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A Concise Synthesis of Forskolin

Ondřej Hylse, Lukáš Maier, Roman Kučera, Tomáš Perečko, Aneta Svobodová, Lukáš Kubala, Kamil Paruch, and Jakub Švenda*

Abstract: *We report a 24-step synthesis of (±)-forskolin, which delivered hundred-milligram quantities of this complex diterpene in one pass. Transformations key to our approach include: (a) a strategic allylic transposition, (b) stepwise assembly of a sterically encumbered isoxazole ring, and (c) citric acid-modified Upjohn dihydroxylation of a resilient tetrasubstituted olefin. We demonstrate that the developed route has an exciting potential for the preparation of new forskolin analogs inaccessible by semisynthesis.*

Forskolin (**1**) is a complex natural product that has become a standard research tool in biology.[1] This labdane diterpene was shown to be an allosteric stimulator of cAMP-generating membrane-bound adenylyl cyclases (ACs).[2] These enzymes are involved in action of numerous small-molecule drugs, most notably those affecting G-protein coupled receptors (GPCRs). Direct targeting of ACs for therapeutic applications is complicated, in part, by their structural similarity and the lack of isoform-selective modulators.[3]

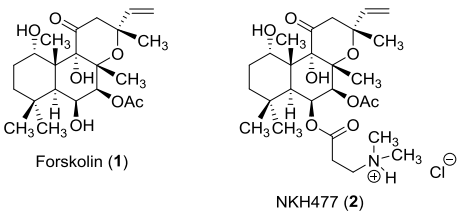

Figure 1. Structure of forskolin (**1**) and drug NKH477 (**2**) derived through semisynthesis.

Semisynthesis was explored extensively in a search of forskolin analogs with improved properties[4] and these efforts led to the identification of NKH477 (**2**, Figure 1), a heart failure drug approved in Japan.[5]

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Fully synthetic approaches often enable structural modifications that would be difficult to address by semisynthesis. With its densely grouped array of quaternary carbons and stereocenters, forskolin (**1**) remains a daunting target, however. Three early syntheses reported by the groups of Ziegler, Corey (enantioselective), and Ikegami, published within one year of each other (31–40 step sequences),[6] remain landmark achievements in the field. More recently, Lett and co-workers disclosed a 32-step synthesis of racemic forskolin (**1**).[7] Formal syntheses and numerous approaches toward forskolin (**1**) have also been described in the literature.[8] Herein, we detail a 24-step synthesis, which intercepted a late-stage intermediate of Lett[7b] and enabled preparation of hundred-milligram quantities of (±)-forskolin [(±)-**1**]. The route gave also access to fully synthetic forskolin analogs.

In our synthetic planning, we considered *cis*-decalin **3** (Scheme 1) as an early intermediate, because its ring systems map onto the decalin substructure of forskolin (**1**) and it is readily prepared on a multigram scale using a flexible Diels–Alder strategy developed by researchers at Hoffmann-La Roche (8 steps from the commercially available materials).[9] Although decalin intermediates of this type were contemplated already some 30 years ago,[10] their conversion into forskolin (**1**) eluded efficient synthetic solution. As a strategic objective, we therefore targeted concise construction of the γ-pyrone ring of forskolin (**1**) from *cis*-decalin **3**. An isoxazole-based route described below emerged as attractive due to the minimum of redox and protecting group manipulations required by such an approach.

Reactivities of the two carbonyl groups in *cis*-decalin **3** differ dramatically, as shown previously.[9b,11] Following precedent, a highly selective addition of monolithium acetylide (2.0 equiv, −78 °C) occurred with convex-face approach of the reagent.[12] Subsequent epimerization in the presence of aluminum oxide (Brockmann grade IV, toluene, reflux) provided *trans*-decalin **4** (82% over 2 steps). We observed that the efficiency of the epimerization was sensitive to the form of the reagent and, strongly, to the substitution of the *cis*-decalin (see SI).[9b] A subsequent sequence of diastereoselective enone 1,2-reduction (diisobutylaluminum hydride, −78 °C) and benzoylation proceeded smoothly to give monoprotected allylic diol **5** in 74% yield (2 steps).

At this stage, we performed an oxidative 1,3-transposition of the tertiary allylic alcohol within **5** (excess pyridinium chlorochromate, sodium acetate, 4Å molecular sieves),[13] followed by highly diastereoselective 1,2-reduction of the resulting enone (**6**) using diisobutylaluminum hydride (5.0 equiv); the latter conditions also removed the benzoyl ester. The isolated 1,2-diol (not shown) was protected as the corresponding acetonide (**7**, 61% over 3 steps). This strategic sequence worked well and solved many of our initial problems with installation of the syn-1,2-diol found in forskolin.

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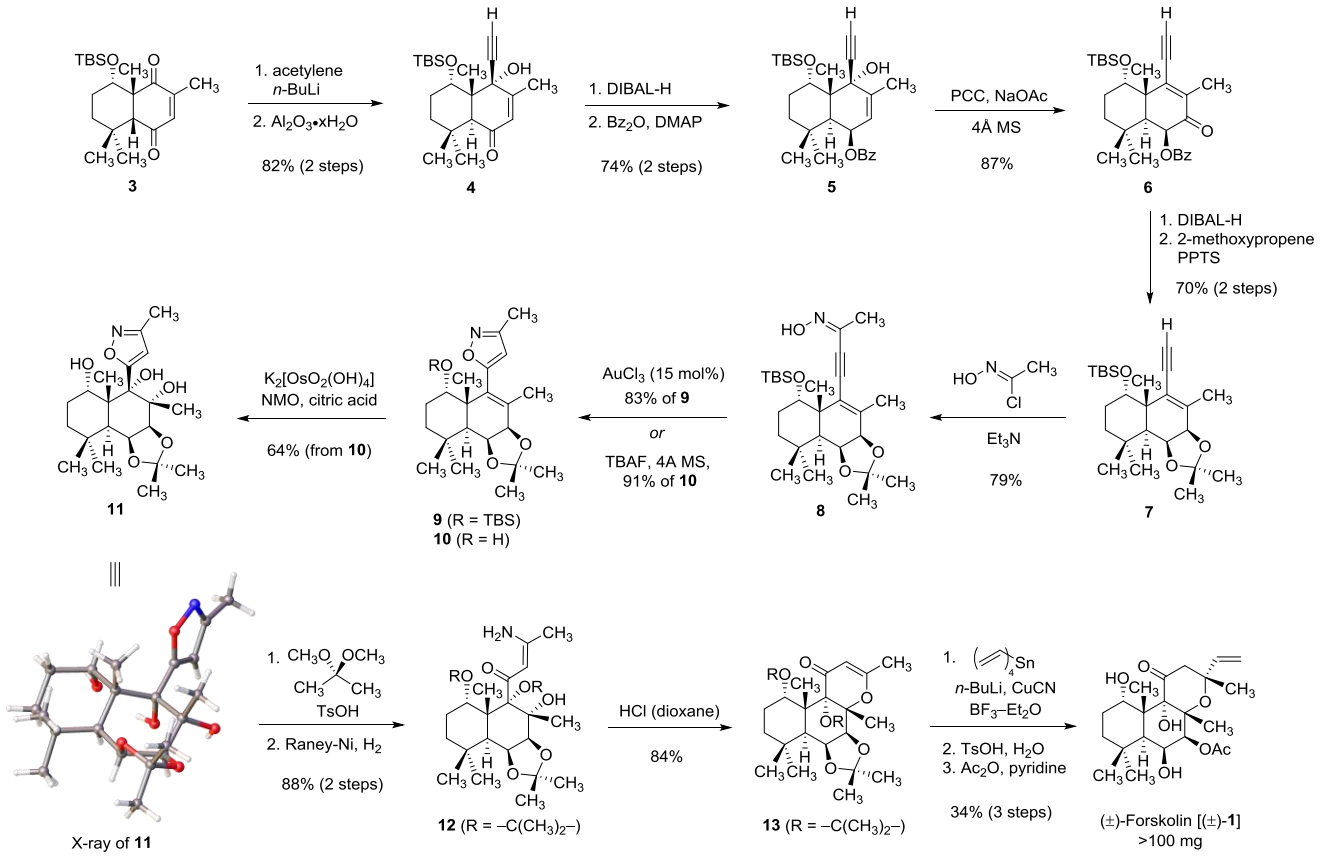
With the alkyne-containing intermediate **7** available in multigram quantities (>4 g), we approached construction of the isoxazole ring with [3+2] cycloaddition chemistry.[14] Exposing the alkyne (**7**) to excess acetonitrile oxide (3.0 equiv, generated *in situ* from freshly prepared *N*-hydroxyacetimidoyl chloride) led to a single isomer of alkynyl oxime **8** (79% isolated yield) as the principal component of the product mixture (ca. 5% of the desired isoxazole detected by ^{1}H NMR). This highly unusual outcome corresponds, formally, to an interrupted cycloaddition process.[15] Alkynyl oximes can be isomerized to isoxazoles however and exposure of **8** to catalytic quantities of gold(III) chloride[16] (15 mol%, 1,2-dichloroethane, 45 °C) induced efficient cyclization to isoxazole **9** (83%). A simpler and even more efficient protocol involved treatment of **8** with excess tetrabutylammonium fluoride (4.0 equiv, 23 °C), conditions that triggered not only formation of the isoxazole but also the desired *tert*-butyldimethylsilyl ether cleavage (product **10**, 91%). Overall, the two-step sequence (alkyne **7** → isoxazole **10**) provided a highly effective solution to the difficult problem of crowded isoxazole assembly (see discussion below).[17]

The projected dihydroxylation of tetrasubstituted alkene **10** was expected to be challenging from the start but was pursued because of reasonable precedent in the forskolin literature.[6b,6h,18] We learned only late in the synthesis that none of these previously described conditions worked on our substrate (intermediate **10**). After a screening of conditions (see SI), we found that the citric acid-modified Upjohn protocol described by Sharpless and colleagues[19] was uniquely effective and did provide the dihydroxylation product (triol **11**) in acceptable yield (64% after 3 cycles, gram scale), with good mass recovery of the unreacted material.[20] An X-ray crystal structure of **11** unambiguously confirmed the relative stereochemistry.

Selective protection of the 1,3-diol function in **11** (*p*-toluenesulfonic acid, 2,2-dimethoxypropane) and hydrogenolysis of the isoxazole ring (Raney-Ni, H_2) gave cleanly β-amino enone **12** (88% over 2 steps). Exposure of **12** to an anhydrous solution of hydrochloric acid (2.0 equiv, dioxane–toluene, 23 °C) resulted in an intramolecular cyclization to known γ-pyrone **13**[7b] (84%). By subtle but important modification of the previously published three-step sequence (see SI),[6d,7b] we converted the γ-pyrone (**13**) into (±)-forskolin [(±)-**1**, >100 mg prepared in one pass] that was structurally identical to the natural product (see SI).

It is worth mentioning that the dense array of substituents and functional groups found in forskolin, and many of its synthetic precursors, often complicated seemingly straightforward chemistry operations.[21] For example, application of the standard dipolar cycloaddition methodology for construction of the isoxazole system was initially challenging. Numerous advanced alkyne intermediates (e.g. **16**–**19**, Scheme 2) were virtually unreactive in the cycloaddition process with acetonitrile oxide.[22] We attribute this behavior primarily to the sterically hindered environment around the alkyne. The single example of a successful and high yielding cycloaddition we observed with oxacyclic alkyne **14**[9b] supports this idea (Scheme 2, top).



Scheme 1. Synthetic pathway to (±)-forskolin [(±)-**1**] featuring stepwise construction of a crowded isoxazole intermediate.

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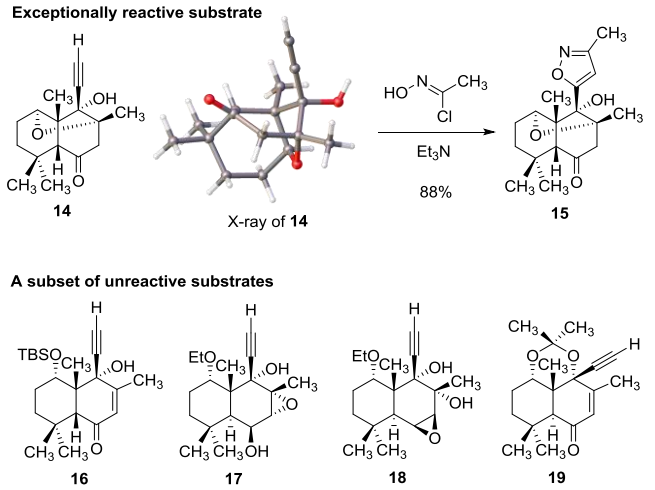

Scheme 2. A subset of terminal alkynes examined as substrates in the [3+2] dipolar cycloaddition with acetonitrile oxide.

In contrast stands the unusual reactivity of alkyne **7** (predominant formation of alkynyl oxime, ≤5% of isoxazole) exploited in our synthesis of forskolin (see Scheme 1), which may stem from presence of the enyne unit within the substrate.[15] It is interesting to add that electronic characteristics of the nitrile oxide can alter the relative product distribution (see SI).

The enantiomers of synthetic forskolin were obtained separately through efficient resolution of the racemic material either by semi-preparative HPLC – first-eluting enantiomer: **1**(ent-1), second-eluting enantiomer: **1**(ent-2) – or through diastereomeric camphanyl esters (see SI). **1**(ent-2) stimulated cAMP production with potency equal to that of the natural forskolin as determined in the cAMP-based membrane assay (Figure 2).

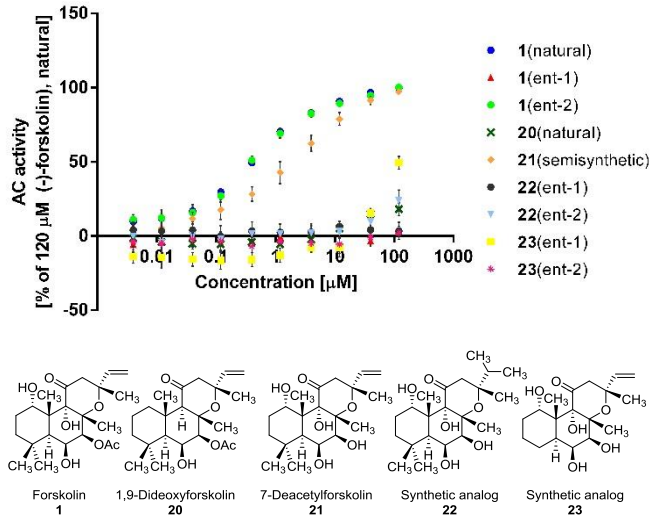

Figure 2. Modulation of activity of ACs by forskolin (**1**), 1,9-dideoxyforskolin (**20**), 7-deacetylforskolin (**21**), and fully synthetic analogs **22** and **23**. The AC activity was determined in a cAMP-based membrane assay (wild-type HEK293 cells-derived) as described in the Supporting Information with increasing concentrations of the diterpenes (0.004–400 μM). Data shown are means from 3–5 independent experiments. AC activity was normalized relative to the maximum stimulation observed with 120 μM of natural forskolin.

The other enantiomer, **1**(ent-1), showed no stimulatory effects up to 100 μM and behaved, in this sense, analogously to 1,9-dideoxyforskolin (**20**, commonly used negative control). It is worth noting that activity of the unnatural enantiomer of forskolin had not been examined previously.

To demonstrate the potential of our route in modifying positions of forskolin that would be difficult or impossible to address by semisynthesis, we have assembled two fully synthetic analogs **22** and **23** (see SI). While neither of these two analogs was more active than natural forskolin in the membrane assay (see Figure 2), our preliminary data in HEK293 cells with individually overexpressed AC isoforms indicate that the level of stimulation varies significantly (including stimulation equipotent to forskolin) depending on the specific isoform (data not shown). Improved isoform selectivity of analogs resulting from structural changes at new positions of forskolin has important implications.

In summary, we have developed a concise synthetic route to (±)-forskolin [(±)-**1**] that produced hundred-milligram quantities of this complex target in one pass. Transformations key to the success of our approach include: (i) a strategic oxidative transposition–reduction sequence to setup the 1,2-diol function of forskolin, (ii) stepwise construction of a densely substituted isoxazole intermediate, and (iii) citric acid-modified Upjohn dihydroxylation of a hindered tetrasubstituted olefin. In contrast to the natural product, the unnatural enantiomer of forskolin [**1**(ent-1)] was found to not have stimulatory activity toward ACs below 100 μM. We have also demonstrated that our route provides access to fully synthetic forskolin analogs modified at new positions. The ongoing studies seek to explore this potential in combination with biological profiling of analogs against a panel of human AC isoforms.

Acknowledgements

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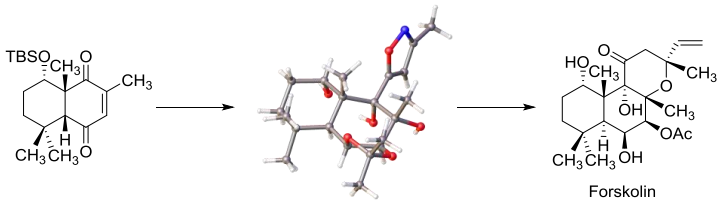

Forskolin

Forskolin is a complex diterpene commonly used in biomedical research to raise levels of cAMP. Previous strategies to access forskolin analogs with improved properties focused primarily on semisynthesis. To complement these approaches, we have streamlined the chemical synthesis of forskolin and applied it to the preparation of fully synthetic forskolin analogs.

Keywords: forskolin, adenylyl cyclases, natural product synthesis, structural analogs