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Large-Scale Asymmetric Synthesis of the Bioprotective Agent JP4-039 and Analogs

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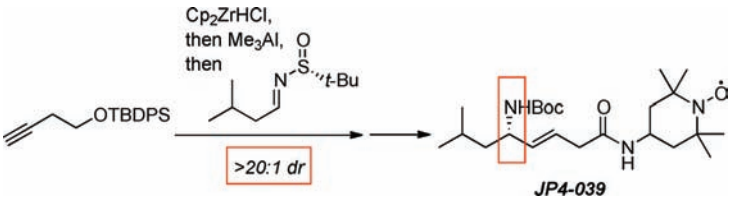
ABSTRACT



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 the 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 rigidity 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

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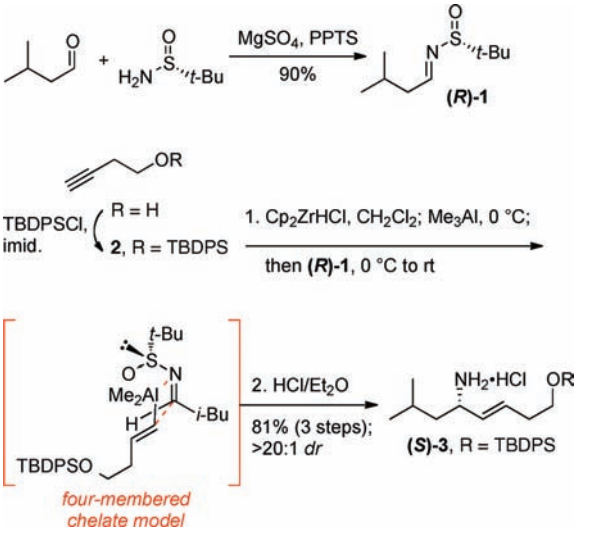
developed to generate these α-chiral allylic amines.[4] For example, Overman developed the rearrangement of allylic trichloroacetimidates,[4b] Berkowitz described an approach which relies on a Ni(0)-mediated allylic amination,[4c] and Krische reported a C−C bond-forming hydrogenation for this purpose.[4k] 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]

Scheme 1. Synthesis of the Common Amine Intermediate (*S*)-**3**

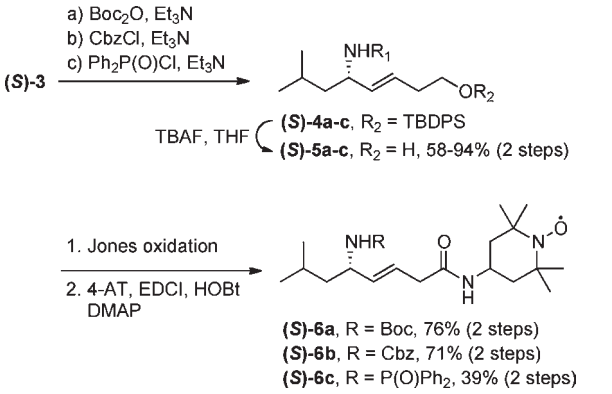


Our group has previously developed an efficient Zr-based method for the asymmetric synthesis of allylic amines.[5] Hydrozirconation of alkyne **2**[6] with Cp_2ZrHCl,[7] followed by transmetalation to trimethylaluminum and subsequent addition to chiral sulfinimine[8] (*R*)-**1**, provided a diastereomerically pure (> 20:1 *dr*) allylic amine according to ^1H NMR analysis of the crude reaction mixture (Scheme 1). A four-membered chelate model has been proposed to account for this excellent diastereoselectivity.[4a,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 three 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 and chiral SFC 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

Scheme 2. Synthesis of the (*E*)-Alkene Isosteres (*S*)-**6a**−**c**



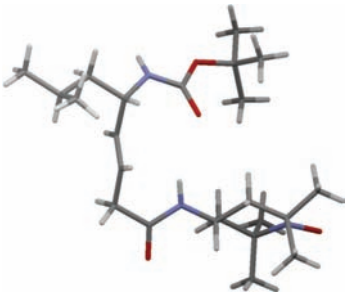


Figure 1. X-ray structure of JP4-039.

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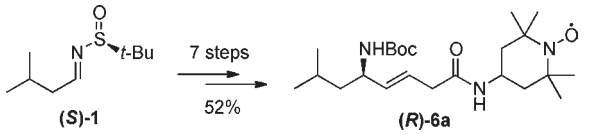
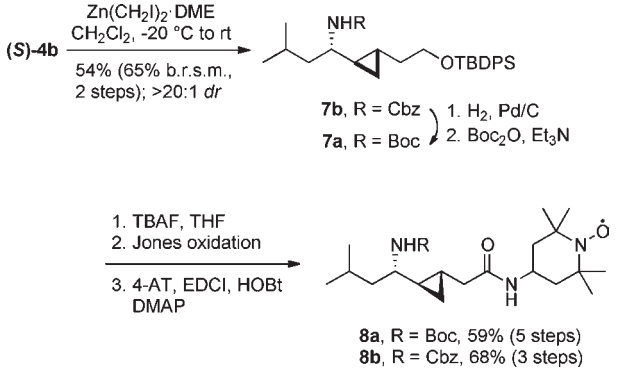
3.30 Å for the i to $i + 3$ intramolecular hydrogen bond between the carbonyl oxygen of the Boc function and the nitrogen atom of 4-AT is indicative of a weak covalent component in this H-bond.

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**.

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

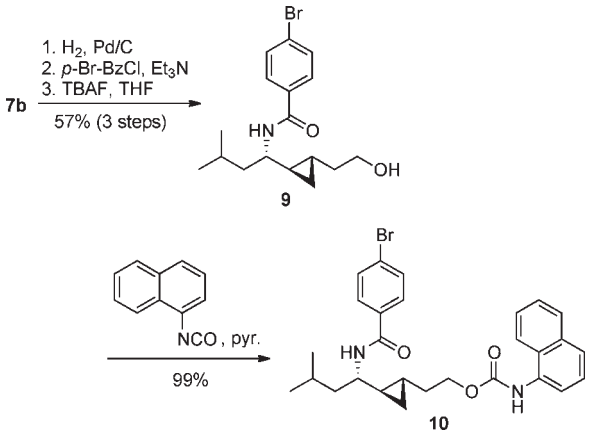


Scheme 4. Synthesis of the Cyclopropyl Isosteres **8a**–**b**



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 the $Zn(CH_2I)_2$•DME complex[11] provided the cyclopropyl analog **7b** on gram scale in 54% yield (65% based on recovered intermediate (***S*)-4b**) over two steps (Scheme 4). Only one diastereomer was isolated after chromatography on SiO_2 (> 20:1 *dr* by ^1H NMR). Since **7b** was not crystalline and spectroscopic analysis did not allow an unambiguous assignment of its configuration, we resorted to

Scheme 5. Synthesis of **10**



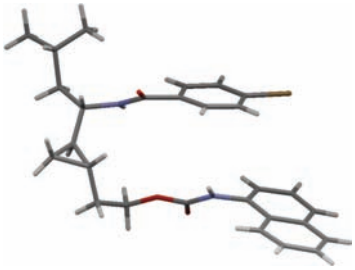


Figure 2. X-ray structure of **10**.

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

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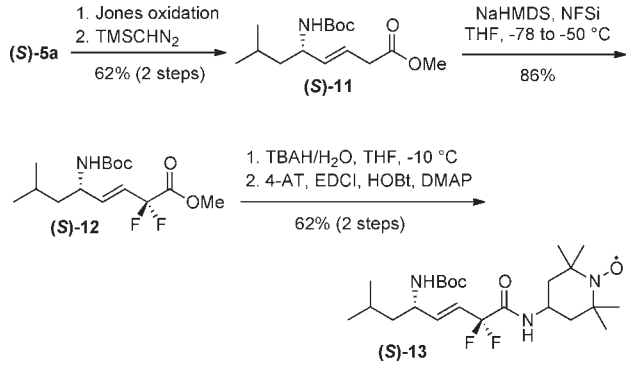
Scheme 6. Synthesis of the Difluoro Analog (*S*)-13



(*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 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.

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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.

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