



Assembling the carbon skeleton of A-74528†

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The 2'-phosphodiesterase inhibitor A-74528, which combines an intriguing biosynthesis with unusual biological activity, is one of the most complex type II polyketides. As a synthetic target, it represents a significant challenge due to its size but also due to a unique carbon skeleton that features a hexacarbocyclic core with an appended pyrone. Here we report our efforts toward the synthesis of A-74528, which culminated in the construction of the full carbon skeleton and the correct installation of all but one stereocenter. Our strategy employs a molybdenum-catalyzed branched allylation to establish the central quaternary carbon and relies on establishing the remaining stereocenters in a substrate-controlled manner. Carbocycles were established using a spiro epoxide annulation, a 1,3-dipolar cycloaddition, followed by an aldol condensation, and a gold-catalyzed hydroarylation. The pyrone was appended to an aldehyde branching off the quaternary stereocenter by a one-carbon homologation and Mukaiyama aldol addition.

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Introduction

In 2004, a research group at the Sankyo company reported their findings¹ on a previously elusive 2'-phosphodiesterase (2'-PDE). They reported that the natural product A-74528, isolated from a streptomyces strain, acts as an inhibitor of 2'-PDE. Thereby, A-74528 modulates the activity of the 2',5'-oligoadenylate (2-5A) system involved in anti-viral and anti-tumor immune responses in human cells. The 2-5A system triggers the enzyme RNase L, which degrades both viral and cellular RNAs and thus shuts down the protein biosynthesis in virus-infected cells. Remarkably, the natural product showed dose-dependent reduction of viral replication and no cytotoxic effects to the host cell. As such, it could serve as a lead compound for the development of mechanistically distinct antiviral and antitumor compounds. Structure elucidation based on extensive NMR studies² showed that A-74528 is a highly fused type II polyketide with an unprecedented carbon skeleton and that it has a rather unusual structure compared to biosynthetically related polyketides^{3a} (Scheme 1).

A-74528 consists of a pyrone side-chain that is connected *via* a methylene bridge to a hexacyclic core, which adopts an L-shaped conformation and consist of two resorcinols that are flanking four annealed six-membered rings. These non-

aromatic rings feature a benzylic carbonyl and an enolized 1,3-diketone as well as a sequence of six contiguous stereocenters, one of which is a quaternary carbon and one of which bears secondary alcohol. Biosynthetically, the positioning of the secondary alcohol is intriguing, as it does not follow the standard oxidation pattern of a nascent polyketide chain. Detailed investigations of the biosynthetic pathway were performed by the group of Khosla,³ who identified and characterized the gene cluster responsible for the production of A-74528. Consequently, several sequences that could transform a C30 polyketide precursor into A-74528 were suggested and it was proposed that the secondary alcohol would result from the opening of an epoxide. Furthermore, Khosla also pointed out the biosynthetic connections between A-74528 and fredericamycin, which are the two largest known compounds within the type II polyketides.

Despite its intriguing structure, A-74528 has received relatively little attention from the synthetic community. In 2011 our group reported a biomimetic approach based on a putative dearomatizing double Michael-addition sequence.⁴ Although this strategy featured novel condensation chemistry to furnish highly functionalized biaryls, it did not result in the establishment of the hexacyclic core. To the best of our knowledge there have been no further attempts to synthesize A-74528, apart from initial exploratory work by the Pronin group⁵ and A-74285 being mentioned as a potential target for applying a new photo-enolization Diels–Alder methodology.⁶ We now disclose an approach toward A-74528 that is not based on biosynthetic speculations and their biomimetic execution, but rather on transition metal catalyzed reactions, which has resulted in the successful establishment of the complex carbon skeleton of our target molecule.

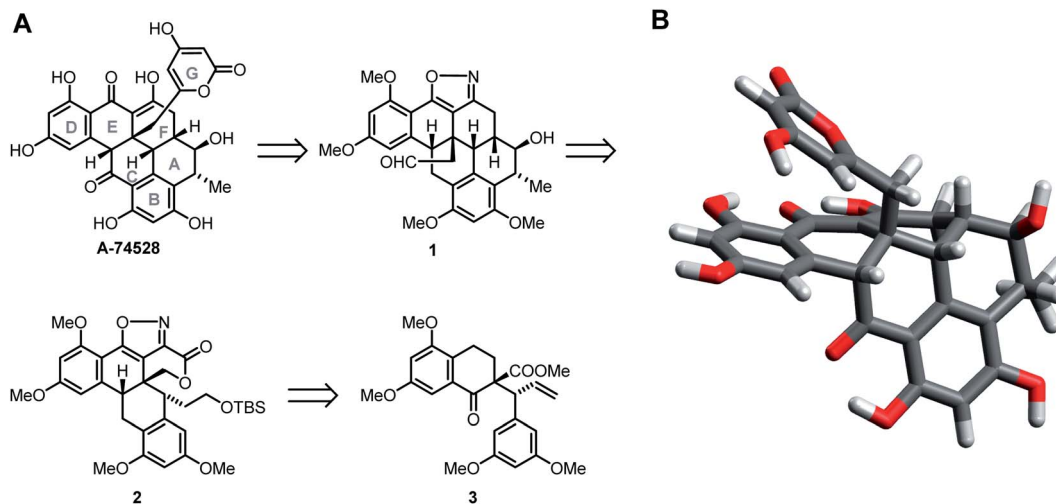
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† Electronic supplementary information (ESI) available: Synthetic procedures and analytical data, X-ray crystallography, NMR spectra. CCDC 2156714 and 2156715. For ESI and crystallographic data in CIF or other electronic format see <https://doi.org/10.1039/d2sc01366e>

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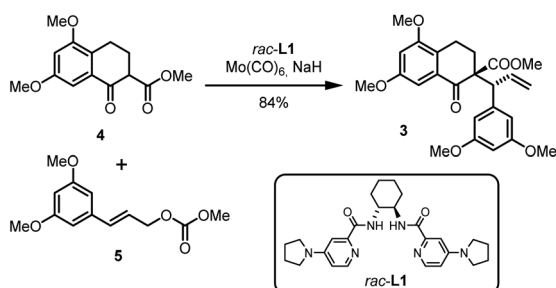




Scheme 1 (A) Structure and retrosynthetic analysis of A-74528. (B) Structural model of A-74528.

Results and discussion

Our retrosynthetic analysis of A-74528 (Scheme 1A) centered on establishing the quaternary carbon early in the synthesis and then approach the remaining stereochemical challenges in a substrate-controlled fashion. We presumed that it would be prudent to both establish the C-ring ketone by a benzylic oxidation and attach the pyrone moiety at a late stage of the synthesis, while the sensitive 1,3-diketone would best be masked as an isoxazole. These considerations led us aldehyde **1**, which we further simplified to lactone **2**, based on the expectation that the A-ring could be closed by an electrophilic aromatic substitution and the F-ring would be formed in an aldol condensation. Lactone **2** would be derived in a multi-step sequence from ketoester **3**, with a 1,3-dipolar cycloaddition as the key reaction. Ketoesters similar to **3** have been prepared enantio-, diastereo- and regioselectively in high yield through iridium-catalyzed allylation by the group of Stoltz.⁷ However, we were also aware of the possibility to use other metals, such as molybdenum, as introduced by Trost and coworkers.⁸ Consequently, we prepared the tetralone ester **4** and the known cinamyl carbonate **5**, the optimized synthesis of which is given in the ESI.†



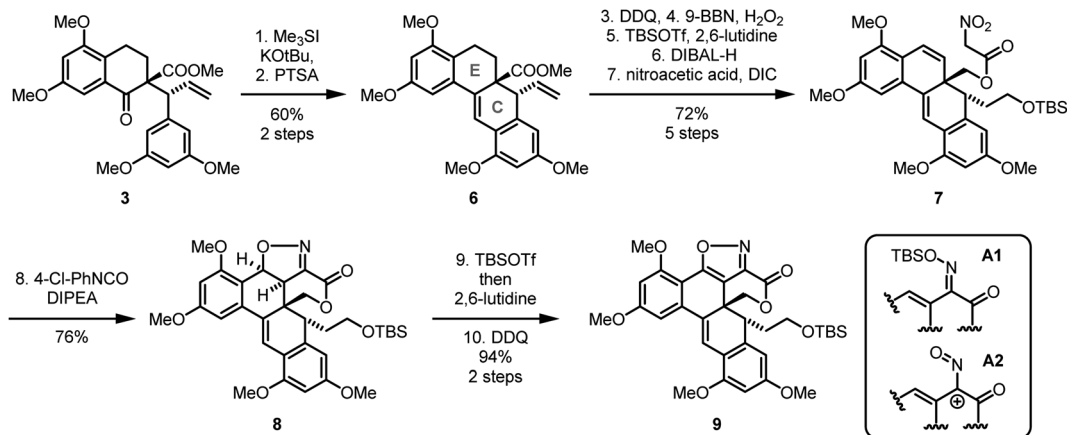
Scheme 2 Fragment coupling.

The coupling of these two fragments is shown in Scheme 2. Employing a catalyst preparation method by Palucki⁹ and an electron-rich bis-pyridine ligand (**L1**) reported by Moberg,¹⁰ we found that ketoester **3** was formed with high diastereo- and regioselectivity by reaction of a slight excess (1.3 eq.) of the sodium enolate of tetralone **4** with carbonate **5**. Thus, we established the quaternary stereocenter and adjacent tertiary stereocenter of A-74528 in a single operation. The desired product **3** was obtained in good yield (84%) after trituration with ethanol, which proved suitable to remove most of the minor regioisomeric and diastereomeric side-products. Regarding the enantioselectivity of this reaction, we performed initial tests without further optimization of the reaction and already observed an enantiomeric excess of 68%. However, as the more easily available racemic material was suitable to explore the following steps of the synthesis, the enantioselective allylation was not further examined.

Having established a practical approach to ketoester **3**, we then turned towards the C-ring of A-74528 (Scheme 3). To forge this ring, it was necessary to introduce one missing carbon atom, which we achieved *via* a Corey–Chaykovsky epoxidation.¹¹ Treatment of ketoester **3** with trimethylsulfonium iodide and potassium *tert*-butoxide was followed directly by treatment with *p*-toluenesulfonic acid, which resulted in an electrophilic aromatic substitution and subsequent elimination of water to afforded stilbene **6** in decent yield (60% over two steps). We speculate that this reaction proceeds *via* formation of a spiro epoxide (see ESI, structure MeSI-10†), followed by acid catalyzed Meinwald rearrangement, electrophilic aromatic substitution, and elimination. However, a direct nucleophilic attack of the electron rich arene into the spiro epoxide cannot be ruled out.

With tetracycle **6** at hand, we next installed the isoxazole *via* intramolecular 1,3-dipolar cycloaddition. To prepare for this step, we needed to install a double bond in the E-ring, which could be achieved in high yield by treatment with DDQ. A highly chemo- and regioselective hydroboration gave a primary alcohol that was protected as a TBS ether. Reduction of the ester and



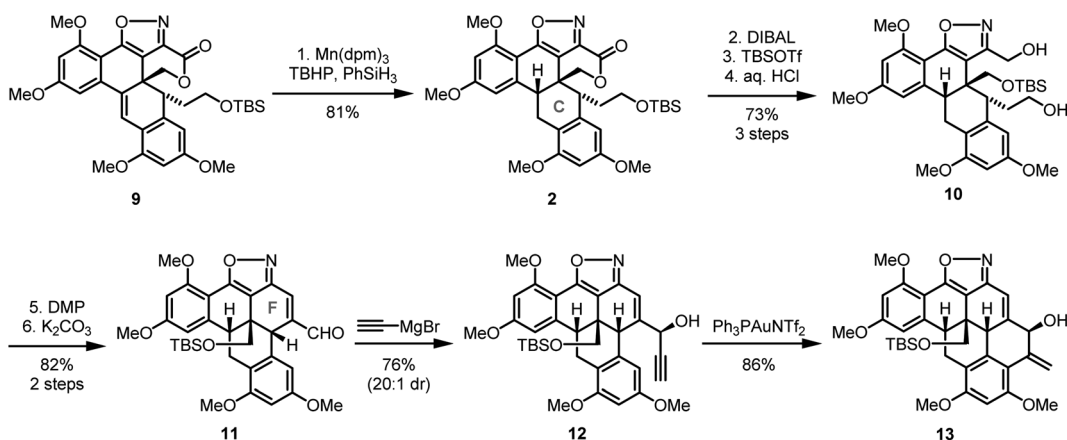


Scheme 3 C-Ring formation and isoxazole installation.

acylation then gave nitroacetic acid ester **7** in good overall yield (72% for 5 steps).

The subsequent cycloaddition was enabled by using conditions similar to Mukaiyama's original work.¹² Thus, isoxazoline **8** was formed by dehydration with *p*-chlorophenyl isocyanate in the presence of catalytic amounts of Hünig's base in hot toluene. In contrast to the previous benzylic unsaturation, the subsequent aromatization of **8** to yield isoxazole **9** proved quite challenging and suffered from sluggish conversion. However, in an attempt to increase the reactivity of a quinone oxidant we observed that isoxazolines like **8** are highly prone to acid-mediated ring-opening to the corresponding unsaturated oxime. This finding enabled us to develop a two-step method for this challenging isoxazoline oxidation: first, the isoxazoline was exposed to *tert*-butyldimethylsilyl trifluoromethanesulfonate, converting it into a *O*-silyl oxime (see Scheme 3, substructure **A1**). Second, this *O*-silyl oxime was oxidized with DDQ, which directly transformed it to the desired isoxazole.¹³ We presume that this sequence, which afforded isoxazole **9** with a yield of 94%, involves a nitrosoallyl cation (see Scheme 3, substructure **A2**) that undergoes a Nazarov-type 4π -electrocyclization.

With isoxazole **9** at hand, we proceeded with installing the next stereocenter through a hydrogenation of the stilbene moiety (Scheme 4). Using a modified version of Shenvi's hydrogen atom transfer (HAT) hydrogenation method,¹⁴ the desired product **2** was obtained in very good yield (86%) and as a single diastereomer without affecting the rather sensitive isoxazole N–O bond. At this point, we suspected that transforming the C-ring methylene to a carbonyl group could result in reduced stability and would limit our future choices of reaction conditions. Thus, we decided to defer this benzylic oxidation to a later stage in the synthesis. Instead, we set the stage for closing the F-ring. Reduction of the lactone moiety of **2**, followed by protecting group manipulations, afforded diol **10** and oxidation with Dess–Martin periodinane,¹⁵ followed by treatment with potassium carbonate in methanol, led to an aldol condensation. This smoothly formed the F-ring and gave enal **11** (82% yield). Now, only two carbon atoms of the hexacyclic core of A-74528 were missing, which we delivered in the form of ethynylmagnesium bromide. Reaction of enal **11** with this Grignard reagent exhibited excellent diastereoselectivity and we exclusively observed the desired diastereomer **12**. The last ring of the core was then established in an intramolecular



Scheme 4 Formation of A-ring and F-ring.



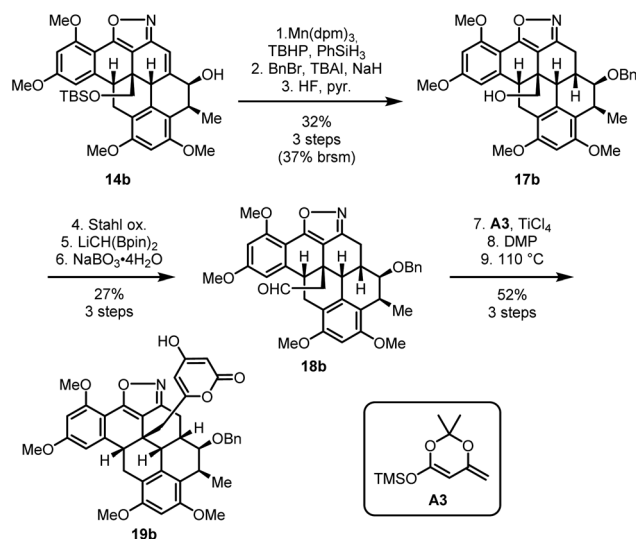
gold-catalyzed hydroarylation, as treatment of propargylic alcohol **12** with triphenylphosphine gold triflimide gave bis-allyl alcohol **13** in very good yield. Considering the sensitive nature of the hydroarylation product **13**, this reaction exemplifies the power of gold-catalyzed reactions under mild conditions.¹⁶

The installation of the remaining stereocenters would require diastereoselective hydrogenation of the two alkenes in **13**. However, this proved to be more challenging than anticipated and we were not able to reduce the *exo*-methylene moiety with satisfactory stereoselectivity. Molecular modeling suggested that the F-ring of **13** adopts a twist-boat conformation (Scheme 5B), which leads to preferential reductions from the undesired face. Thus, despite extensive experimentation (*e.g.* Crabtree's catalyst, Wilkinson's catalyst, palladium on carbon, Adam's catalyst, HAT hydrogenation), we were only able to achieve a 1 : 2.5 mixture of **14a** : **14b** in a combined yield of 80% by reaction with diimide (generated from *o*-nitrobenzenesulfonylhydrazide).¹⁷ After separation by silica gel chromatography, we initially continued with minor diastereomer **14a**, but found that further reduction of the remaining trisubstituted alkene also proved problematic. The HAT hydrogenation, which had worked very well at an earlier stage, now only proceeded to **15a** in disappointing yield, as a similar reactivity of the alkene and isoxazole gave undesired by-products (mainly by N–O cleavage). Fortunately, benzyl protection of the secondary alcohol **15a** gave crystalline ether **16a**, which was suitable for X-ray analysis. Its result unambiguously confirmed all our stereochemical assignments (Scheme 5C).

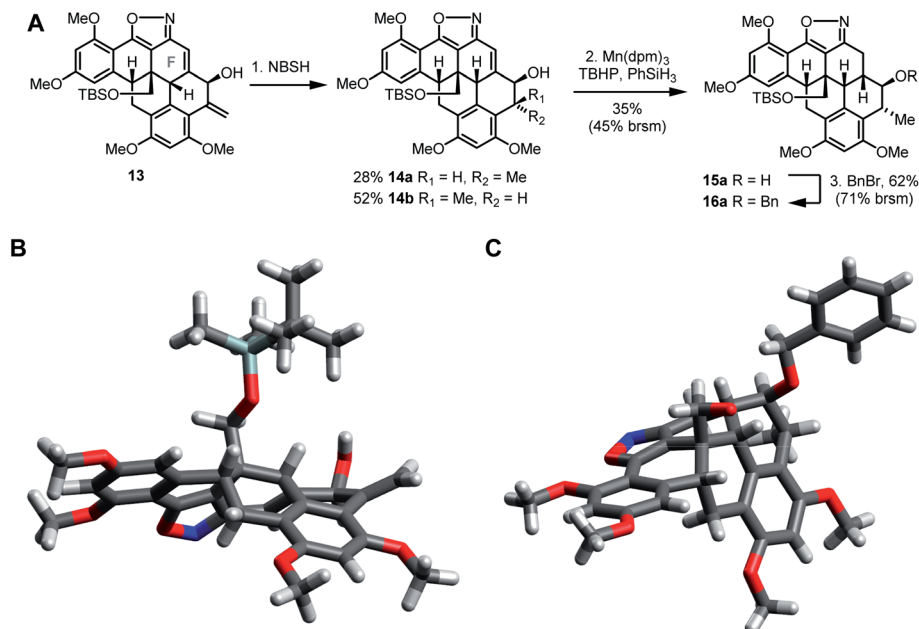
Due to the limited supply of material at this stage of the synthesis we then switched to using the major product of the diimide reduction for our further investigations (Scheme 6). HAT hydrogenation and protecting group manipulations

converted **14b** into neopentyl alcohol **17b**, which was oxidized using Stahl's method¹⁸ and homologated to aldehyde **18b**. For this homologation, Morcken's boron–Wittig reaction¹⁹ proved uniquely efficient and reliable. Next, we introduced the pyrone moiety in a three-step sequence²⁰ including a vinylogous Mukaiyama aldol addition of silyl dienolate **A3** to aldehyde **18b** and oxidation of the intermediary alcohol with Dess–Martin periodinane, followed by thermal cyclization to provide compound **19b**.

Compound **19b** contains all the carbons of the natural product and five of six stereocenters in the correct configuration. Completion of the synthesis would require a satisfactory



Scheme 6 Finishing the carbon skeleton of A-74528.



Scheme 5 (A) Final modifications of the A-ring and F-ring. (B) Structural model of intermediate **13**. (C) X-ray structure of ether **16a** (disordered TBS group not shown).



solution of the alkene reduction, C-ring benzylic oxidation, unmasking of the 1,3-dicarbonyl by cleavage of the isoxazole, and global demethylation. Model studies with an advanced intermediate indicated that the latter two transformations should be straightforward.

In our studies towards the double reduction of the penta-dienol moiety in **13** with Adams' catalyst we had observed the formation of enaminone **20b** albeit with undesired stereochemical outcome (Scheme 7). This shows that the isoxazole can be cleaved under these conditions and that the enaminone does not spontaneously hydrolyze to afford the 1,3-diketone. After some experimentation, however, we found that treatment of **20b** with *tert*-butyl nitrite in DMSO in the presence of triflic acid²¹ gave the enaminone hydrolysis product **21b** (61% yield). This reaction is believed to proceed *via* formation of diazonium intermediate **A4** followed by attack of water and expulsion of nitrogen. Finally, treatment of **21b** with BBr₃ led to the deprotection of all four methyl ethers and cleavage of the silyl ether with an unoptimized yield of 36%. This demonstrates that the secondary hydroxy group and the enolized 1,3-diketone are compatible with such demethylation conditions.

Conclusions and outlook

In sum, we have synthesized compounds that closely resemble the natural product A-74528, bear most of its functional groups and stereocenters, and could share its biological activity. The key steps of our pathway include a molybdenum-catalyzed allylic alkylation to establish the central quaternary stereocenter, the installation of a masked 1,3-diketone *via* a (3 + 2)-cycloaddition, the formation of the A-ring by gold-mediated alkyne hydroarylation, and a late-stage pyrone attachment *via* a vinylogous Mukaiyama aldol reaction. Our experiments have provided insights into chemical stabilities and chemoselectivities as well as diastereoselectivities, that will inform future synthetic work. While it may be possible to finish the synthesis from compound **16a** with brute force, ignoring the unfortunate diastereoselectivity of the *exo*-methylene double bond reduction, our study also provides a plethora of readily

accessible intermediates that could eventually be forwarded to A-75428.

Data availability

Synthetic procedures, characterization and X-ray crystallography data and NMR spectra are provided in the ESI† accompanying this paper.

Author contributions

The manuscript was written through contributions of all authors.

Conflicts of interest

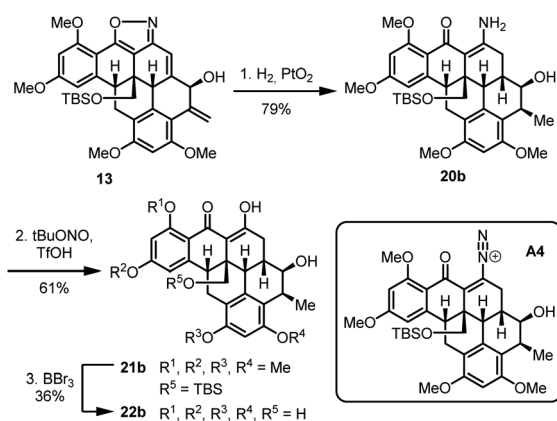
There are no conflicts to declare.

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Scheme 7 Enaminone hydrolysis and global demethylation studies.



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