JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

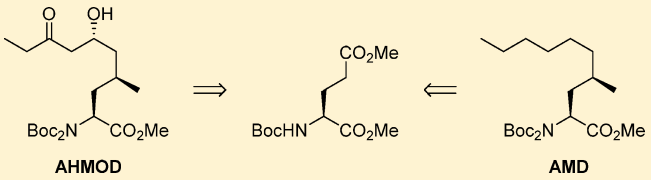
Improved Synthesis of the Unnatural Amino Acids AHMOD and AMD, Components of the Anticancer Peptaibol Culicinin D

Kwang-Yoon Ko, Sarah Wagner, Sung-Hyun Yang, Daniel P. Furkert, and Margaret A. Brimble*

Maurice Wilkins Centre for Molecular Biodiscovery. School of Chemical Sciences, University of Auckland, 23 Symonds Street, Auckland 1142, New Zealand

Supporting Information

ABSTRACT: An improved second-generation synthesis of the unnatural amino acid components of the anticancer peptaibol culicinin D has been developed. With a protected glutamic acid derivate as the starting material, the process readily delivered the Fmoc-protected free acid derivatives of AHMOD ((2*S*)-amino-(6*R*)-hydroxy-(4*S*)-methyl-8-oxodecanoic acid) and AMD ((2*S*)-amino-(4*S*)-methyldecanoic acid) required to support solid phase peptide synthesis (SPPS) for structure−activity studies of the natural product. The same approach also provides improved access to pipecolic acid derivatives. A novel Wittig reagent for one-carbon homologation of aldehydes, developed during this work, is also reported.



INTRODUCTION

Culicinins A−D are linear peptides, isolated in 2006 by He et al. from the fungus *Culicinomyces clavisporus*.[1] The major compound, culicinin D (**1**, Figure 1), was found to exhibit potent antitumor activity, selectively suppressing proliferation of phosphatase tensin (PTEN) negative MDA468 breast tumor cells (IC_{50} 6 nM). Culicinin D belongs to the wider peptaibol family of polypeptide natural products that range between 5 and 20 amino acids in length and possess an acylated N terminus, a C-terminal amino alcohol, and a high abundance of α,α-dialkylated amino acids.[2] The culicinins contain the unnatural amino acids 2-amino-(6*R*)-hydroxy-(4*S*)-methyl-8-oxodecanoic acid (AHMOD) and 2-((2-aminopropyl)amino)-ethanol (APAE). In addition, they also possess a 2-amino-(4*R*)-methyldecanoic acid (AMD) residue that has not previously been identified in other peptaibols. This unique combination of amino acid structures and potent antitumor properties make culicinin D an attractive target for synthesis and structure−activity studies.

Our group recently reported the total synthesis of culicinin D and confirmed the stereochemistry of the hydroxyl group in the AHMOD unit.[3] The key unnatural amino acids, Fmoc-AMD-OH (**2**) and Fmoc-AHMOD-OH (**3**), were prepared in 15% (13 steps) and 3% (12 steps) yields, respectively (Schemes 1 and 2), starting from propionyl (1*S*,2*S*)-pseudoephedrine (**4**). In this study we established that installation of the side chain methyl group could be achieved by stereoselective Myers alkylation of propionate **4**,[4] followed by reductive removal of the auxiliary and conversion to the iodide **5**.[5] Alkylation of the Belokon nickel glycinate complex **6** (Scheme 1, inset)[6] with **5** using sodium hydroxide in acetonitrile, followed by acidic cleavage of the proline-based template, stereoselectively delivered the required α-amino acid nucleus **7**. *N*-Boc protection, esterification, and oxidative cleavage of the olefin then afforded aldehyde **8**, which served as the common precursor for both unnatural amino acids. AMD and AHMOD were prepared via a Wittig (Scheme 1) or asymmetric aldol sequence (Scheme 2), respectively. The di-Boc methyl ester amino acids **9** and **11** were finally converted to the corresponding Fmoc-protected free acids, ready for use in solid-phase peptide synthesis (SPPS).

This first-generation approach proved more efficient than earlier literature syntheses[7] and enabled successful completion of the first total synthesis of culicinin D. Difficulties encountered during scale-up, however, limited access to AMD and in particular the more complex AHMOD. We report here the results of our synthetic investigations that resulted in a more practical and efficient synthetic approach, to provide a reliable platform for ongoing comprehensive SAR studies.

RESULTS AND DISCUSSION

We reasoned that use of a chiral pool starting material might enable diastereoselective installation of the chiral methyl group in the side chain, in contrast to the sequential installation of the chiral centers used in the first -generation synthesis of AHMOD and AMD. Specifically, methylation of *N*-Boc glutamic acid dimethyl ester **12** has been previously shown to selectively afford the 4*S* configuration required.[8] In order to successfully apply this chemistry, it would be necessary to subsequently carry out regioselective reduction of the distal ester and then a one-carbon homologation, to intersect with our previously established synthetic route. It appeared that if these later steps could be efficiently realized, this route might be sufficiently robust to support ongoing synthetic studies, due to the reduced

Received: June 4, 2015
Published: August 7, 2015

DOI: 10.1021/acs.joc.5b01265
J. Org. Chem. 2015, 80, 8631−8636

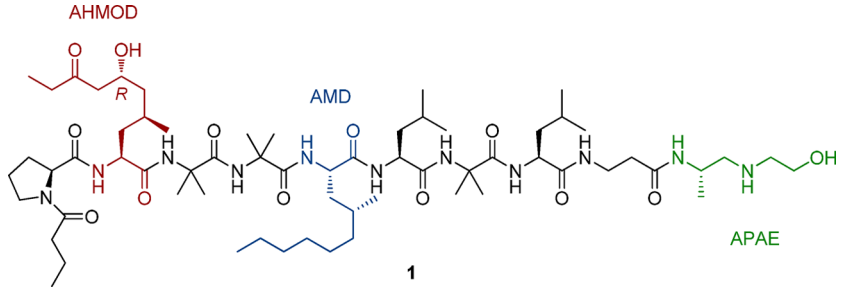
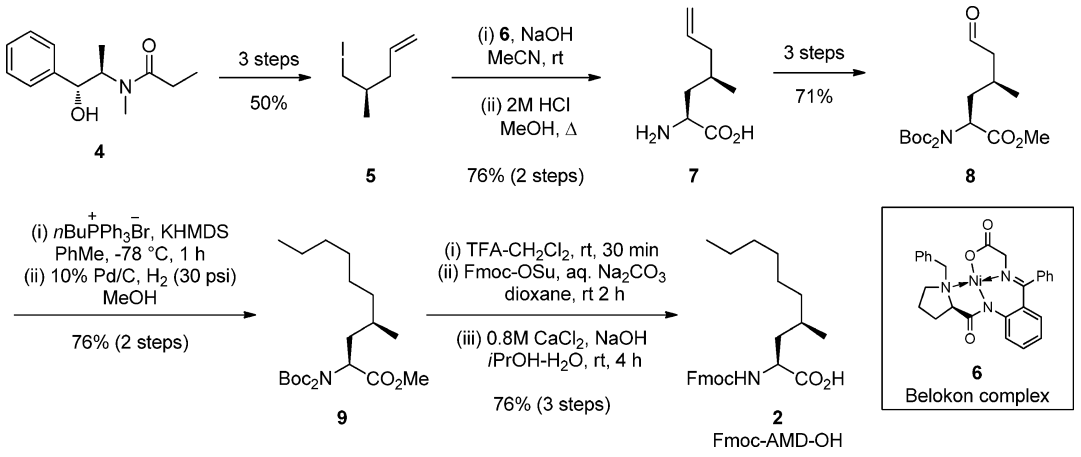
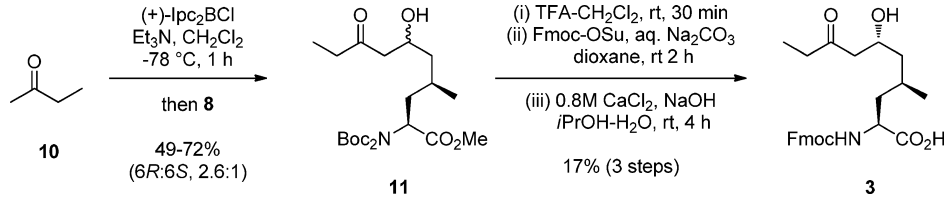


Figure 1. Structure of culicinin D (**1**), highlighting the unnatural amino acids AHMOD (red), AMD (blue), and APAE (green).

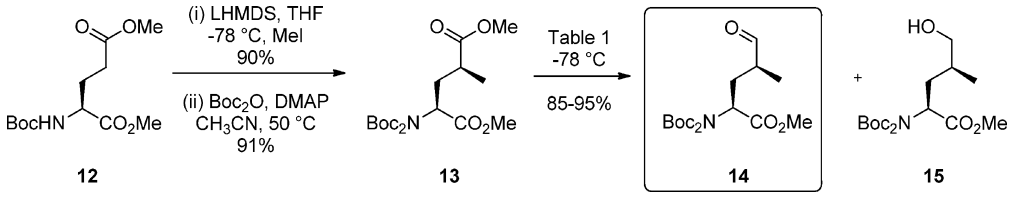
Scheme 1. First-Generation Synthesis of Key Intermediate Aldehyde 8, via Alkylation of Chiral Belokon Ni^{II} Complex 6 (Inset), and Final Conversion to (6*R*)-Fmoc-AMD-OH (2)[5]



Scheme 2. First-Generation Aldol Route for Conversion of Aldehyde 8 to (6*R*)-Fmoc-AHMOD-OH (3)[5]



Scheme 3. Synthesis of Key Aldehyde 14, via Diastereoselective Methylation of Glutamate 12



number of synthetic steps and the ready availability of glutamate **12**, already possessing the amino acid α stereocenter.

Accordingly, treatment of *N*-Boc glutamic acid dimethyl ester **12** with 3 equiv of LHMDS at −78 °C (Scheme 3), followed by addition of methyl iodide, was found to afford the previously reported (4*S*)-methyl glutamic acid diester in excellent yield, as a single diastereoisomer by ^{1}H NMR. Pleasingly, this reaction proved robust to scale-up and could be reliably conducted on up to a 30 mmol scale without erosion of either yield or diastereoselectivity. In order to avoid the formation of byproducts arising from nucleophilic attack of the nitrogen in later steps, a second *N*-Boc group was then introduced, affording **13** in 91% yield, before addressing the selective reduction of the distal ester.

As some difficulty was anticipated in achieving robust partial reduction, **13** was treated with 3 equiv of diisobutyaluminum hydride at −40 °C, to cleanly give alcohol **15** in 86% yield (Table 1, entry 1). Oxidation of **15** proved somewhat unreliable, using a variety of standard reagents (IBX/EtOAc, IBX/DMSO, PCC, Parikh−Doering), but could be achieved on a small scale (<200 mg) under Swern conditions to afford the desired aldehyde **14** in 88% yield. Ultimately, however, optimization of the DIBAL partial reduction step parameters proved to be the more reliable alternative. The optimal conditions (entry 7) reliably delivered aldehyde **14** via

DOI: 10.1021/acs.joc.5b01265
J. Org. Chem. 2015, 80, 8631−8636

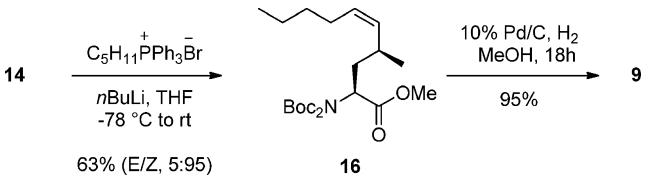
Table 1. Regioselective Partial Reduction of Glutamic Ester 13

entry	DIBAL (equiv)	solvent	time (h)	**13:14:15**
1	3.0	THF	1	0:0:100[a]
2	1.1	Et_2O	1	63:27:0
3	1.1	Et_2O	2	55:45:0
4	1.5	Et_2O	5	31:69:0
5	1.5	CH_2Cl_2	5	66:34:0
6	2.0	Et_2O	2	0:69:31
7	**2.0**	**Et_2O**	**1**	**1:99:0**[b]

[a]Reaction conducted at −40 °C, 86% yield. [b]95% yield.

regioselective partial reduction, in an excellent 95% yield on the gram scale required to support our studies. With aldehyde **14** in hand, the AMD unit was then readily synthesized using chemistry similar to the previously established route (Scheme 4). Wittig reaction of **14** with the ylide of *n*-pentyl triphenylphosphonium bromide to give **16**, followed by reduction of the olefin, afforded our known intermediate **9**. After protecting group adjustment and saponification to the ester using optimized conditions (see Scheme 1), *N*-Fmoc-AMD-OH (**2**) was reliably obtained in 45% yield over the five-step sequence from **14**.

Scheme 4. Elaboration of Aldehyde 14 to *N*-Boc$_2$-AMD-OMe (9)

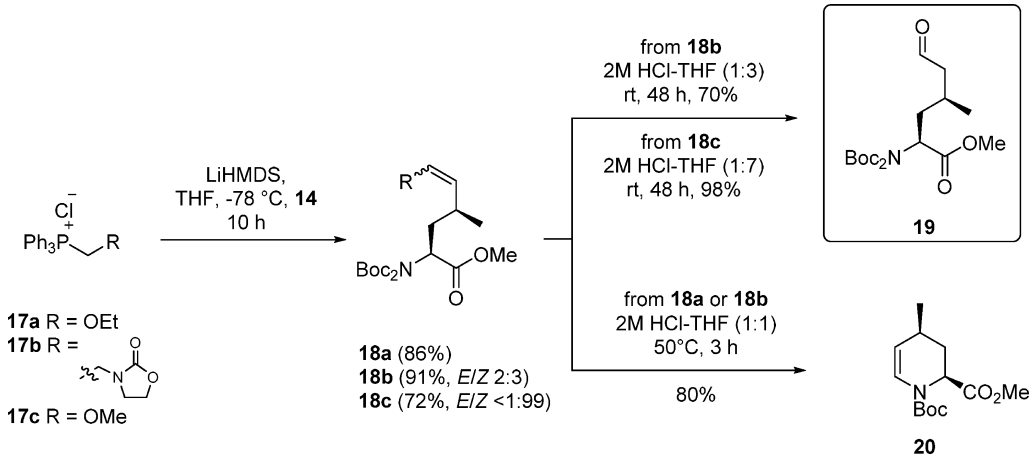


In order to access the AHMOD fragment, one-carbon homologation of aldehyde **14** was required. Initially, a Wittig reaction with the ylide prepared from (ethoxymethyl)-triphenylphosphonium bromide (**17a**; Scheme 5) proceeded uneventfully, to afford enol ether **18a**. Disappointingly, however, despite a thorough survey of hydrolysis conditions (acids, cosolvents, mercury salts, temperatures) conversion of **18a** to the desired homologated aldehyde **19** was found to be capricious and low yielding (20−40%). Alternatively, the Wittig reaction of **14** with the ylide derived from the novel 2-oxazolidinone-derived phosphonium salt **17b**,[9] or (methoxymethyl)triphenylphosphonium chloride (**17c**), proceeded to give the expected alkenes **18b** (91%, *E*/*Z* 2:3) and **18c** (72%, *E*/*Z* < 1:99), respectively. The preparation and use of phosphonium salt **17b** has not previously been reported for the homologation of aldehydes; however, Wittig reactions using related reagents have been shown to proceed readily.[10] The mild hydrolysis of an oxazolidinyl enecarbamate similar to **18b**, prepared via a different route, has also been demonstrated.[11]

After some experimentation, we were pleased to find that hydrolysis of either **18b** or **18c**, with specific aqueous acid−THF mixtures, provided the expected homologation product **19** in good to excellent yields. Interestingly, under slightly more forcing conditions, mono-Boc deprotection also occurred concomitantly with enol ether hydrolysis, to afford small amounts of the cyclized pipecolic acid derivative **20** in the product mixture. On the basis of this observation, after optimization of an alternative set of reaction conditions, it proved possible to deliver **20** as the sole product, in 80% yield. Using this approach, either hydrolysis product **19** or **20** was readily prepared on a gram scale, representing a significant improvement over the previous syntheses of both aldehyde **19** and pipecolate **20**, reported earlier by our group.[5]

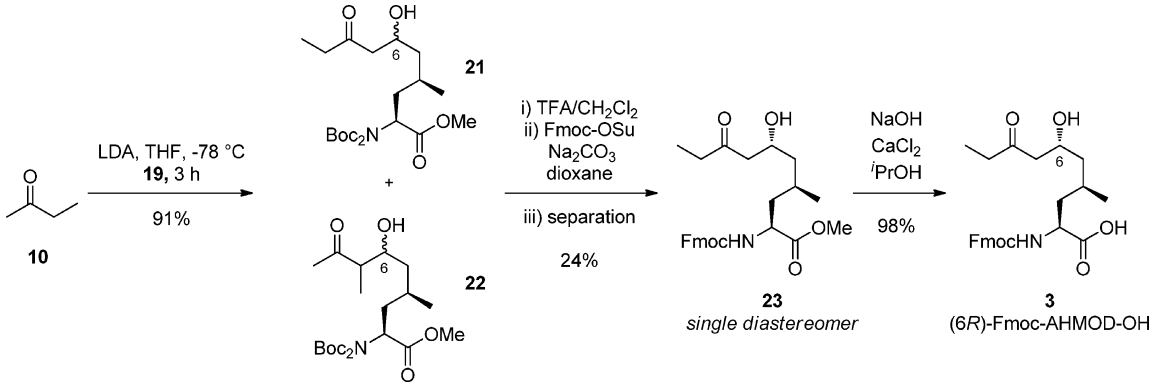
Final transformation of aldehyde **19** to the unnatural amino acid AHMOD had previously been accomplished using an Ipc$_2$BCl-mediated aldol reaction, to give a mixture of the C6 epimers (2:1 6*R*:6*S*) in moderate yield, favoring the desired 6*R* diastereoisomer. Although this method did previously enable assignment of the (6*R*)-AHMOD stereochemistry in culicinin D (**1**), the reaction proved to be unreliable and could not be productively scaled up. It was subsequently observed that, following introduction of the Fmoc group, the C6 epimers were separable by chromatography. Accordingly, the aldol reaction between aldehyde **19** and the enolate generated by treatment of 2-butanone (**10**) with LDA at −78 °C was carried out, in excellent yield (Scheme 6). The inseparable product mixture contained predominantly the desired aldol products **21**, as a 1:1 mixture of C6 epimers, contaminated with a small amount of **22**, presumably arising from an aldol reaction of the thermodynamic enolate of **10**. Conversion of the *N*-Boc group to an *N*-Fmoc group by exposure to 10% trifluoroacetic

Scheme 5. One-Carbon Homologation of Aldehyde 14 with Wittig Reagents 17b,c, To Selectively Deliver Aldehyde 19 or Pipecolate 20



DOI: 10.1021/acs.joc.5b01265
J. Org. Chem. 2015, 80, 8631−8636

Scheme 6. Elaboration of Aldehyde 19 to Homochiral (6*R*)-*N*-Fmoc-AHMOD-OH (3)



acid in dichloromethane, followed by Fmoc protection with Fmoc-OSu, allowed the isolation of homochiral **23** by careful column chromatography, in 24% yield over the two steps. Final saponification of **23** according to the established conditions,[5] using sodium hydroxide in 0.8 M $CaCl_2$ solution to avoid protecting group cleavage, afforded *N*-Fmoc-AHMOD-OH (**3**) in 98% yield. Although only moderate yields were obtained in this final aldol sequence, largely due to the difficult separation of (6*R*)- and (6*S*)-**23**, this achiral lithium aldol approach was successful in reliably delivering (6*R*)-Fmoc-AHMOD-OH (**3**) and its 6*S* epimer in sufficient quantity and purity to support our ongoing culicinin D SAR studies using solid-phase peptide synthesis.

CONCLUSION

In summary, a new chiral pool approach to the unnatural amino acid residues AMD and AHMOD required for synthesis of the anticancer peptaibol culicinin D (**1**) has been developed, on the basis of regio- and diastereoselective alkylation of a protected glutamic acid derivative. Using this methodology, the *N*-Fmoc free acids of AMD (**2**) and AHMOD (**3**), required to support total synthesis of **1**, and related biological activity studies, were prepared from *N*-Boc glutamic diester **12**, in overall yields of 40% over seven steps and 11% over eight steps, respectively. An improved synthesis of methyl pipecolate **20** from the same starting material (**12**) was also identified. In the course of this study, Wittig reagent **17b** was employed for one-carbon homologation of aldehydes, with potential wider application in organic synthesis. Overall, this new synthetic approach provides an efficient and scalable route to the unnatural amino acid components required for assembly of the selective cytotoxin culicinin D (**1**) and related natural products. Synthetic and SAR studies based around these promising therapeutic peptaibol leads are ongoing in our laboratory and will be reported in due course.

EXPERIMENTAL SECTION

General Information. NMR spectra were recorded at room temperature in $CDCl_3$ solution at either 300 MHz for ^{1}H nuclei and 75 MHz for ^{13}C nuclei or 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei. Chemical shifts are reported in parts per million (ppm) on the δ scale, and coupling constants, *J*, are in hertz (Hz). Multiplicities are reported as "s" (singlet), "d" (doublet), "dd" (doublet of doublets), "t" (triplet), and "m" (multiplet). Where appropriate, resonances due to minor isomers or rotamers are denoted by an asterisk (*). ^{1}H and ^{13}C NMR resonances were assigned using 2D COSY and HSQC spectra. High-resolution mass spectra (HRMS) were obtained using a spectrometer operating at a nominal accelerating voltage of 70 eV or on a TOF-Q mass spectrometer.

Dimethyl (2*S*,4*S*)-*N*,*N*-Bis(*tert*-butoxycarbonyl)-4-methylglutamate (13). To a stirred solution of (2*S*)-dimethyl-*N*-*tert*-butoxycarbonylglutamate (**12**) (5.0 g, 18.2 mmol) in anhydrous THF (100 mL) at −78 °C was added dropwise LHMDS (57 mL, 0.67 M in THF, 38.1 mmol). The reaction mixture was stirred at −78 °C for 30 min, and then MeI (3.4 mL, 54.6 mmol) was added. The reaction mixture was further stirred at −78 °C for 6 h, and then 2 M HCl (25 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 3/1) afforded dimethyl (2*S*,4*S*)-*N*-(*tert*-butoxycarbonyl)-4-methylglutamate (4.73 g, 90%) as a yellow oil; R_f 0.49 (hexanes/EtOAc, 3/1); $[\alpha]_D^{23}$ + 21.1°° (*c* 0.87 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.94 (d, 1H, *J* = 7.8 Hz), 4.36−4.33 (m, 1H), 3.72 (s, 3H), 3.67 (s, 3H), 2.60−2.52 (m, 1H), 2.10−1.80 (m, 2H), 1.43 (s, 9H), 1.22 (d, 3H, *J* = 6.9 Hz). Spectroscopic data were in agreement with that reported in the literature.[8]

To a stirred solution of dimethyl (2*S*,4*S*)-*N*-(*tert*-butoxycarbonyl)-4-methylglutamate (0.28 g, 0.97 mmol) in CH_3CN (2.5 mL) was added DMAP (24 mg, 0.19 mmol), followed by di-*tert*-butyl dicarbonate (0.53 g, 2.42 mmol). The reaction mixture was heated at 50 °C for 18 h and concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 3/1) afforded **13** (0.34 g, 91%) as a pale yellow oil; R_f 0.57 (hexanes/EtOAc, 3/1); $[\alpha]_D^{23}$ −14.6° (*c* 0.96, CH_2Cl_2); δ_H (400 MHz, $CDCl_3$) 4.96 (dd, 1H, *J* = 9.2 and 4.8 Hz), 3.71 (s, 3H), 3.66 (s, 3H), 2.54 (sextet, 1H, *J* = 7.0 Hz), 2.34 (ddd, 1H, *J* = 14.5, 9.5, and 7.1 Hz), 2.18 (ddd, 1H, *J* = 14.4, 7.0, and 5.1 Hz), 1.50 (s, 18H), 1.20 (d, 3H, *J* = 6.8 Hz). Spectroscopic data were in agreement with those reported in the literature.[8]

Methyl (2*S*,4*S*)-(Bis(*tert*-butoxycarbonyl)amino)-4-methyl-5-oxopentanoate (14). To a stirred solution of **13** (1.00 g, 0.26 mmol) in anhydrous Et_2O (30 mL) at −78 °C was added dropwise DIBAL-H (0.52 mL, 1.0 M in toluene, 0.52 mmol). The reaction mixture was stirred at −78 °C for 1 h before methanol (1 mL) was added dropwise, followed by saturated aqueous Rochelle's salt (2 mL). The reaction mixture was warmed to room temperature and stirred for 1 h. The layers were separated, and the aqueous layer was extracted with EtOAc (3×). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 3/1) afforded **14** (0.88 g, 95%) as a yellow oil: R_f 0.56 (hexanes/EtOAc, 3/1); $[\alpha]_D^{23}$ −19.5° (*c* 0.90 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 9.61 (d, 1H, *J* = 1.2 Hz), 4.97 (dd, 1H, *J* = 9.2 and 4.8 Hz), 3.73 (s, 3H), 2.46−2.38 (m, 2H), 2.10−2.04 (m, 1H), 1.50 (s, 18H), 1.16 (d, 3H, *J* = 6.8 Hz). Spectroscopic data were in agreement with those reported in the literature.[8]

Methyl (2*S*,4*S*)-(Bis(*tert*-butoxycarbonyl)amino)-5-hydroxy-4-methylpentanoate (15). To a solution of **13** (13.4 g, 34.4 mmol) in THF (80 mL) at −40 °C was added DIBAL-H (100 mL, 1 M in toluene) dropwise, and stirring was continued for 1 h at this temperature. The reaction was quenched by addition of saturated

DOI: 10.1021/acs.joc.5b01265
J. Org. Chem. 2015, 80, 8631−8636

$NaHCO_3$ solution. The organic solvents were removed in vacuo, and CH_2Cl_2 (100 mL) and saturated Rochelle's solution (50 mL) were added to the residue. After the mixture was stirred for 1 h at room temperature, the layers were separated and the aqueous phase was further extracted with CH_2Cl_2 (3×). The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo. Purification by chromatography (hexanes/EtOAc, 3/1) gave **15** (10.4 g, 86%) as a yellow oil: R_f 0.40 (hexanes/EtOAc, 2/1); $[\alpha]_D^{23}$ −42.2° (*c* 1.0, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.98 (dd, 1H, *J* = 8.6 and 5.7 Hz), 3.71 (s, 3H), 3.54−3.52 (m, 2H), 2.03−1.91 (m, 2H), 1.72−1.65 (m, 2H), 1.49 (s, 9H), 0.96 (d, 3H, *J* = 6.6 Hz).[8]

Methyl (2*S*,4*S*,5*E*)-(Bis(*tert*-butoxycarbonyl)amino)-4-methyldec-5-enoate (16). To a suspension of pentylphosphonium bromide (0.23 g, 0.56 mmol) in THF (2 mL) at −78 °C was added dropwise *n*BuLi (0.35 mL, 1.2 M in hexanes, 0.42 mmol). The reaction mixture was stirred at 0 °C for 15 min and then cooled to −78 °C and a solution of aldehyde **14** (0.10 g, 0.28 mmol) in THF (1 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 10 h. Saturated aqueous NH_4Cl (1 mL) was added, and the layers were separated. The aqueous phase was extracted with CH_2Cl_2 (2 × 2 mL), and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification by column chromatography (hexanes/EtOAc, 9/1) afforded **16** (72 mg, 63%) as a yellow oil: R_f 0.53 (hexanes/EtOAc, 9/1); $[\alpha]_D^{22.5}$ −23.5° (*c* 0.40 in $CHCl_3$); ν_{max} (neat)/cm^{-1} 2933, 1747, 1702, 1367, 1224, 1126 and 856; δ_H (400 MHz, $CDCl_3$) 5.38−5.30 (m, 1H), 5.18−5.11 (m, 1H), 4.84 (t, 1H, *J* = 6.9 Hz), 3.70 (s, 3H), 2.72−2.62 (m, 1H), 2.23−2.13 (m, 1H), 2.09−1.89 (m, 2H), 1.67−1.59 (m, 1H), 1.49 (s, 18H), 1.33−1.25 (m, 4H), 0.98 (d, 3H, *J* = 6.6 Hz), 0.88 (t, 3H, *J* = 7.1 Hz); δ_C (100 MHz, $CDCl_3$) 171.8, 152.2 (2C), 135.1, 129.8, 83.1 (2C), 56.6, 52.2, 38.3, 32.2, 29.1, 28.1 (6C), 27.2, 22.5, 21.1, 14.1; HRMS [ESI, $(M + Na)^+$] *m*/*z* calcd for $C_{22}H_{39}NNaO_6$ 436.2670, found 436.2670.

(2*S*,4*R*)-Methyl 2-(Bis(*tert*-butoxycarbonyl)amino)-4-methyldecanoate (9). To a solution of **16** (50 mg, 0.12 mmol) in MeOH (2 mL) was added 10% Pd/C (13 mg). The reaction mixture was stirred under an atmosphere of H_2 for 18 h. The reaction mixture was filtered through Celite and concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 9/1) afforded **9** (48 mg, 95%) as a yellow oil: R_f 0.53 (hexanes/EtOAc, 9/1); $[\alpha]_D^{23}$ −20.1° (*c* 0.20 in $CHCl_3$), lit. $[\alpha]_D^{20}$ − 27.0° (*c* 8.91 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 4.94 (dd, 1H, *J* = 10.5 and 4.5 Hz), 3.71 (s, 3H), 2.00 (ddd, 1H, *J* = 14.4, 10.5, and 3.4 Hz), 1.77 (ddd, 1H, *J* = 14.3, 10.1, and 4.5 Hz), 1.50 (s, 18H), 1.25 (m, 10H), 0.90 (d, 3H, *J* = 6.5 Hz), 0.86 (t, 3H, *J* = 7.0 Hz). Spectroscopic data were in agreement with those reported in the literature.[3]

((2-Oxooxazolidin-3-yl)methyl)triphenylphosphonium Chloride (17b). To a stirred suspension of 2-oxazolidinone (0.10 g, 1.15 mmol) and paraformaldehyde (40 mg, 1.33 mmol) in $CHCl_3$ (3 mL) was added dropwise TMSCl (0.44 mL, 3.45 mmol). The reaction mixture was heated at reflux for 3 h and then concentrated in vacuo. The resultant oil was dissolved in CH_3CN (4 mL), PPh_3 (0.30 g, 3.45 mmol) was added, and the reaction mixture was stirred at 50 °C for 10 h. The solvent was removed in vacuo, and the resultant solid was washed with EtOAc to afford **17b** (0.42 g, 91%) as a colorless solid; mp 205−208 °C; ν_{max} (neat)/cm^{-1} 3358, 1744, 1435, 1111, 743, 688; δ_H (400 MHz, $CDCl_3$) 8.00−7.70 (m, 15H), 6.23 (d, 2H, *J* = 3.0 Hz), 4.20 (t, 2H, *J* = 7.5 Hz), 3.79 (t, 2H, *J* = 7.4 Hz); δ_C (100 MHz, $CDCl_3$) 158.8, 135.6 (3C), 134.6 (3C), 130.6 (3C), 116.8 (3C), 63.0, 46.5, 41.9, 41.3; HRMS [ESI, $(M)^+$] *m*/*z* calcd for $C_{22}H_{21}NO_2P$ 362.1304, found 362.1316.

Methyl (2*S*,4*S*)-(Bis(*tert*-butoxycarbonyl)amino)-4-methyl-6-(2-oxooxazolidin-3-yl)hex-5-enoate (18b). To a stirred suspension of **17b** (3.20 g, 8.04 mmol) in anhydrous CH_2Cl_2 (40 mL) at −78 °C was added LHMDS (12 mL, 0.67 M in THF, 8.04 mmol) dropwise. The reaction mixture was stirred at 0 °C for 10 min and then cooled again to −78 °C and a solution of aldehyde **14** (1.45 g, 4.03 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 10 h. Saturated aqueous NH_4Cl (15 mL) was added, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 3/1) afforded **18b** (1.62 g, 91%, *E*/*Z*, 2:3) as a yellow oil: R_f 0.21 (hexanes/EtOAc, 3/1); ν_{max} (neat)/cm^{-1} 2980, 1742, 1367, 1230, 1124, 757; δ_H (400 MHz, $CDCl_3$) 6.64* (d, 1H, *J* = 14.3 Hz) 6.31 (d, 1H, *J* = 9.8 Hz) 4.92−4.89* (m, 1H) 4.84 (t, 1H, *J* = 7.0 Hz) 4.67* (dd, 1H, *J* = 14.3 and 8.4 Hz) 4.55 (dd, 1H, *J* = 10.8 and 9.8 Hz) 4.44−4.38* (m, 2H), 4.37−4.32 (m, 2H) 4.02−3.83 (m, 2H) 3.70* (s, 3H) 3.69 (s, 3H) 2.91−2.80 (m, 1H), 2.40−2.29* (m, 1H) 2.28−2.15* (m, 2H) 1.82−1.72* (m, 2H) 1.70−1.62 (m, 2H) 1.48 (s, 18H) 1.06* (d, 3H, *J* = 6.7 Hz) 1.05 (d, 3H, *J* = 6.6 Hz); δ_C (100 MHz, $CDCl_3$) 171.6, 156.9, 152.2 (2C), 123.5*, 122.0, 118.6, 116.5*, 83.3 (2C), 62.2, 56.8, 56.6*, 52.3, 45.2, 38.7, 38.3*, 32.4, 28.1 (6C), 22.5, 21.5*; HRMS [ESI, $(M + Na)^+$] *m*/*z* calcd for $C_{21}H_{34}N_2NaO_8$ 465.2207, found 465.2225.

Methyl (2*S*,4*S*,5*Z*)-(Bis(*tert*-butoxycarbonyl)amino)-6-methoxy-4-methylhex-5-enoate (18c). To a stirred suspension of (methoxymethyl)triphenylphosphonium chloride (0.73 g, 2.14 mmol) in anhydrous THF (17 mL) at −78 °C was added dropwise LiHMDS (1.93 mL, 1.0 M in THF, 1.93 mmol). The reaction mixture was stirred at 0 °C for 10 min and then cooled again to −78 °C, and aldehyde **14** (0.39 g, 1.07 mmol) in anhydrous THF (10 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred overnight. Saturated aqueous NH_4Cl (15 mL) was added, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 3/1) afforded **18c** (0.30 g, 72%) as a yellow oil: R_f 0.73 (hexanes/EtOAc, 3/1); $[\alpha]_D^{23.4}$ −25.0° (*c* 0.10 in $CHCl_3$); ν_{max} (neat)/cm^{-1} 2982, 2954, 1745, 1699, 1653, 1367, 1209, 1136, 1115; δ_H (300 MHz, $CDCl_3$) 6.17 (d, 1H, *J* = 12.7 Hz), 4.81 (dd, 1H, *J* = 7.8 and 6.0 Hz), 4.44 (dd, 1H, *J* = 12.7 and 8.4 Hz), 3.59 (s, 3H), 3.37 (s, 3H), 2.18−2.00 (m, 2H), 1.67−1.55 (m, 1H), 1.38 (s, 18H), 0.92 (d, 3H, *J* = 6.3 Hz); δ_C (75 MHz, $CDCl_3$) 171.3, 151.7, 146.3, 107.9, 82.5 (2C), 56.3, 55.3, 51.7, 38.2, 30.3, 27.6 (6C), 21.9; HRMS [ESI, $(M + Na)^+$] *m*/*z* calcd for $C_{19}H_{33}NNaO_7$ 410.2149, found 410.2139.

Methyl (2*S*,4*S*)-(Bis(*tert*-butoxycarbonyl)amino)-4-methyl-6-oxohexanoate (19). *Method A.* To a solution of **18b** (0.20 g, 0.45 mmol) in THF (25 mL) was added 2 M HCl (8 mL). The reaction mixture was stirred at room temperature for 40 h before saturated aqueous $NaHCO_3$ (15 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 3/1) afforded **19** (0.12 g, 70%) as a colorless oil.

Method B. To a solution of **18c** (0.81 g, 2.1 mmol) in THF (70 mL) was added 2 M HCl (10 mL). The reaction mixture was stirred at room temperature overnight before saturated aqueous $NaHCO_3$ (15 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 3/1) afforded **19** (0.77 g, 98%) as a colorless oil: R_f 0.59 (hexanes/EtOAc, 3/1); $[\alpha]_D^{21.8}$ −27.1° (*c* 0.50 in $CHCl_3$), lit $[\alpha]_D^{20}$ −32.0° (*c* 1.08 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 9.73 (t, 1H, *J* = 2.1 Hz), 4.98−4.94 (m, 1H), 3.72 (s, 3H), 2.45−2.29 (m, 2H), 2.15−2.07 (m, 1H), 2.01−1.96 (m, 2H), 1.50 (s, 18H), 1.02 (d, 3H, *J* = 6.5 Hz). Spectroscopic data were in agreement with those reported in the literature.[3]

1-*tert*-Butyl 2-Methyl (2*S*,4*S*)-4-Methyl-1,2,3,4-tetrahydropyridine-1,2-dicarboxylate (20). To a solution of **18b** (50 mg, 0.11 mmol) in THF (5 mL) was added 2 M HCl (5 mL). The reaction mixture was heated at 50 °C for 3 h before saturated aqueous $NaHCO_3$ (5 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 3/1) gave pipecolate **20** (23 mg, 80%) as a colorless oil: R_f

DOI: 10.1021/acs.joc.5b01265
J. Org. Chem. 2015, 80, 8631−8636

0.78 (hexanes/EtOAc, 3/1); δ_H (400 MHz, $CDCl_3$) 6.89 (d, 1H, J = 8.1 Hz), 6.77 (d, 1H, J = 8.3 Hz), 4.89 (m, 1H), 4.80 (m, 1H), 4.64 (t, 1H, J = 5.3 Hz), 4.51 (t, 1H, J = 5.4 Hz), 3.72 (s, 3H), 2.27 (m, 1H), 2.17−1.95 (m, 2H), 1.50 (s, 18H), 1.44 (s, 18H), 0.94 (d, 3H, J = 7.2 Hz). Spectroscopic data were in agreement with those reported in the literature.[5]

Methyl (2*S*,4*S*)-2-(Bis(*tert*-butoxycarbonyl)amino)-6-hydroxy-4-methyl-8-oxodecanoate (21). To a solution of diisopropylamine (41 μL, 0.30 mmol) in anhydrous THF (8 mL) at −78 °C was added dropwise *n*BuLi (0.24 mL, 1.21 M in hexanes, 0.30 mmol). The reaction mixture was warmed to 0 °C and stirred for 10 min and then cooled to −78 °C. 2-Butanone (**10**; 24 μL, 0.27 mmol) was added dropwise, and after 5 min, a solution of **19** (0.10 g, 0.27 mmol) in anhydrous THF (2 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 3 h, and saturated aqueous NH_4Cl (2 mL) was added. The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 2 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 3/1) afforded an inseparable isomeric mixture of **21** and **22** (0.11 g, 91%) that was used directly in the next step without further purification: R_f 0.26 (hexanes/EtOAc, 3/1); HRMS [ESI, $(M + Na)^+$] m/z calcd for $C_{22}H_{39}NNaO_8$ 468.2568, found 468.2564.

Methyl (2*S*,4*S*,6*R*)-2-(((9*H*-Fluoren-9-yl)methoxycarbonyl)-amino)-6-hydroxy-4-methyl-8-oxodecanoate (23). To the crude mixture of **21** and **22** obtained above (0.10 g, 0.22 mmol) in CH_2Cl_2 (1.8 mL) was added trifluoroacetic acid (0.2 mL) dropwise, and the reaction mixture was stirred at room temperature for 30 min. The solvent was removed in vacuo to afford a crude oil, which was taken up in 1,4-dioxane (4 mL). A portion of 10% $NaHCO_3$ (3 mL) was added followed by Fmoc-OSu (83 mg, 0.25 mmol) in 1,4-dioxane (2 mL) dropwise. The reaction mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the resultant solid was partitioned between a mixture of H_2O (5 mL) and CH_2Cl_2 (5 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by careful chromatography (hexanes/EtOAc, 1/1) afforded **23** (25 mg, 24%) as a colorless oil, as a single diastereoisomer: R_f 0.35 (hexanes/EtOAc, 1/1); $[\alpha]_D^{21}$ +13.3° (*c* 0.03 in $CHCl_3$), lit. $[\alpha]_D^{20}$ +9.5° (*c* 1.22 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.76 (d, 2H, J = 7.6 Hz), 7.60 (t, 2H, J = 6.3 Hz), 7.39 (t, 2H, J = 7.4 Hz), 7.31 (t, 2H, J = 7.4 Hz), 5.48 (d, 1H, J = 8.1 Hz), 4.47−4.34 (m, 3H), 4.23 (t, 1H, J = 7.1 Hz), 4.19−4.10 (m, 1H), 3.74 (s, 3H), 2.53−2.48 (m, 2H), 2.43 (q, 2H, J = 7.3 Hz), 1.89−1.75 (m, 2H), 1.60−1.48 (m, 2H), 1.30−1.23 (m, 1H), 1.06 (t, 3H, J = 7.4 Hz), 1.00 (d, 3H, J = 6.3 Hz). Spectroscopic data were in agreement with those reported in the literature.[3]

(2*S*,4*S*,6*R*)-2-(((9*H*-Fluoren-9-yl)methoxycarbonyl)amino)-6-hydroxy-4-methyl-8-oxodecanoic Acid (3). To a solution of **23** (0.23 g, 0.47 mmol) in isopropyl alcohol (8 mL) was added 0.8 M $CaCl_2$ (4 mL) and NaOH (22 mg, 0.54 mmol), and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by the addition of acetic acid (32 μL), and the solvent was removed in vacuo to afford **3** (0.21g, 98%) as a white solid: δ_H (400 MHz; DMSO-d_6) 7.91 (d, 2H, J = 7.5 Hz), 7.73 (d, 2H, J = 7.4 Hz), 7.53 (d, 1H, J = 8.2 Hz), 7.43 (t, 2H, J = 7.4 Hz), 7.36−7.31 (m, 2H), 4.55 (d, 1H, J = 5.5 Hz), 4.32−4.21 (m, 3H), 4.05−3.98 (m, 2H), 2.48−2.40 (m, 4H), 1.83−1.66 (m, 2H), 1.43−1.21 (m, 3H), 0.92 (t, 3H, J = 7.5 Hz), 0.86 (d, 3H, J = 6.3 Hz). Spectroscopic data were in agreement with those reported in the literature.[3]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01265.

^{1}H and ^{13}C NMR spectra for novel compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*M.A.B.: fax, +64(9)3737422; e-mail, m.brimble@auckland.ac.nz.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) He, H.; Janso, J. E.; Yang, H. Y.; Bernan, V. S.; Lin, S. L.; Yu, K. *J. Nat. Prod.* **2006**, *69*, 736−741.

(2) (a) Degenkolb, T.; Kirschbaum, J.; Brückner, H. *Chem. Biodiversity* **2007**, *4*, 1052−1067. (b) Wiest, A.; Grzegorski, D.; Xu, B.; Goulard, C.; Rebuffat, S.; Ebbole, D. J.; Bodo, B.; Kenerley, C. *J. Biol. Chem.* **2002**, *277*, 20862−20868. (c) Brückner, H.; Maisch, J.; Reinecke, C.; Kimonyo, A. *Amino Acids* **1991**, *1*, 251−257.

(3) Hung, K.; Harris, P. W. R.; Brimble, M. A. *Org. Lett.* **2012**, *14*, 5784−5787.

(4) Myers, A. G.; Yang, B. H.; Chen, Y. H.; McKinstry, L.; Kopecky, D. J.; Gleason, J. L. *J. Am. Chem. Soc.* **1997**, *119*, 6496−6511.

(5) Hung, K.; Harris, P. W. R.; Brimble, M. A. *J. Org. Chem.* **2010**, *75*, 8728−8731.

(6) Belokon, Y. N.; Zeltzer, I. E.; Bakhmutov, V. I.; Saporovskaya, M. B.; Ryzhov, M. G.; Yanovsky, A. I.; Struchkov, Y. T.; Belikov, V. M. *J. Am. Chem. Soc.* **1983**, *105*, 2010−2017.

(7) (a) El Hadrami, M. E.; Lavergne, J.-P.; Viallefont, P.; Itto, M. Y. A.; Hasanoui, A. *Tetrahedron Lett.* **1991**, *32*, 3985−3988. (b) Degenkolb, T.; Berg, A.; Gams, W.; Schlegel, B.; Gräfe, U. *J. Pept. Sci.* **2003**, *9*, 666−678. (c) Zhang, W.; Sun, T. − T.; Li, Y.-X. *J. Pept. Sci.* **2009**, *15*, 366−368. (d) Zhang, W.; Li, X.; Ding, N.; Li, Y. *J. Pept. Sci.* **2012**, *18*, 163−169.

(8) Ardá, A.; Soengas, R. G.; Nieto, M. I.; Jiménez, C.; Rodriguez, J. *Org. Lett.* **2008**, *10*, 2175−2178.

(9) Prepared from 2-oxazolidinone using a one-pot procedure. See the Experimental Section.

(10) (a) Lorion, M.; Couture, A.; Deniau, E.; Grandclaudon, P. *Synthesis* **2008**, *2008*, 2141−2147. (b) Aciro, C.; Davies, S. G.; Garner, A. C.; Ishii, Y.; Key, M.-S.; Ling, K. B.; Prasad, R. S.; Roberts, P. M.; Rodriguez-Solla, H.; O'Leary-Steele, C.; Russell, A. J.; Sanganee, H. J.; Savory; Smith, A. D.; Thomson, J. E. *Tetrahedron* **2008**, *64*, 9320−9344.

(11) Liu, L.; Floreancig, P. E. *Org. Lett.* **2009**, *11* (14), 3152−3155.

DOI: 10.1021/acs.joc.5b01265
J. Org. Chem. 2015, 80, 8631−8636