Organic LETTERS

Letter

pubs.acs.org/OrgLett

Synthesis and Bioactivity of a Brasilicardin A Analogue Featuring a Simplified Core

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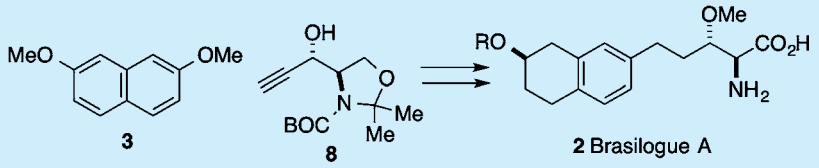
Supporting Information



ABSTRACT: An analogue **2** of Brasilicardin A, **1** (BraA), a potent immunosuppressive and cytotoxic agent, was synthesized in which the natural tricyclic skeleton was replaced with a synthetically more accessible substituted tetrahydronaphthalene core. BraA, this analogue (BraL), and cyclosporine A were tested for their ability to inhibit the proliferation of human T cells upon CD3/CD28 activation. Although BraL did not impact T cell activation over the dose range tested, this study shows the inhibitory activity of BraA on human T cells for the first time.

Brasilicardin A (BraA), **1** (Figure 1), is a terpenoid natural product isolated from the pathogenic bacteria *Norcardia brasiliensis* that features a disaccharide and amino acid moieties linked to a central tricyclic core.[1] In addition to the unusual and synthetically challenging *anti*−*syn*−*anti* configuration of the perhydrophenanthrene skeleton,[2] BraA is a cytotoxic agent with highly potent immunosuppressive activity (IC_{50}, 0.057 μg/mL, mouse mixed lymphocyte assay)[3] that has been shown to act via a different mechanism than the current immunosuppressive agents,[4] which can cause severe off-target side-effects, i.e., cyclosporine A (CsA) and tacrolimus (FK506),[5] or rapamycin.[6] Clinical investigation of BraA, however, has been hampered by a low-yielding fermentation process and an unsolved total synthetic route that, even if completed, is unlikely to be able to deliver clinically useful amounts of material. Herein we describe the preparation of a BraA analogue (BraL), **2**, that connects the natural sugar and amino acid components of BraA to the tetrahydronaphthalene (tetralin) core structure (Figure 1). We also report the results of biological testing of these compounds, as well as those of cyclosporine A, for their immunosuppressive effects.

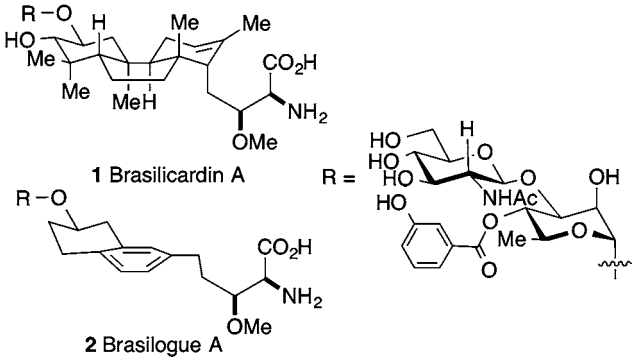
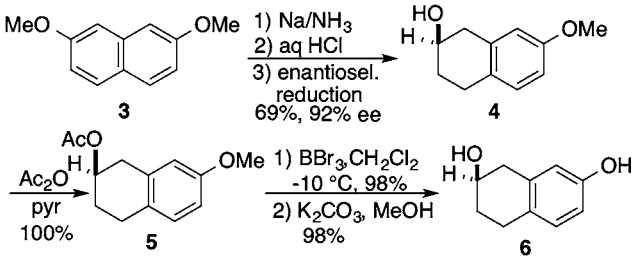


Figure 1. Structure of BraA, **1**, and the tetralin analogue, BraL, **2**.

Scheme 1. Synthesis of (*R*)-7-Hydroxytetralin-2-ol, 6



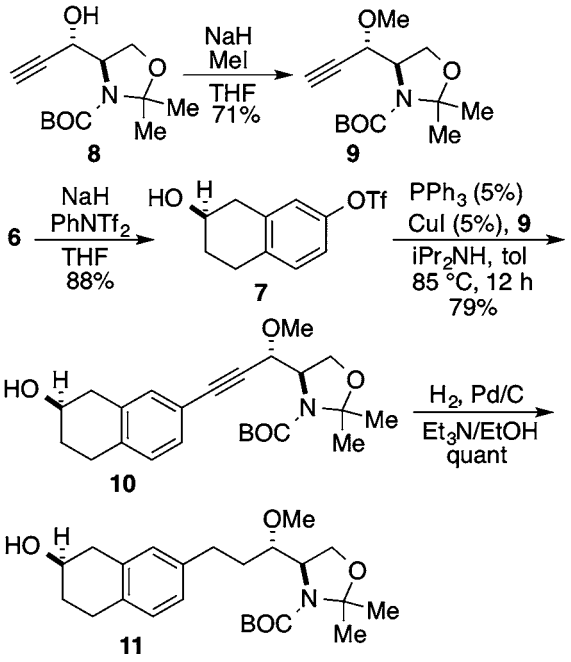
BraL, **2**, was designed to retain the natural disaccharide and amino acid portions of the natural compound based on structure−activity relationship (SAR) data of related natural products[7] and a series of derivatized BraA compounds.[8] Several candidate core structures were built using Dreiding models, and they were compared with a model of the natural core to see which best approximated the relative distance and orientation of the sugar and amino acid side chain. Ultimately, a chiral tetralin core was shown to mimic the natural tricyclic core and to also provide for relatively straightforward assembly.

The synthesis of **2** was accomplished in 15 steps from known intermediates as shown in Schemes 1−4. Initially we explored a direct route to the chiral 7-hydroxytetralin-2-ol, **6**, via superacid-mediated reduction of 2,7-dihydroxynaphthalene,[9] followed by

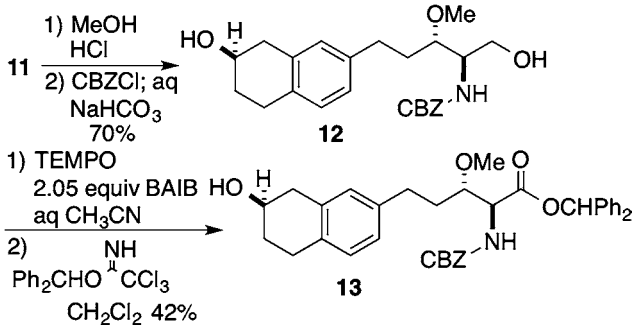
Received: June 11, 2015
Published: July 6, 2015

DOI: 10.1021/acs.orglett.5b01712
Org. Lett. 2015, 17, 3608−3611

Scheme 2. Synthesis of the Alcohol, 11



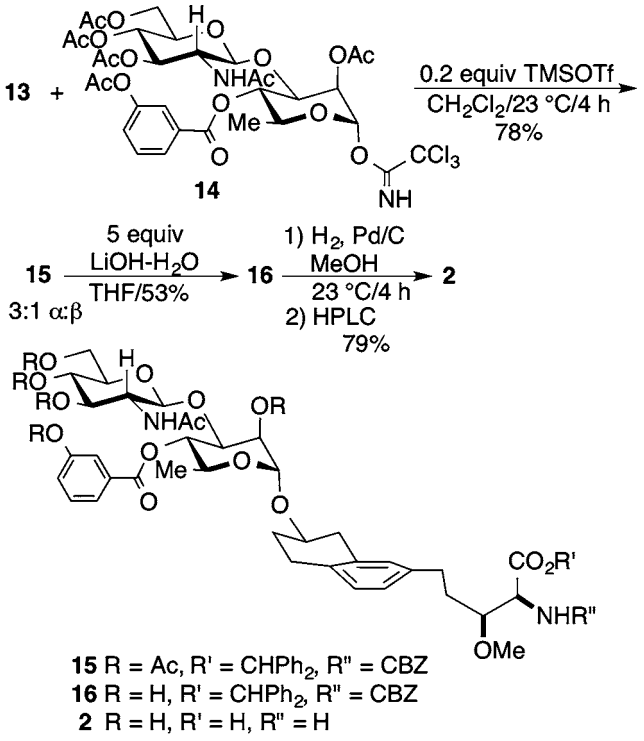
Scheme 3. Synthesis of BraA Analogue Core Structure 13



asymmetric reduction using the $[RuCl_2(benzene)]_2$ catalyst with the ligand (*R*,*R*)-*N*-(2-amino-1,2-diphenylethyl)-*p*-toluenesulfonamide and KOH in iPrOH according to the method of Noyori as reported by Node.[10] Although this reaction sequence, via the reduction of 7-hydroxytetralin-2-one, proceeded in 88% ee as determined by methylation of the phenol followed by Mosher's ester formation, in our hands the superacid reduction gave unpredictable conversions and was problematic on the multigram scale. Alternatively, dissolving metal reduction of the 2,7-dimethoxynaphthlene **3**[11] followed by Noyori asymmetric reduction[12] gave the chiral (*R*)-7-methoxytetralin-2-ol, **4**, in good yield and excellent ee (92%; determined by chiral SFC). Direct demethylation of **4** with BBr_3 was unsuccessful, but protection of the alcohol as the acetate gave **5**, which was demethylated using BBr_3. The acetate was then cleaved in base to give **6** in excellent yield.

The synthesis of the key alcohol intermediate **11** is depicted in Scheme 2. Selective triflation of the phenol of **6** over the secondary alcohol was achieved in 88% yield using NaH and *N*-phenyltriflimide in THF to provide **7** in 88% yield. Methylation of the known propargylic alcohol **8**,[13] which is readily prepared from D-serine,[14] afforded the desired methyl ether **9** in good yield. Sonogashira coupling of **7** with **9** proceeded in 79% yield on a gram scale to provide the aryl alkyne **10**. A possible alternate

Scheme 4. Synthesis of BraA Analogue (BraL) 2



route, namely, formation of the lithium anion of the 7-ethynyltetralin-2-ol TBS ether, via treatment with BuLi in THF/HMPA, followed by addition of the well-known Garner aldehyde[15] did not provide the TBS-protected hydroxy analogue of **10**, even though compound **8** is prepared by just such an addition. Hydrogenation of **10** proceeded uneventfully to give the saturated compound **11** in quantitative yield. The final transformations to produce the compound to couple with the protected disaccharide unit are shown in Scheme 3. Concomitant acetonide opening and Boc removal in methanolic·HCl gave the diol amine, the amine of which was protected as the benzyl carbamate (Cbz) to give **12** in two steps and 70% overall yield. Chemoselective oxidation of the primary alcohol using TEMPO in aqueous acetonitrile with bis(acetoxy)iodobenzene (BAIB) as the primary oxidant gave the carboxylic acid,[16] which was not isolated but rather directly converted to the benzhydryl ester **13** under neutral conditions using benzhydryl trichloroacetimidate in DCM[17] (two steps, 42% yield).

We had previously accomplished a very concise synthesis of a fully protected and activated BraA disaccharide, which began with D-glucosamine and L-rhamnose and required only 9 steps to produce the crystalline trichloroacetimidate **14**.[18] At that time, we also demonstrated that a cyclic secondary alcohol, e.g., cholesterol as a model, could be successfully coupled to **14** using TMSOTf as an activator (i.e., a Schimdt glycosylation[17]), which provided the desired α-anomeric linkage in a 9:1 α/β ratio and in good yield.[18] When we carried out this key coupling reaction of the BraA analogue core structure **13** with the trichloroacetimidate **14** using TMSOTf as the promoter, we obtained the desired glycoside **15** in 78% yield (Scheme 4). The anomeric selectivity was somewhat diminished since **15** was shown to be an approximate 3:1 α/β ratio. This assignment was confirmed by the $^1J_{CH}$ coupling constants.[19] Interestingly, the anomeric selectivity here, and in our previous study,[18] could be improved by elevating the reaction temperature from −78 to 22 °C.

DOI: 10.1021/acs.orglett.5b01712
Org. Lett. 2015, 17, 3608−3611

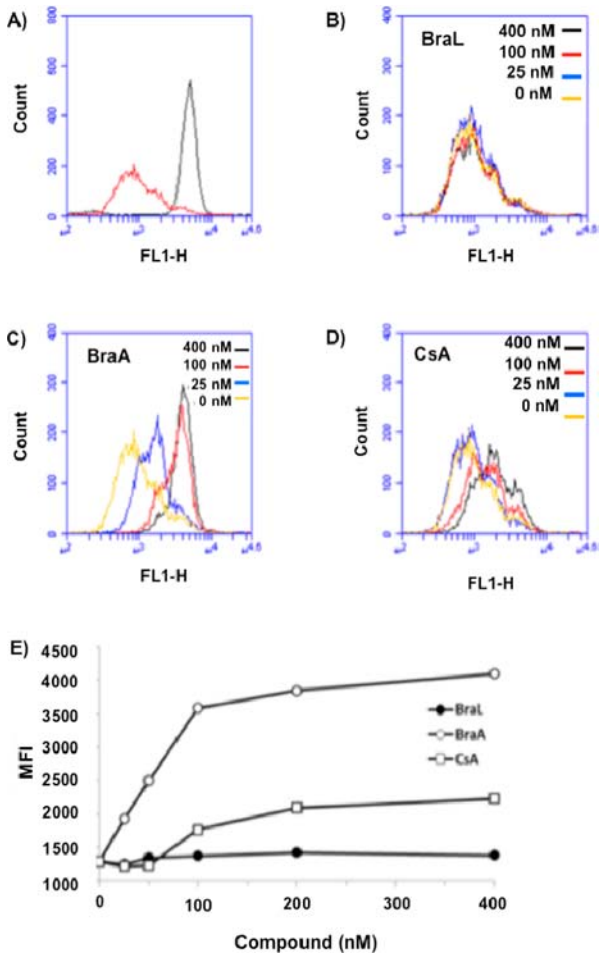


Figure 2. Characterization of BraL **2** activity in inhibiting CD4+ T cell proliferation. (A) Comparison of T cell cellular fluorescence (FL1-H) in the absence (black histogram) or presence (red histogram) of anti-CD3/CD28 bead stimulation. Flow cytometric evaluation of T cell proliferation in the presence of increasing concentrations of (B) BraL **2**, (C) BraA **1**, and (D) cyclosporine A (CsA). (E) Mean fluorescence intensity (MFI) comparison of anti-CD3/CD28 bead stimulated CD4+ T cells in the presence of increasing concentrations of BraL **2** (open circles), BraA **1** (closed circles), and CsA (open squares).

Although the anomers could not be separated at this stage by silica gel chromatography, final HPLC purification of **2** was effective in isolating the desired anomer. Deprotection of all of the five acetate protecting groups of **15** was achieved in 53% yield with a stoichiometric amount of $LiOH{\cdot}H_2O$ in $THF{:}H_2O$, which left the benzhydryl ester and the significantly more stable benzoate moieties intact, **16**. It is important to note that these conditions did not cause any racemization or elimination of the protected β-methoxy amino acid derivative. We had already reported on selective hydrolysis of the acetates without affecting the hindered benzoate functionality of the disaccharide unit.[18] Final deprotection of both the Cbz and benzhydyl esters using catalytic hydrogenation with palladium on charcoal in methanol proceeded without complication, and the BraA analogue, the brasililogue BraL, **2**, was isolated by preparative HPLC chromatography in 79% yield as a single anomer as the TFA salt. Thus, this compound was synthesized in only 10 steps from the optically active diol **6** in fair overall yield (~10%).

The immunosuppressive activity of BraA was previously studied using a one-way mouse mixed lymphocyte reaction

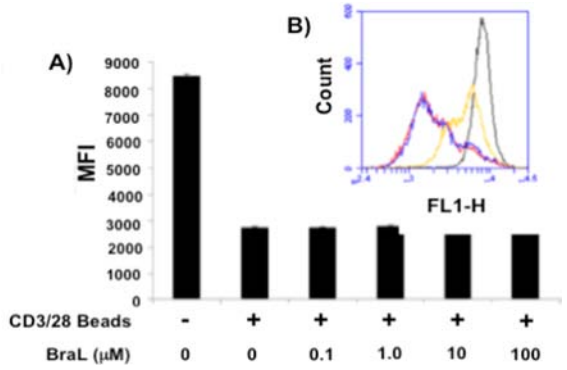


Figure 3. Evaluation of T cell anti-CD3/CD28-mediated proliferation in the presence of high doses of synthetic BraL **2**. (A) Expanded dose titration of BraL **2** on T cell proliferation as determined by quantification of mean fluorescence intensity (MFI). (B) Inset depicting fluorescence levels of unstimulated T cells (black histogram), bead-stimulated alone T cells (red histogram), bead-stimulated T cells in the presence of 100 mM BraL **2** (blue histogram), or bead-stimulated T cells in the presence of 100 nM BraA **1** (mustard histogram).

(MLR) and demonstrated an IC_{50} of approximately 63 nM.[3] Here, in order to evaluate the efficacy of BraA **1** and BraL **2** in human cells, an in vitro CD4+ T cell activation system was developed based upon anti-CD3/CD28 bead stimulation and CFSE fluorescent signal dilution as a means of monitoring cellular proliferation. In this system, bead stimulation of normal donor human CD4+ T cells activates the vast majority to replicate (Figure 2a, red histogram) compared to unstimulated cells (black histogram). Incubation of activated cells with BraL **2** (up to 400 nM) did not prevent proliferation (Figure 2b), while cells incubated in the presence of natural BraA **1** were significantly inhibited at concentrations as low as 25 nM (Figure 2c). Similar to previous reports, cyclosporine A treatment demonstrated modest activity at concentrations approximating 100 nM in this assay system (Figure 2d).[20] Dose titration analysis of mean fluorescence intensity (MFI) as a function of compound concentration estimates the IC_{50} of BraA **1** as approximately 65 nM, which is consistent with the previously reported results in mouse cells (Figure 2e). BraL **2** was further tested at concentrations between 100 nM to 100 μM in the assay system described above in order to estimate an IC_{50} (Figure 3A). While BraL **2** did not inhibit T cell proliferation at these concentrations, the addition of 100 nM natural BraA **1** was found to be inhibitory ($p < 0.0064$), reproducing our earlier observations (Figure 3B). The inhibition of a CD3/CD28-dependent activation signal by natural BraA **1** is quite significant since this is one of the most potent in vitro activation signals available to immunologists. Using only a CD3 activation, which induces some proliferation and can be inhibited by CsA, activation by both a primary (via CD3) and a secondary signal (via CD28) results in massive proliferation and autocrine secretion of the T cell growth factor IL-2 as well as its receptor.

In conclusion, we have synthesized a BraA mimic in which the natural tricyclic core structure was replaced with a chiral tetralin unit. Although the BraA analogue, BraL, **2**, like the natural BraA, possesses the same amino acid side chain and the complex disaccharide, it did not display the immunosuppressive activity of BraA **1**, shown here for the first time in human T cells. This result demonstrates that merely approximating the distance between the sugar and the amino acid portions of BraA is not sufficient for bioactivity. Additionally, this work presents a model system that

DOI: 10.1021/acs.orglett.5b01712
Org. Lett. 2015, 17, 3608−3611

suggests a protecting group strategy that could be useful in solving the total synthesis of BraA. Although BraL did not mimic the desired immunosuppressive bioactivity of BraA, this study provides a meaningful entry into the BraA SAR, serves as a useful model study for total synthesis of the natural product, and demonstrates for the first time the immunosuppressive activity of BraA in human T cells.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and proton and carbon NMR for all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01712.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Joel Smith and the Prof. Neil Garg laboratory (UCLA) for assistance with the chiral SFC analysis of **4**, Tristan Rose and the Prof. Patrick Harran laboratory (UCLA) for assistance with the HPLC purification of **2**, and Prof. Jun'ichi Kobayashi (Hokkaido University) for donation of an authentic sample of BraA, **1**. We also gratefully thank partial funding for this work from California Capital and NantWorks LLC.

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DOI: 10.1021/acs.orglett.5b01712
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