CH\(_2\)Cl, RT; d) 1-Me-AZADO, NaOCl, KBr, nbuNBr, CH\(_2\)Cl\(_2\), 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.

### 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\(_2\)Cl\(_2\), RT (37%), EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

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

| Entry | 6 | Temperature | Time | Yield of \((+)-6\) | Yield of \((-)-6\) |
|-------|---|-------------|------|-------------------|-------------------|
| 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\textsuperscript{[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 = benzylxyloxy-methyl 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.\textsuperscript{[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).\textsuperscript{[29]}

Selectivity of optical resolution can be evaluated in terms of $E$ value.\textsuperscript{[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 synthetically intermediate. This should enable us to obtain optically active DEFGH-ring compounds. Further work, aimed at the total synthesis of physalins, is underway.

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