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Stereoselective synthesis of 4-amino-3-hydroxy-2-methylpentanoic acids: stereochemistry of the amino acid occurring in the marine toxin janolusimide

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Abstract

Four diastereomers of 4-amino-3-hydroxy-2-methylpentanoic acid, an amino acid constituent of the hexapeptide portion of the antitumor antibiotic bleomycin A_2, have been stereoselectively synthesized by crotylboration of *N*-Boc-L-alaninal. The synthesis allowed the assignment of the stereochemistry as 2*R*,3*S*,4*S* to the 4-amino-3-hydroxy-2-methylpentanoic acid occurring in the tripeptide marine toxin janolusimide.

(2*S*,3*S*,4*R*)-4-Amino-3-hydroxy-2-methylpentanoic acid **1** is an amino acid constituent of the hexapeptide portion of bleomycin A_2, the major naturally occurring component of the clinical antitumor drug blenoxane. Extensive studies are currently reported, chiefly by Boger's group,[1–3] on the synthesis of bleomycin A_2 analogues with the aim of understanding the structural features necessary for the sequence-selective cleavage of duplex DNA exerted by this antitumor drug. Inter alia, in the continued efforts to define the fundamental functional roles of the individual subunits, Umezawa and coworkers have shown that the presence and absolute configuration of the C-4 methyl group in the subunit containing **1** potentiates the cytotoxicity and DNA cleavage efficiency of bleomycin A_2.[4] More recently, the definition of the effect and role of the bleomycin A_2 valerate substituents has been reported.[3]

Moreover, the presence of 4-amino-3-hydroxy-2-methylpentanoic acid of unknown stereochemistry has been reported in another naturally occurring peptide, the tripeptide marine toxin janolusimide **2**.[5] In order to elucidate the stereochemistry of the janolusimide amino acid we have decided to develop a synthetic method capable of affording stereoselectively all of the 4-amino-3-hydroxy-2-methylpentanoic acid stereoisomers.

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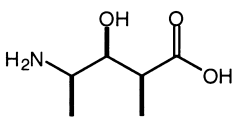
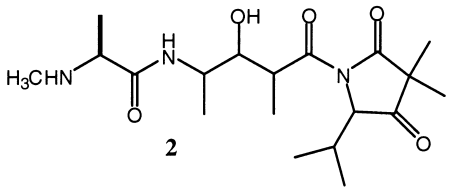


1



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The previously reported syntheses of 4-amino-3-hydroxy-2-methylpentanoic acid were either not stereoselective[6] or capable of affording stereoselectively only one or two stereoisomers.[7–10] The synthetic approach reported here is based on the crotylboration of α-chiral aldehydes with diisopinocampheylborane as the chiral auxiliary[11] and could afford all the eight stereoisomers of **1**. The sequence reported here starts from L-alanine and has afforded the diastereomers **16**–**19**; their enantiomers could be prepared by the same sequence starting from D-alanine.

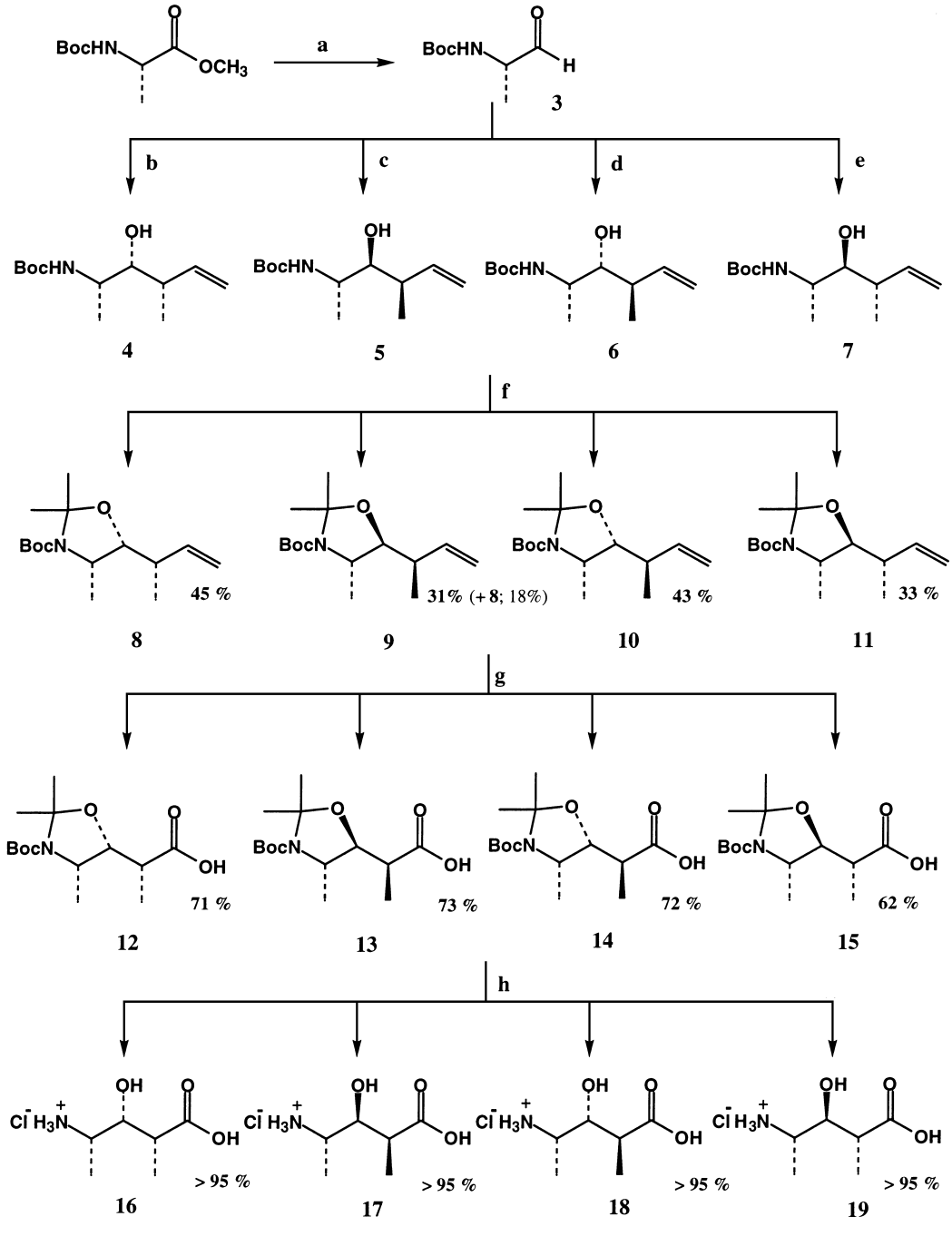
N-Boc-L-alaninal **3**, obtained by reduction of *N*-Boc-L-alanine methyl ester,[12] was reacted with the organoborane reagents prepared from (+)- or (−)-*B*-methoxydiisopinocampheylborane and (*Z*)- and (*E*)-2-butene[11,13] to afford the diastereomeric homoallylic alcohols **4**–**7** (Scheme 1). The diastereomeric purity of compounds **4**, **6** and **7** was >95%, as judged by the NMR spectra of crude reaction mixtures, while compound **5** was accompanied by a comparable amount of the *syn–syn* diastereomer **4**. From these data it is apparent that in the case of compound **4** the intrinsic diastereofacial selectivity of the two reagents is matched, while it is mismatched in the case of compound **5**. The poor performance of the reagents affording **5** parallels a previous observation with other aldehydes of the poor stereoselectivity of (*Z*)-crotylboronates in the mismatched series.[14,15] On the other hand, in the case of **6** and **7** the enantioselectivity of the chiral auxiliary overrides the diastereofacial preference of the chiral aldehyde substrate in both the matched and mismatched series.

The stereochemistry of **4**–**7** can be easily predicted via cyclic transition states,[13] since conceivably the behavior of α-amino aldehydes should strictly parallel that of α-hydroxy aldehydes whose stereochemical outcome is well documented.[11] Furthermore, since α-amino aldehydes have been rarely utilized in this reaction,[16] the absolute configuration at the carbinol carbons of **4**–**7** was independently determined by the advanced Mosher method[17] and the overall stereochemistries were later confirmed by comparison of the spectral and optical properties of the derived amino acid **16** with an authentic sample of its enantiomer **1**.

We encountered difficulties in separating **4**–**7** in acceptable yields from the isopinocampheol arising from the reaction, even when using an improved workup procedure involving 8-hydroxyquinoline.[18] Crude **4**–**7** were thus converted into the oxazolidine derivatives **8**–**11** which were easily separated from isopinocampheol by silica gel column chromatography. The yields of **8**–**11** over three steps and after isolation ranged from 31% for compound **9** to 45% for compound **8**. Both the ^{1}H and ^{13}C NMR spectra of compounds **8**–**11** ($CDCl_3$) showed two sets of signals at ambient temperature. Upon raising the probe temperature to 100°C ($CDCl_2$–$CDCl_2$) these signals merged into one set, thus suggesting the presence of a slow conformational equilibrium at ambient temperature, as previously noted for other oxazolidine derivatives.[19]

Ruthenium tetraoxide catalyzed oxidation[20] of compounds **8**–**11** afforded, in satisfactory yields, the protected amino acids **12**–**15**, which once again showed the presence of a mixture of conformers in the NMR spectra run at ambient temperature. Finally, deprotection of **12**–**15** with 4N HCl afforded the desired amino acids **16**–**19**.

We have found that ^{1}H and ^{13}C NMR chemical shifts, the ^{1}H NMR coupling constants and the specific rotations of compounds **16**–**19** are highly dependent upon the concentration and the acidity of the D_2O solution in which the spectra were taken. The values for compound **1** reported in the literature[7–9] are



a: DIBALH; b: $^{l}Ipc_2B$-(Z)-crotyl; c: $^{d}Ipc_2B$-(Z)-crotyl; d: $^{l}Ipc_2B$-(E)-crotyl; e: $^{d}Ipc_2B$-(E)-crotyl; f: $(CH_3)_2C(OCH_3)_2$, p-TsOH (cat.); g: $NaIO_4$, $RuCl_3$ (cat.); h: 4N HCl/AcOEt

Scheme 1.

inconsistent and we had serious problems in the identification of the amino acid **16** from the chemical shift and J values reported for its enantiomer **1**. Finally, the problem was solved by running ^{1}H and ^{13}C NMR spectra in D_2O solution in which approximately the same amount of **1** and **16** were dissolved and observing only a single set of signals. Analogously, the relative stereochemistry of 4-amino-3-hydroxy-2-methylpentanoic acid arising from degradation of the tripeptide toxin janolusimide[5] was assigned as 2*R*,3*S*,4*S* (or its enantiomer) by mixed NMR spectra of **19** with a sample obtained from hydrolysis of the tripeptide. The absolute configuration was assigned as 2*R*,3*S*,4*S* by the positive value of the optical rotation ($[\alpha]_D$ +5.4; c 1.7; H_2O. Lit.[5]=+10; c 0.8; H_2O). To overcome the above problems we report here, as reference data, the NMR spectra of **16**–**19** taken in 2N DCl in D_2O and optical rotations measured in 2N HCl in H_2O.[21]

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21. NMR data (2N DCl; 400 MHz) and optical rotation (2N HCl) for compounds **16**–**19**. **16** ^{1}H NMR δ: 0.53 (3H, d, J=6.0), 0.55 (3H, d, J=5.7), 1.89 (1H, m), 2.82 (1H, m), 3.21 (1H, m). ^{13}C NMR δ: 11.9, 14.5, 43.1, 50.2, 72.2, 178.5. $[\alpha]_D$=−9.4 (c=1.6). **17** ^{1}H NMR δ: 0.47 (3H, d, J=6.0), 0.48 (3H, d, J=7.5), 1.81 (1H, m), 3.24 (1H, m), 3.42 (1H, m). ^{13}C NMR δ: 13.0, 14.1, 44.8, 53.8, 75.8, 181.6. $[\alpha]_D$=−16.3 (c=1.2). **18** ^{1}H NMR δ: 0.43 (3H, d, J=6.9), 0.53 (3H, d, J=6.6), 1.94 (1H, m), 2.93 (1H, m), 3.28 (1H, m). ^{13}C NMR δ: 11.1, 13.7, 43.9, 49.3, 72.4, 179.1. $[\alpha]_D$=−2.0 (c=1.3). **19** ^{1}H NMR δ: 0.50 (3H, d, J=7.0), 0.64 (3H, d, J=6.6), 2.12 (1H, m), 2.87 (1H, m), 3.02 (1H, m). ^{13}C NMR δ: 14.2, 15.9, 42.2, 50.6, 74.4, 178.2. $[\alpha]_D$=−6.8 (c=1.2).