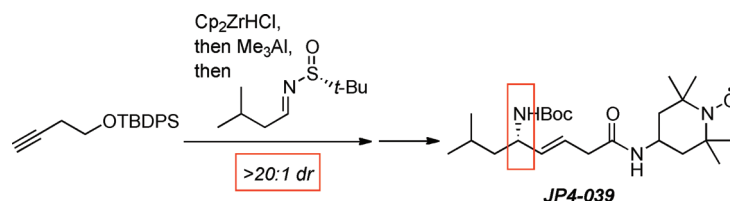


Large-Scale Asymmetric Synthesis of the
Bioprotective Agent JP4-039 and AnalogsMarie-Céline Frantz,[†] Joshua G. Pierce,[†] Joan M. Pierce,[†] Li Kangying,[‡]
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ABSTRACT



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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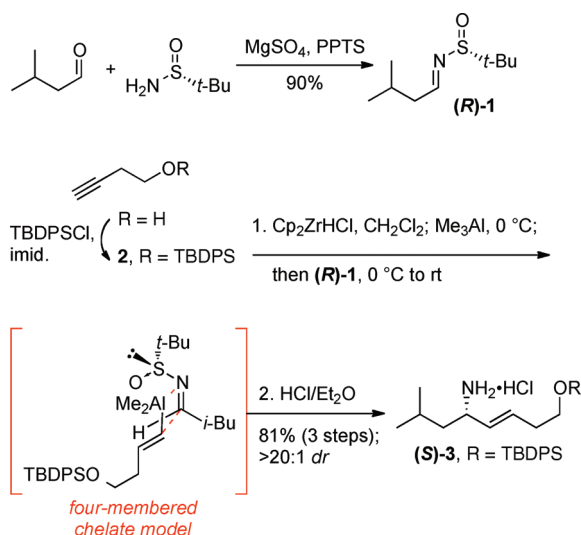
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developed to generate these α -chiral allylic amines.⁴ For example, Overman developed the rearrangement of allylic trichloroacetimidates,^{4b} Berkowitz described an approach which relies on a Ni(0)-mediated allylic amination,^{4c} and Krische reported 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 sulfinimine⁸ (*R*)-**1**, provided a

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

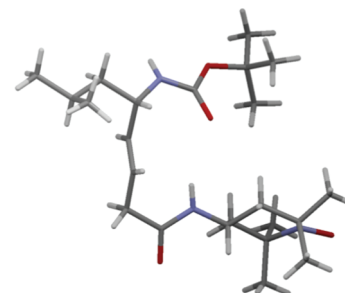
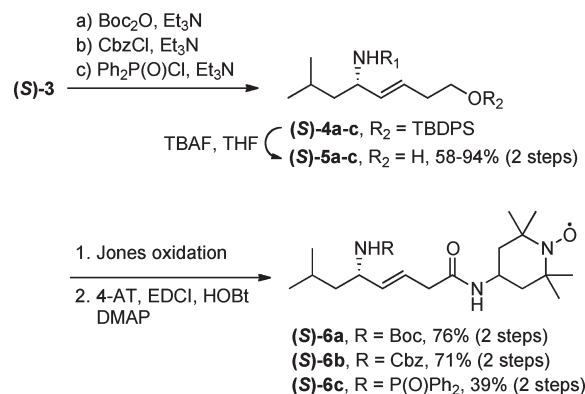


Figure 1. X-ray structure of JP4-039.

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-butyne-1-ol. The *N*-*tert*-butylsulfinimine (*R*)-**1** could be easily obtained from isovaleraldehyde in 90% yield and proved remarkably stable to storage.

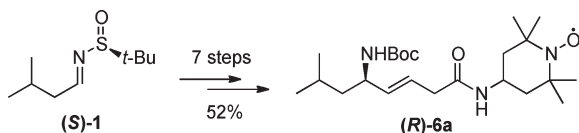
We were able to use the allylic sulfinylamine (*S*)-**3** as a linchpin intermediate for the preparation of JP4-039 as well as a first series of analogs. Thus, acylation of (*S*)-**3** with either a Boc, Cbz, or diphenylphosphinoyl group, followed by TBAF-mediated deprotection of the silyl ether, afforded 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

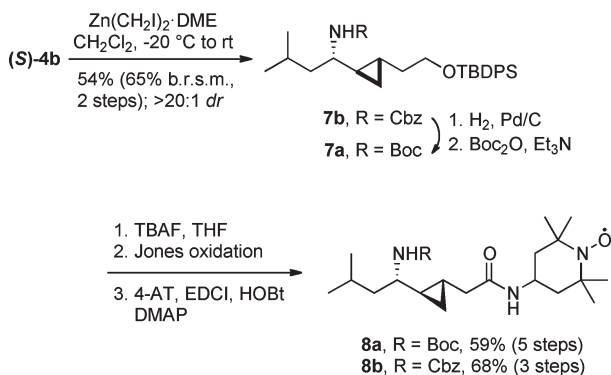
3.30 Å for the i to $i + 3$ intramolecular hydrogen bond between the carbonyl oxygen of the Boc function and the nitrogen atom of 4-AT is indicative of a weak covalent component in this H-bond.

The (*R*)-enantiomer of JP4-039 (**R**)-**6a** was obtained in 52% overall yield according to an analogous synthetic route starting from (*S*)-sulfimine (**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 cyclopropanation¹⁰ of the Cbz-protected allylic amine (**S**)-**4b** with the $\text{Zn}(\text{CH}_2\text{I})_2 \cdot \text{DME}$ complex¹¹ 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

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Scheme 5. Synthesis of **10**

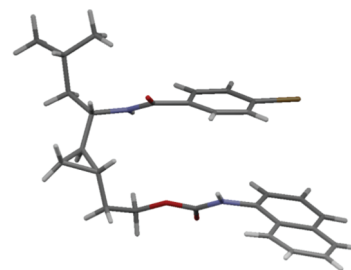
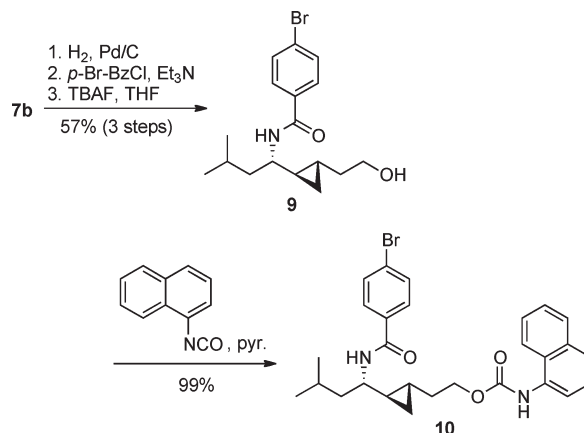


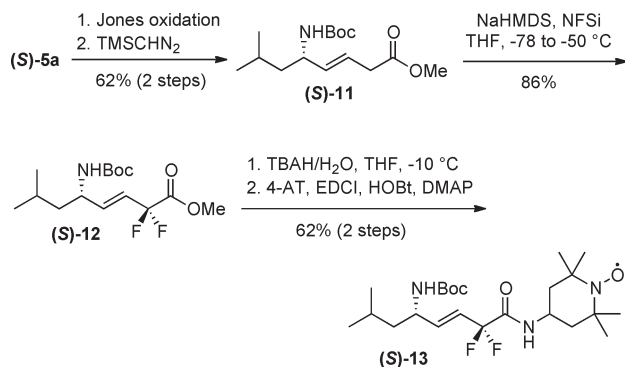
Figure 2. X-ray structure of **10**.

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 prepara-

tion 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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