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Large-scale asymmetric synthesis of the bioprotective agent JP4-039 and analogs

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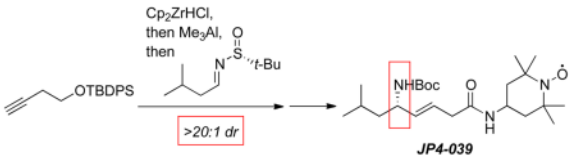
Abstract



JP4-039 is a novel nitroxide conjugate capable of crossing lipid bilayer membranes and scavenging reactive oxygen species (ROS). An efficient and scalable one-pot hydrozirconation-transmetalation-imine addition methodology has been developed for its asymmetric preparation. Furthermore, this versatile methodology allows for the synthesis of cyclopropyl and fluorinated analogs of the parent lead structure.

The 4-amino-Tempo (4-AT) derivative JP4-039 (**(*S*)-6a**, Scheme 2) is a lead structure among a new generation of peptidomimetic conjugates targeted to mitochondria and effective at scavenging reactive oxygen species (ROS) such as the superoxide radical anion.[1,2] In particular, JP4-039 has been shown to protect cells from radiation damage[2b] and to prolong survival of mice subjected to high levels of ionizing irradiation.[2c] Moreover, the ameliorating effects of JP4-039 in irradiation-induced delay of bone wound healing were demonstrated in a murine model of combined bone wound/irradiation injury.[2d] JP4-039 and its larger congener, XJB-5-131, are found to enrich in mitochondria by a factor of 30–600 times over the cytosolic concentration, at least in part due to their affinity to the mitochondrial lipid, cardiolipin.[1b,2a] There, they serve to scavenge ROS and prevent hydroperoxidation of cardiolipin by cycling between nitroxide, hydroxylamine, and nitroxonium redox states.[3] Their design was based on the structure of the antibiotic gramicidin S; the alkene peptide isostere replaces a polar internal amide bond and thus increases conformational regidity and membrane permeability.[1a,g] In order to further explore the therapeutic potential of JP4-039, a robust synthetic route was required to prepare multigram quantities of highly pure material.

JP4-039 contains a single asymmetric carbon atom as part of an alkene isostere dipeptide moiety composed of leucine and glycine residues. Numerous methods have been developed to generate these α-chiral allylic amines.[4] For example, Overman developed the rearrangement of allylic trichloroacetimidates,[4b] Berkowitz described an approach which

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Supporting Information Available. Experimental procedures, x-ray and complete spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

relies on a Ni(0)-mediated allylic amination,[4c] and Krische reported a C-C bond-forming hydrogenation for this purpose.[4k] Overall, vinyl addition to imines remains the most commonly used strategy to prepare chiral allylic amines.[4d-k] Highly effective protocols for this route are based on the reductive coupling of alkynes,[4d-f] the diastereoselective addition of vinylorganometallic species,[4g-i] and the acylvinyl anion addition.[4j]

Our group has previously developed an efficient Zr-based method for the asymmetric synthesis of allylic amines.[5] Hydrozirconation of alkyne **2**[6] with Cp_2ZrHCl,[7] followed by transmetalation to trimethylaluminum and subsequent addition to chiral sulfinimine[8] **(*R*)-1** provided a diastereomerically pure (>20:1 *dr*) allylic amine according to ^{1}H NMR analysis of the crude reaction mixture (Scheme 1). A four-membered chelate model has been proposed to account for this excellent diastereoselectivity.[5a,c] Treatment of the crude reaction mixture with HCl in diethyl ether yielded the amine hydrochloride **(*S*)-3** on multigram scale and in 81% yield over 3 steps from 3-butyn-1-ol. The *N-tert*-butylsulfinimine **(*R*)-1** could be easily obtained from isovaleraldehyde in 90% yield and proved remarkably stable to storage.

We were able to use the allylic sulfinylamine **(*S*)-3** as a linchpin intermediate for the preparation of JP4-039 as well as a first series of analogs. Thus, acylation of **(*S*)-3** with either a Boc, Cbz or diphenylphosphinoyl group, followed by TBAF-mediated deprotection of the silyl ether, afforded the alcohols **(*S*)-5a-c** (Scheme 2). Jones oxidation to the corresponding carboxylic acids and final coupling with 4-AT using the EDCI/HOBt/DMAP protocol provided the desired alkene isosteres **(*S*)-6a-c** in moderate to good yields.

This methodology was further adapted for a >150 g scale preparation of JP4-039 (**(*S*)-6a**), obtained in 32% overall yield, 99% purity and >99% *ee* based on HPLC analysis. At this scale, the target compound was isolated from a 20:3 mixture of *n*-hexane/EtOAc as a crystalline solid. The X-ray structure of **(*S*)-6a** is in good agreement with a type II' β-turn (Figure 1). The distance of 3.30 Å for the *i* to *i*+3 intramolecular hydrogen bond between the carbonyl oxygen of the Boc function and the nitrogen atom of 4-AT is indicative of a weak covalent component in this H-bond.

The (*R*)-enantiomer of JP4-039 **(*R*)-6a** was obtained in 52% overall yield according to an analogous synthetic route starting from (*S*)-sulfinimine **(*S*)-1** (Scheme 3). Chiral SFC on a Chiralpak-IC phase was used to determine an *ee* of 96.6% for alcohol **(*S*)-5a** and 98.0% for its enantiomer **(*R*)-5a**.

An isosteric replacement of the (*E*)-alkene bond with a cyclopropyl moiety was also investigated as part of our medicinal chemistry program.[9] Charette-modified Simmons-Smith cyclopropanation[10] of the Cbz-protected allylic amine **(*S*)-4b** with $Zn(CH_2I)_2$•DME complex[11] provided the cyclopropyl analog **7b** on gram scale in 54% yield (65% based on recovered intermediate **(*S*)-4b**) over 2 steps (Scheme 4). Only one diastereomer was isolated after chromatography on SiO_2 (>20:1 *dr* by ^{1}H NMR). Since **7b** was not crystalline and spectroscopic analysis did not allow an unambiguous assignment of its configuration, we resorted to an X-ray analysis of a suitable crystalline derivative. Hydrogenolysis, coupling with *p*-bromobenzoyl chloride, TBAF-desilylation and treatment with 1-naphthylisocyanate afforded urea **10**, which gave fine colorless needles suitable for X-ray diffraction (Scheme 5). The X-ray analysis confirmed the *anti*-configuration of the cyclopropane vs the C-N bond (Figure 2). This diastereoselectivity had been previously noted by our group for the dimethylzinc-mediated addition of alkenylzirconocenes to *N*-diphenylphosphinoyl imines, which provided diastereomerically pure *C*-cyclopropylalkylamines.[5b,9f] The high level of *anti*-selectivity is also consistent with diastereoselectivities observed in the Simmons-Smith cyclopropanation of allylic ethers.[12]

Somewhat surprisingly, the Boc group on **(*S*)-4a** was not compatible with the Simmons-Smith conditions. The desired intermediate **7a** was therefore prepared by hydrogenolysis of **7b** followed by Boc protection of the resulting amine (Scheme 4). Subsequent TBAF-desilylation of **7a** and **7b**, Jones oxidation, and coupling with 4-AT afforded the cyclopropyl isosteres **8a** and **8b**.

Finally, a difluorinated analog of JP4-039 was envisioned to enhance the bioavailability of the agent. The methyl ester **(*S*)-11**, prepared from alcohol **(*S*)-5a** by Jones oxidation and esterification of the acid with TMS-diazomethane, was treated with 3 equiv. of the fluorinating agent *N*-fluoro-*N*-(phenylsulfonyl)benzene-sulfonamide (accufluor, NFSi)[13] and 2.3 equiv. of NaHMDS in THF at −78 °C to afford the desired α,α-difluoroester **(*S*)-12** in 86% yield (Scheme 6).[14] Saponification with *tetra*-butylammonium hydroxide (TBAH)[15] and condensation with 4-AT provided the difluorinated JP4-039 analog **(*S*)-13** in good yield. The biological activities of **(*S*)-13** as well as **(*R*)-6a** are currently under investigation.

In summary, the hydrozirconation-transmetalation-imine addition methodology was extended to the preparation of both enantiomers of a chiral allylic amine and the corresponding dipeptide alkene and cyclopropane isosteres. This straightforward approach enabled the synthesis of the bioprotective Tempo-conjugate JP4-039 on a 160 g scale. Noteworthy is also the ready access to diverse analogs of the parent compound, including the *β*,*γ*-cyclopropylamine isosteres and difluoromethylene derivatives. SAR studies of the JP4-039 scaffold are ongoing, and the synthesis of further derivatives will be reported in due course, along with their antioxidant and biological activities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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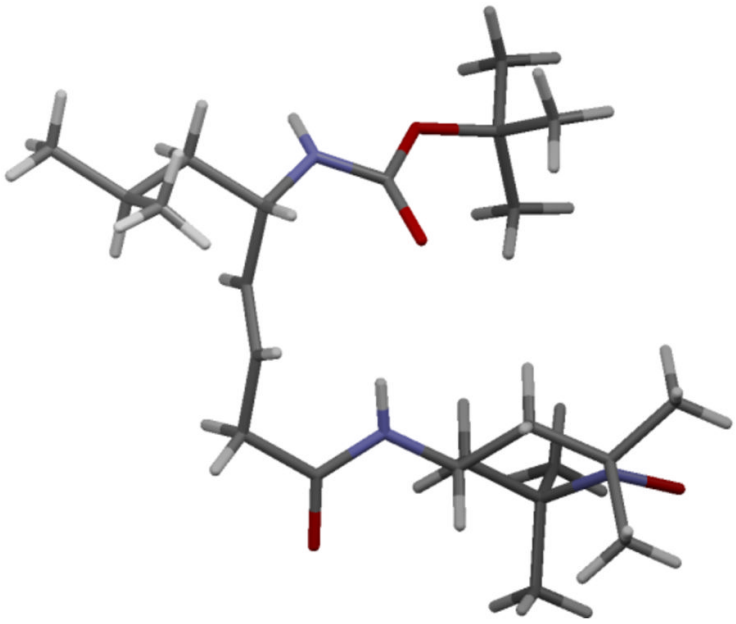


Figure 1.
X-ray structure of JP4-039.

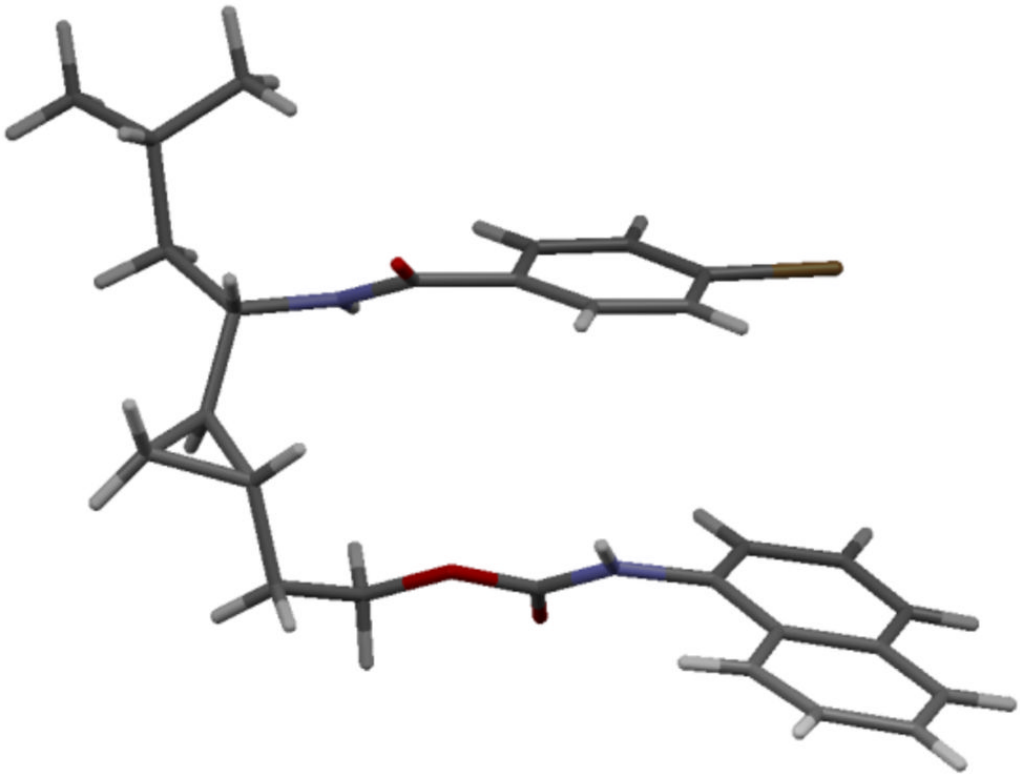
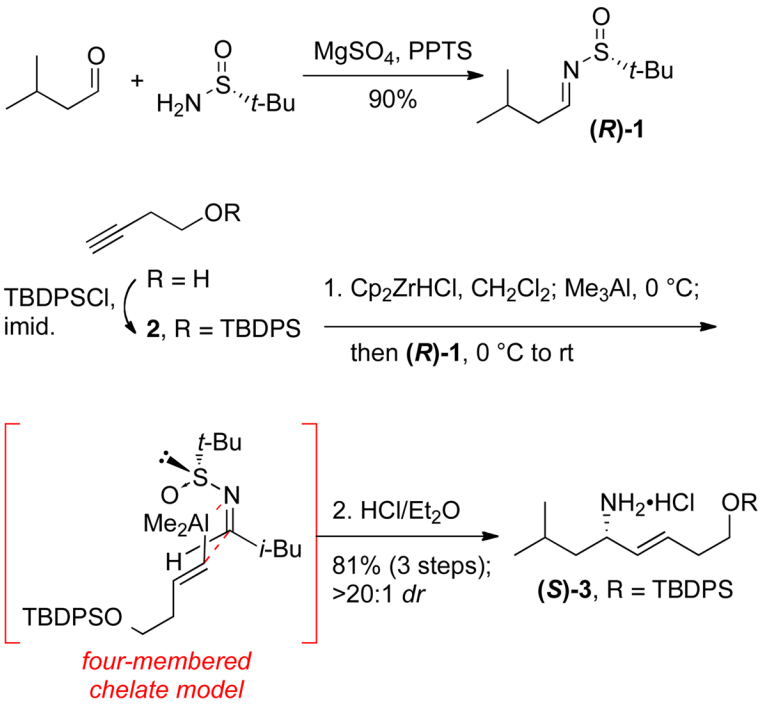
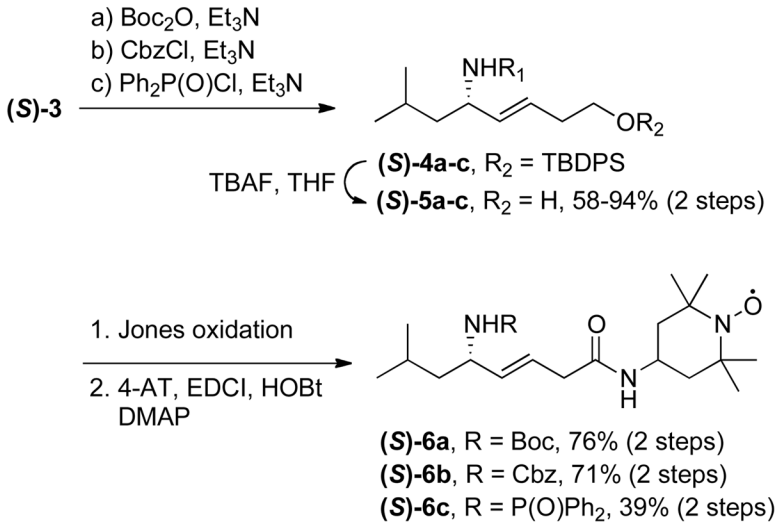


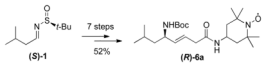
Figure 2.
X-ray structure of **10**



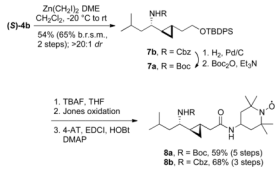
Scheme 1.
Synthesis of the common amine intermediate **(*S*)-3**



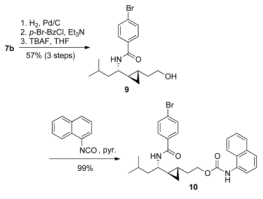
Scheme 2.
Synthesis of the (*E*)-alkene isosteres **(*S*)-6a-c**



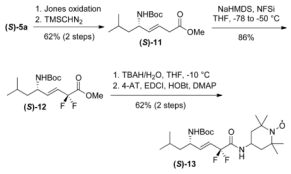
Scheme 3.
Synthesis of **(*R*)-6a**



Scheme 4.
Synthesis of the cyclopropyl isosteres **8a-b**



Scheme 5.
Synthesis of **10**



Scheme 6.
Synthesis of the difluoro analog **(*S*)-13**