Assignment 1

Professor: Dr. Louis Barriault

Student Name(s):

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Deadline: Electronic submission of the assignment to lbarriau@uottawa.ca (subject CHM-8304 assignment 1) by February 3, 2017 11:59 pm. After the deadline, there will be a penalty of 5 pts per day. N.B. No more than 4 students per assignment. Please provide all the necessary reference (if needed)

1. By applying the principles of retrosynthetic analysis, show how each of the indicated target molecules could be prepared from the starting material given. No more than four or five separate transformations are necessary in any of the syntheses (20 pts)

1.1

1.2

1.3

2. Read the attached paper on the total synthesis of (+)-ryanodol by Reisman et al. Provide a valuable reaction mechanism for each steps displayed in Figure 2 and explain the diastereoselectivity when applicable (80 pts)
A 15-step synthesis of (+)-ryanodol

Kangway V. Chuang,* Chen Xu,* Sarah E. Reisman†

(+)-Ryanodine and (+)-ryanodol are complex diterpenoids that modulate intracellular calcium ion release at ryanodine receptors, ion channels critical for skeletal and cardiac muscle excitation-contraction coupling and synaptic transmission. Chemical derivatization of these diterpenoids has demonstrated that certain peripheral structural modifications can alter binding affinity and selectivity among ryanodine receptor isomers. Here, we report a short chemical synthesis of (+)-ryanodol that proceeds in only 15 steps from the commercially available terpene (S)-pulegone. The efficiency of the synthesis derives from the use of a Pauson-Khand reaction to rapidly build the carbon framework and a SeO₂-mediated oxidation step to install three oxygen atoms in a single step. This work highlights how strategic C–O bond constructions can streamline the synthesis of polyhydroxylated terpenes by minimizing protecting group and redox adjustments.

Terpenes are a large and structurally diverse family of natural products that range from simple hydrocarbons associated with flavors and fragrances, to complex, highly oxidized polycyclic molecules such as the antimalarial drug artemisinin, and the anticancer compounds ingenol and taxol (7). Although terpenes are isolated from natural sources, it can be challenging to translate their biological activity into a practical application (2). In some cases, the hurdle is low natural abundance; at other times, it is the difficulty encountered by chemists seeking to precisely edit a terpene’s molecular structure to improve its drug-like properties or interrogate its role in modulating disease pathways. The development of concise chemical syntheses of terpenes can transform our ability to use these molecules and their synthetic derivatives as biological probes or as lead compounds for the development of new medicines (3–5). Furthermore, these scientific efforts often innovate chemical reactivity or synthetic design concepts (6).

The natural product ryanodine (1) (7, 8) and its hydrolysis product ryanodol (2) (8, 9) are among the most highly oxidized and synthetically challenging diterpenoids reported to date (Fig. 1A). Isolated from the tropical shrub Rya nia indica Vahl in connection with its insecticidal properties, ryanodine is the namesake ligand of the ryanodine receptors (RyanRs) (10), an important family of ion channels that regulate intracellular Ca²⁺ release and play a critical role in signal transduction (11). In mammalian cells, these receptors exist in multiple isoforms (RyanR1, RyanR2, and RyanR3) that serve to mediate both movement and cognitive function. Mutations of RyRs are associated with genetic diseases such as malignant hyperthermia and central core disease (12), whereas altered expression of RyR2 and RyR3 has been associated with the pathogenesis of neurodegenerative disorders such as Alzheimer’s disease (13). Ryanodine binds with high affinity to the conducting state of RyanRs, exerting concentration-dependent modulation of Ca²⁺ release: At nanomolar concentrations, ryanodine locks RyanRs in an open, subconductance state, whereas at higher concentrations, ryanodine causes closure of the channels (14). The deacylated compound ryanodol binds with lower affinity than 1 to mammalian RyanRs; however, it still induces a subconductance state and has been reported as a reversible probe of RyR-mediated Ca²⁺ release in cells (15).

Since the initial reports describing the isolation of ryanodine from Rya nia, a number of congeners (known as ryanoids) that vary in oxidation patterns have been isolated (16–20). Whereas ryanodol—the compound obtained by hydrolysis of ryanodine—has not yet been isolated directly from a natural source, the closely related compound C3-epi-ryanodol (4) was isolated by González-Coloma from Persea indica (18). Indeed, owing to their structural similarities, C3-epi-ryanodol (4) was initially erroneously reported to be ryanodol (2); however, the structure of the Coloma-González isolate (21) was recently reassigned through the synthetic efforts of Inoue (see below) (22). These subtle differences in structure exert a pronounced effect on RyR binding; C3-epi-ryanodol (5), prepared from 4, binds to RyanRs with an affinity 1/100th that of 1 (23).

Given the biological importance of the RyanRs, the ryanoids have been the focus of both total synthesis and derivatization efforts. In 1979, Deslongchamps and co-workers disclosed a total synthesis of (+)-2, which hinged on a key Diels–Alder cycloaddition and a series of elegantly designed intramolecular aldol reactions to assemble the tetracyclic ABCD framework (Fig. 1B) (24–28). In a classic example of relay synthesis, the degradation product (+)-anhydroryanodol (3) was converted to (+)-ryanodol (2) in a final two-step sequence (28), providing the target molecule in 41 total steps (37 steps in its longest linear sequence). A more recent series of studies from Inoue and co-workers highlighted the utility of several radical-based C–O and C–C bond-forming reactions in a total synthesis that furnishes (+)-2 (29) and (+)-4 (22), each in 35 total steps. These studies allowed Inoue et al. to correctly reassign the structure of the natural product isolated from P. indica (18) as C3-epi-ryanodol (4). Inoue and co-workers subsequently reported that their synthesis could be rendered enantioselective through a catalytic asymmetric desymmetrization process and that an appropriately protected derivative of (+)-2 could be elaborated to (+)-1 in five synthetic steps (30, 31). In addition to these total syntheses, several medicinal chemistry studies have focused on the derivatization of ryanodine and ryanodol (32–34). Here, we disclose a direct and concise strategy to access the central ryanodinyl ring system, which has enabled the chemical synthesis of (+)-ryanodol (2) in only 15 steps from commercially available starting materials. These studies provide a synthetic platform from which it will be possible to prepare previously inaccessible ryanoid derivatives.

Perhaps the most substantial synthetic challenge embedded within the structure of 2 is the highly oxidized five-membered A-ring, which bears an all-carbon quaternary center and four additional stereogenic carbons bonded to oxygen (Fig. 1A). A concise synthesis of 2 requires a carefully choreographed introduction of these moieties to minimize redox, protecting group, and functional group transformations. Guided by the landmark studies of Deslongchamps and co-workers (28), we identified the C1–C15 bond of ryanodol (2) as the most simplifying and strategic initial retrosynthetic disconnection (Fig. 1B). This analysis revealed the tetracyclic lactone anhydroryanodol (3) as our initial synthetic target (Fig. 1C), with the isopropyl group introduced at a late stage through a transition metal–catalyzed cross-coupling reaction of enol triflate 6; this approach would also allow for versatile incorporation of alternative carbon-based fragments to facilitate long-term goals of preparing ryanoid derivatives. We anticipated that the requisite A-ring oxidation pattern could be accessed via chemo- and stereoselective functionalization of cyclopentene 7, whereby the enone would provide a functional group handle to install the critical C3, C4, and C12 alcohols of 2. We envisaged preparing cyclopentene 7 by an intramolecular Pauson-Khand reaction (33), a transformation that would be facilitated by the conformational rigidity of bicyclic lactone 8. Lactone 8 could arise through a series of transformations from (S)-pulegone, a commercially available terpene. By this analysis, the oxygen atoms at C6, C10, and C11 would be incorporated early in the synthesis, whereas the oxygen atoms at C3, C4, and C12 would be introduced at a late stage. With the implementation of appropriate protecting groups, we anticipated that this strategy would minimize synthetic manipulations ancillary to the direct construction of the natural product.
We first investigated the stereoselective oxidation of (S)-pulegone (10) to install the C6 and C10 alcohols of the C-ring (Fig. 2), envisioning functionalization at both carbons via enolates accessed by γ- or α'-deprotonation of the ketone, respectively. Preliminary experiments confirmed that the C6-alcohol could be installed with 2.5:1 diastereomic ratio (dr) through generation of the thermodynamic dienolate with potassium hexamethyldisilazide (KHMDS) followed by quenching with oxaziridine 11 (36). Noting that KHMDS did not appear to directly react with oxaziridine 11 at −78°C, we hypothesized that a one-step protocol involving excess KHMDS and 11 might simultaneously install both alcohols. Indeed, treatment of 10 with 2.5 equivalents (equiv.) of KHMDS at −78°C followed by dropwise addition of 2.4 equiv. of 11 provided α,α'-diol 12, which could be isolated as a single diastereomer in 42% yield (120 mmol scale). Although the yield is modest, this single transformation converts a simple terpene to a valuable building block with the requisite ryanodol C-ring oxidation pattern. We improved the efficiency of this transformation to 50% yield by modification of the standard procedure on a smaller scale (10 mmol, see supplementary materials). However, given the operational simplicity of the former procedure on larger scales, this protocol was preferentially employed for increased material throughput.

Treatment of diol 12 with excess benzyloxymethyl ether effected protection of both alcohols as benzoxymethyl ethers to give 13. At this stage, the D-ring was constructed by an efficient four-step sequence. First, addition of propynylmagnesium bromide to 13 at 0°C proceeded in 5:1 dr, providing the equatorially disposed alkene in 81% isolated yield. Ozonolytic cleavage of the 1,1-disubstituted olefin proceeded in high yield to afford methyl ketone 14. Although a N,N-dicyclohexylcarbodiimide-mediated phosphonoacylation and intramolecular Horner–Wadsworth–Emmons lactone synthesis had proved moderately effective in model studies lacking C10 oxidation, the tertiary alkynol of 14 proved resistant to acylation under a number of conditions. Presumably, the increased steric encumbrance and inductively withdrawing C10-ether sharply decreases the nucleophilicity of the tertiary alkynol. Instead, ketone 14 was efficiently converted to α,β-unsaturated lactone 15 via 1,2-addition of ethoxycarbonylmagnesium bromide followed by an Ag-catalyzed cyclization and elimination cascade (37).

With lactone 15 in hand, 1,4-addition of magnesium divinyl cuprate provided the corresponding enyne 16 as a single diastereomer in 84% yield, forging the critical all-carbon quaternary center at C5 while simultaneously setting the stage for a key Pauson-Khand cyclization. A number of Pauson-Khand procedures were screened for their ability to deliver 17 in both high yield and diastereoselectivity (Table 1). Although standard conditions (38, 39) using stoichiometric amounts of CO2(CO)8 produced the desired product, separation of diastereomeric enone 17 proved challenging and indicated that a more selective protocol was necessary. Mononetallic

Table 1. Evaluation of Pauson-Khand reaction conditions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions*</th>
<th>dr†</th>
<th>Yield (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CO2(CO)8 (1.2 equiv.), THF, 12 hours; then DMSO, 65°C</td>
<td>2.2:1</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>CO2(CO)8 (1.2 equiv.), CH2Cl2, 9 hours; then NMO, 23°C</td>
<td>4.5:1</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>Mo(CO)5 (1.2 equiv.), DMSO, PhMe, 110°C</td>
<td>–</td>
<td>Trace</td>
</tr>
<tr>
<td>4</td>
<td>Mo(CO)5(DMF)3 (1.1 equiv.), CH2Cl2, 23°C</td>
<td>&gt;20:1</td>
<td>67</td>
</tr>
<tr>
<td>5</td>
<td>[RhCl(CO)2]2 (1 mol %), CO (1 atm.), m-xylene, 110°C</td>
<td>&gt;20:1</td>
<td>85</td>
</tr>
</tbody>
</table>

*Reactions conducted on 0.2 mmol scale. THF, tetrahydrofuran; DMSO, dimethylsulfoxide; NMO, N-methylmorpholine N-oxide; DMF, N,N-dimethylformamide. †Determined by 1H-NMR spectroscopy. ‡Isolated yield after purification by silica gel chromatography.

Fig. 1. Ryanodine and selected ryanoids. (A) Chemical structures of (+)-ryanodine (1), (+)-ryanodol (2), (+)-anhydroryanodol (3), 3-epi-ryanodol (4), and 3-epi-ryanodine (5). (B) Carbon numbering and ring system letter assignment. (C) Retrosynthetic analysis of anhydroryanodol.
mediators were found to improve the dr, with Mo(CO)₆(DMF)₃ (40) providing 17 as a single diastereomer in 67% yield. Despite the serviceable yield and scalability of this transformation, we sought a more efficient protocol that could obviate the need for stoichiometric metals and their attendant by-products. Treatment of 16 with 1 mol % [RhCl(CO)₂]₂ (1 mol %) in an atmosphere of carbon monoxide provided the desired product in 85% yield, again as a single diastereomer. This reaction has been conducted in a single pass. Single-crystal x-ray diffraction analysis of 17 confirmed both the absolute configuration and structural assignment of this key tetracyclic intermediate.

The successful realization of the Pauson-Khand cyclization led us to the next phase of our synthetic studies: chemo- and stereoselective functionalization of the A-ring through the introduction of the C3, C4, and C12-alcohols, as well as the C2-isopropyl unit. To this end, initial investigations were aimed at oxidation of the C4 allylic methine of 17, but efforts in this vein were thwarted by undesired reactivity. For example, established methods for allylic oxidation via reactive radical species [e.g., Pd(OH)₂/C/t-BuOOH (42) or Rh₂(capt)/t-BuOOH (43)] were unfruitful owing to competitive functionalization of the C1–C12 olefin (i.e., epoxidation, 1,2-difunctionalization). Although we considered the possibility of advancing these unanticipated products further in the synthesis, it was unclear how to achieve efficient transposition to the requisite oxidation pattern.

Instead, we turned to C3-functionalization of 17 using SeO₂ (44), anticipating that the enone might be readily oxidized to the corresponding α-diketone (Fig. 3). We found that treatment of 17 with excess SeO₂ in wet 1,4-dioxane at 110°C not only effected C3-oxidation, but also the formal hydration of the enone, thereby installing the necessary oxygen atoms at C3, C4, and C12 while simultaneously furnishing the necessary C12-alcohol and providing diosphenol 18, a compound with the C4, C12 syn-venial diol of 2. This single-step transformation installs the necessary oxygen atoms at C3, C4, and C12 while simultaneously providing a functional group handle for incorporation of the C2-isopropyl group.

Because C4-deoxy analog of ryanodine is itself a natural product, deoxyxiptigandine (20), and could be of interest for future biological studies, we optimized procedures to prepare both 18 and 21 (Fig. 5). Given concerns that contamination by H–nuclear magnetic resonance (¹H-NMR) silent, red selenium by-products resulted in artificially inflated isolated yields, our optimization efforts were guided by ¹H-NMR yields determined versus an added standard. Isolated yields were determined after conversion to the requisite oxidation pattern.

Rigorously structurally assigned of this compound revealed it to be the fully oxidized diosphenol 18, a compound with the C4, C12 syn-venial diol of 2. This single-step transformation installs the necessary oxygen atoms at C3, C4, and C12 while simultaneously providing a functional group handle for incorporation of the C2-isopropyl group.

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activated 4 Å MS at 110°C for 9 hours provided 18 in 33 to 35% 1H-NMR yield, and vinyl triflate 19 in 28% isolated yield over two steps. Despite the modest yield, this sequence accomplishes the stereoselective incorporation of three oxygen atoms, proceeds with an average efficiency of ~70% yield per transformation, and fares well in comparison to conceivable multiple step protocols to achieve the same reactivity. Although the precise mechanism of the SeO₂-mediated oxidation remains unclear at this time, investigations are ongoing and should aid the development of a more efficient protocol.

In the final stages of the synthesis, advancement of 19 to (+)-anhdyranoanol was achieved by a three-step sequence. Palladium-catalyzed cross-coupling between 19 and tributyl(2-propenyl) stannane installed the final three carbons, delivering 20 in 64% yield. LiBH₄-mediated 1,2-reduction stereoselectively furnished the C₃ alcohol, which was subjected to H₂ and Pd(OH)₂/C to simultaneously reduce the disubstituted olefin and remove the benzylxymethyl groups, providing (+)-3 in 61% yield over two steps. Using this route, we have prepared >400 mg of (+)-anhdyranoanol to date. Conversion of this material to (+)-ranyanol was achieved via a slight modification of Deslongchamps’s two-step protocol (28). Treatment of 3 with trifluoroacetic acid, freshly prepared from trifluoroacetic anhydride and urea hydrogen peroxide (in place of the originally reported concentrated hydrogen peroxide), cleanly afforded epianhydroyranoanol epoxide in 86% yield. Subjection of this material to Li₃ in NH₃ (distilled from Na₅) at ~78°C resulted in reduction cyclization to produce (+)-2 in 38% yield (lit. 60% yield). In our hands, the reaction profile was highly dependent on the purity of the ammonia. Specifically, independent control reactions conducted with ammonia condensed directly from the gas cylinder, or using redistilled ammonia with either added H₂O (10 equiv.), or exogenous Fe-salts (45), revealed that these parameters all substantially affect the ratio of 2 to carbonyl-reduction products, as well as the formation of minor unidentified degradation products.

The concise synthesis of (+)-ranyanol described here proceeds in only 15 synthetic steps (0.42% overall yield) from (S)-pulegone (10), fewer than half the steps of the previously disclosed syntheses by Deslongchamps et al. (37 linear steps, 0.23% yield) and Inoue et al. (35 linear steps, 0.008% yield). The efficiency of our approach derives from the development of a direct and scalable route to key cyclopentenone 17, which can be prepared on a multigram scale in only eight steps and rapidly elaborated to (+)-anhdyranoanol. The strategic use of C-O bond-forming reactions minimizes redox adjustments and the use of protecting groups. Indeed, the five alcohols found in (+)-3 are incorporated with just two transformations: the dihydroxylation of 10 and the SeO₂-mediated oxidation of enone 17. Moreover, all but the C₃-alcohol are introduced with over, all but the C₃-alcohol are introduced with 14. G. Meissner, M. Nagatomo, et al. (2010). SCIENCE Vol 353 Issue 6302 26 AUGUST 2016 915
Rapid ryanodol route

The plant-derived compound ryanodine and its hydrolyzed cousin ryanodol are biochemically interesting for their calcium-regulating capacity and chemically interesting for their dense tangle of carbon rings brimming with oxygen appendages. Chuang et al. report an efficient 15-step asymmetric synthesis of ryanodol from the structurally much simpler terpene pulegone (see the Perspective by Verdaguer). Key steps include a Pauson-Khand cyclization of a tethered alkene and alkyne with carbon monoxide to set the ring motifs, followed by an oxidation using selenium dioxide that delivers three different oxygen substituents in tandem.

Science, this issue p. 912; see also p. 866