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Total Synthesis and Complete Structural Assignment of Yaku'amide A

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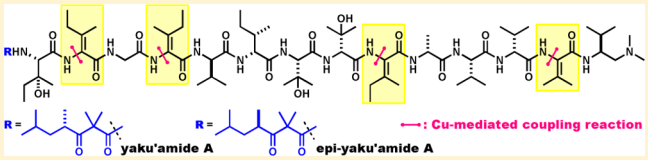
Supporting Information

ABSTRACT: Here we report the first total synthesis and the complete stereochemical assignment of yaku'amide A. Yaku'amide A (**1**) was isolated from a sponge *Ceratopsion* sp. as an extremely potent cytotoxin. Its structure was determined except for the C4-stereochemistry in the N-terminal acyl group (NTA). This tridecapeptide consists of 2 proteinogenic and 11 nonproteinogenic amino acid residues and is capped with NTA and a C-terminal amine (CTA). α,β-Dehydrovaline, *E*- and *Z*-α,β-dehydroisoleucines are the most unusual nonproteinogenic residues of **1** and necessitated development of new methodologies for their assembly. Consequently, Cu-mediated cross-coupling reactions were efficiently employed for *E*/*Z*-selective syntheses of the three dipeptides with the dehydroisoleucines and for construction of the tetrapeptide with the dehydrovaline. The peptide was then elongated from the tetrapeptide in a stepwise fashion to deliver the two possible C4-epimers of **1**. Extensive NMR studies revealed that the natural **1** possessed the C4*S*-stereochemistry, and biological assays using P388 mouse leukemia cells demonstrated that both C4-epimers possessed comparable toxicities. The present synthetic methodologies for construction of the highly unsaturated peptide sequence of **1** will allow studies of the relationships between the conformational properties of dehydro amino acid residues and cytotoxicity.



INTRODUCTION

Marine sponges are rich sources of structurally unusual, biologically active peptides.[1] These peptides exhibit a variety of activities, including insecticidal, antimicrobial, antiviral, antitumor, tumor promotive, antiinflammatory, and immunosuppressive actions. Some of these compounds have served as drugs or as lead compounds in drug development,[2] while others have proven useful in studies directed toward the elucidation of biochemical pathways. This significant pharmacological diversity is a function of peptide structure and conformation, which are in turn dictated by the structurally diverse constituent amino acids. These peptides contain not only the 20 canonical proteinogenic L-amino acids but also numerous nonproteinogenic amino acids, such as N- and C-substituted amino acids of either L- or D-chirality and α,β-dehydro amino acids.[3]

In 2010, two linear tridecapeptides yaku'amide A and B (**1** and **2**, Figure 1) were isolated by Matsunaga from a deep sea sponge *Ceratopsion* sp. as extremely potent cytotoxins.[4] Extensive NMR analyses and chemical derivatization studies revealed the entire stereostructures of **1** and **2** except for the stereochemistry of the C4-methyl group in the N-terminal acyl group (NTA). Yaku'amides consist of 2 proteinogenic and 11 nonproteinogenic amino acid residues and are capped with NTA and a C-terminal amine (CTA). The nonproteinogenic amino acids of **1** are categorized into three structural classes: β-hydroxy L-amino acids (residues 1 and 7), D-amino acids (residues 5, 6, 8, 10, and 12), and dehydro amino acids (residues 2, 4, 9, and 13). The presence of α,β-dehydrovaline (residue 13) and *E*- (residue 4) and *Z*-α,β-dehydroisoleucines (residues 2 and 9) is the most unusual structural feature of yaku'amides, because dehydrovaline and *E*-dehydroisoleucine have only been found in several natural peptides and *Z*-dehydroisoleucine is unprecedented.[5] As the C=C bond prevents rotation of the side chain, these four unsaturated amino acids together have a large impact on the conformational behavior of their proximal residues,[6] thereby potentially influencing the bioactive three-dimensional structure of the entire molecule.[7]

The cytotoxicity assays of **1** using a panel of 39 human cancer cell lines (JFCR39)[8] unveiled its distinct growth-inhibitory profile in comparison to 38 clinically available anticancer drugs.[4] Accordingly, exceptional cytotoxicity and a potentially new mode of action hold great promise for the development of yaku'amides and their related structures as novel therapeutics. However, the natural supply of **1** and **2** has been extremely limited, preventing detailed investigations of their biological activities as well as spectroscopic determination of the intrinsic three-dimensional shape.

The highly unsaturated peptide structure and characteristic cytotoxicity profile motivated us to launch a program toward deciphering the chemical and biological functions of yaku'amides using synthetic organic chemistry.[9,10] Here we report the development of a new assembly methodology of the α,β-unsaturated amino acid residues, and the first total synthesis of **1** as well as the structural elucidation of the *S*-stereochemistry of the C4-stereocenter.[11]

RESULTS AND DISCUSSION

We planned to synthetically construct the two possible C4 isomers of yaku'amide A (**1a** and **1b**, Figure 1) and then to spectroscopically compare the two compounds with the natural **1** for determination of the absolute C4-stereochemistry.

Received: February 8, 2013

   dx.doi.org/10.1021/ja401457h | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

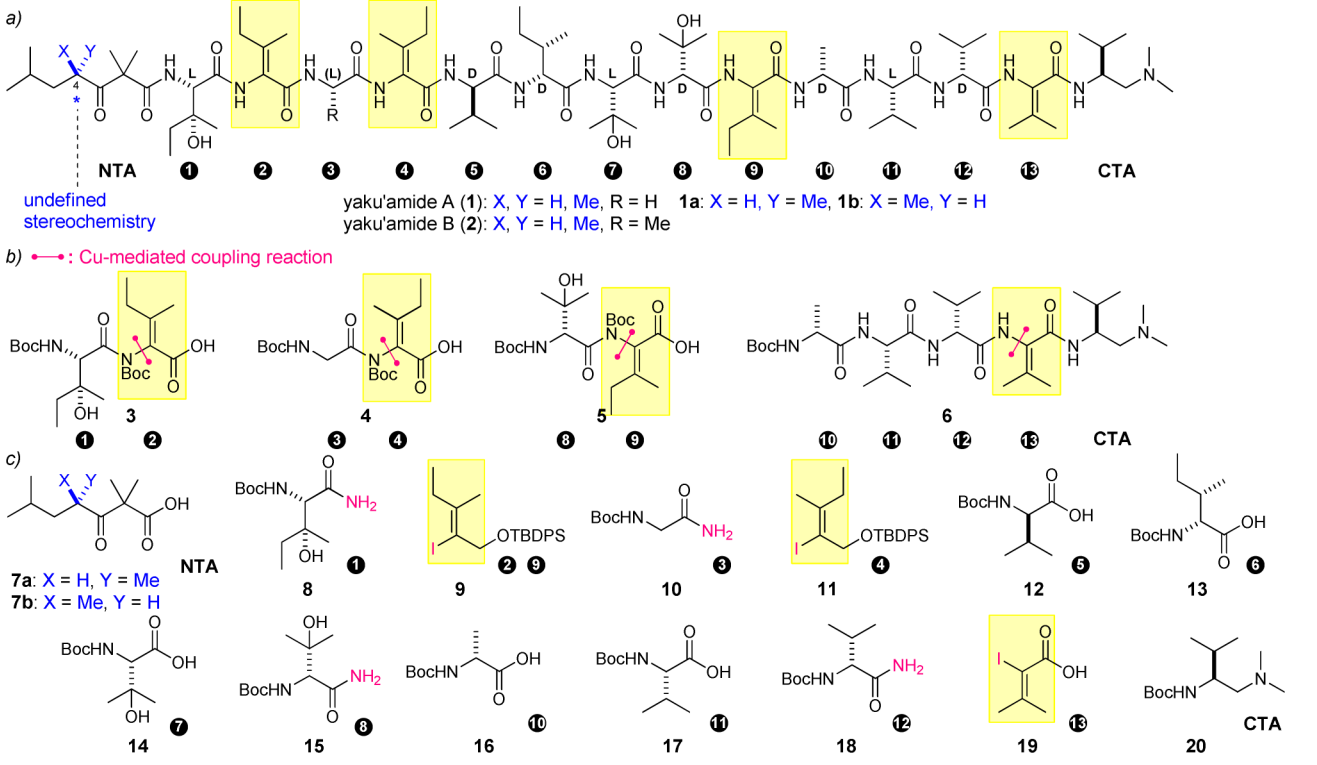
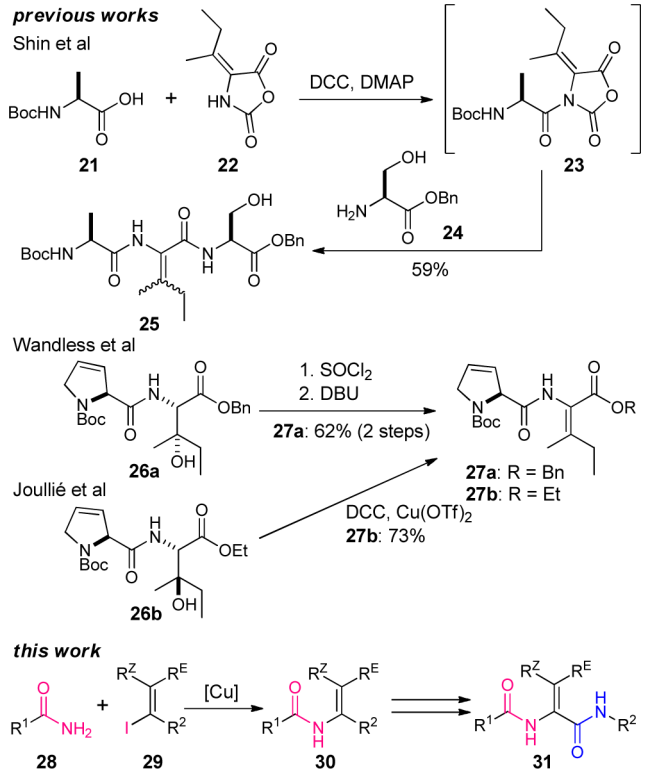


Figure 1. (a) Structures of **1**, **2**, and two possible C4-stereoisomers of yaku'amide A (**1a** and **1b**). (b) Structures of the four fragments **3**–**6** that contain α,β-dehydro amino acid residues. (c) Structures of the fourteen monomers **7**–**20**. Tetrasubstituted olefins are highlighted in yellow. Boc = *t*-butoxycarbonyl; TBDPS = *t*-butyldiphenylsilyl.

First, retrosynthetic removal of the NTA moieties from **1a** and **1b** generated the enantiomers **7a** and **7b**, respectively. Then, the three dipeptides **3** (residue 1–2), **4** (residue 3–4), **5** (residue 8–9), and one tetrapeptide **6** (residue 10–13) were disassembled from **1**. The three compounds **3**–**5** were designed to possess α,β-unsaturated carboxylic acids at their C-termini, because these are non-epimerizable at the C_α carbons upon coupling. In the synthetic direction, the four unsaturated fragments **3**–**6** together with the four monomers **7a**/**7b**, **12** (residue 5), **13** (residue 6), and **14** (residue 7) were to be condensed in a stepwise fashion from the C-terminal tetrapeptide **6** by seven amide bond formations.

The stereoselective introduction of *E*- and *Z*-α,β-dehydroisoleucines within the complex peptide sequence has been the most significant challenge in the development of the route to **1**.[12] Previously, while Shin realized the expeditious and nonstereoselective incorporation of dehydroisoleucine in tripeptide **25** by employing anhydride **22** (Scheme 1),[13] Wandless[14] and Joullié[15] independently developed stereoselective methods to synthesize *E*-dehydroisoleucines **27** via dehydration of the β-hydroxyisoleucine residues of **26**. Alternatively, we envisioned the development of a mild Cu-catalyzed cross-coupling method for stereo- and chemoselective formation of the C(sp^2)–N bond of **30** from primary amide **28** and alkenyl iodide **29**[16–18] and then to establish a nonisomerizable procedure from **30** to peptide **31**. In accordance with this plan, the three peptide fragments **3**–**5** in Figure 1 were retrosynthetically disconnected at the C_α–N bonds to generate the corresponding primary amides **8** (residue 1), **10** (residue 3) and **15** (residue 8) together with *Z*-alkenyl iodide **9** (residues 2 and 9) and its *E*-counterpart **11** (residue 4). Furthermore, tetrapeptide **6** would be cross-coupled at residue 13 and thus was dissected into five monomers: **16** (residue 10), **17** (residue 11), **18** (residue 12), **19** (residue 13) and **20** (CTA).

Scheme 1. Dehydroisoleucine Syntheses[a]



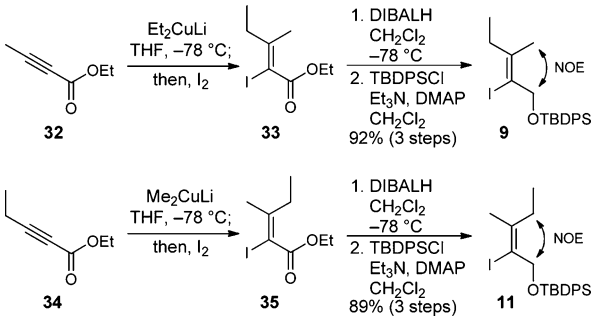
[a]Bn = benzyl; DCC = *N*,*N*′-dicyclohexylcarbodiimide; DMAP = 4-dimethylaminopyridine; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

To prepare for stereoselective synthesis of the *E*- and *Z*-dehydroisoleucine moieties, *E*- and *Z*-alkenyl iodide monomers were first

dx.doi.org/10.1021/ja401457h | J. Am. Chem. Soc. XXXX, XXX, XXX–XXX

synthesized in a geometry-controlled fashion (Scheme 2). A conjugate addition of lithium diethyl cuprate to ethyl 2-butynoate

Scheme 2. Stereoselective Syntheses of *E*- and *Z*-Alkenyl Iodide Monomers[a]



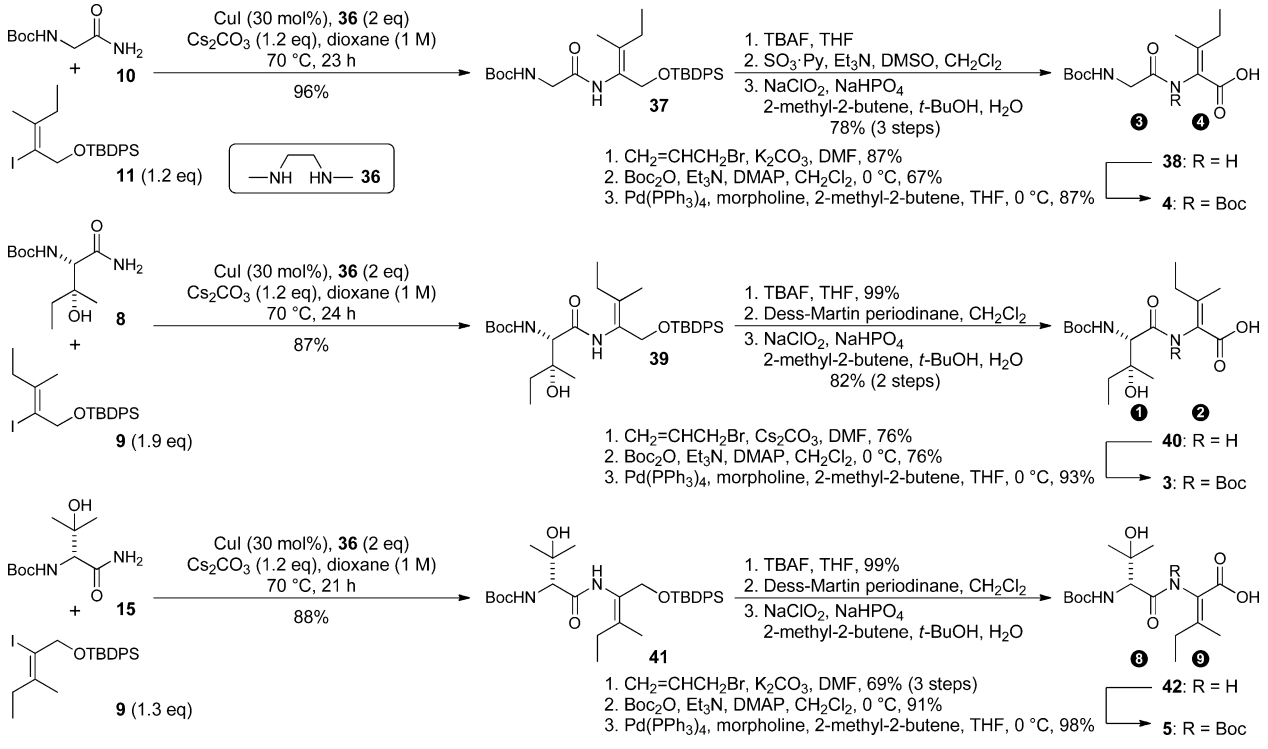
[a]DIBALH = diisobutylaluminum hydride; NOE = nuclear Overhauser effect.

32 and in situ trapping with iodine delivered *Z*-olefin **33**.[19] Reduction of **33** with diisobutylaluminum hydride (DIBALH) and protection of the resultant allylic alcohol with the *t*-butyldiphenylsilyl (TBDPS) group furnished *Z*-alkenyl iodide **9**. Through the same two-step procedure, *E*-alkenyl iodide **11** was produced from **35**, which resulted from 1,4-addition of lithium dimethyl cuprate to ethyl 2-pentynoate **34**. The geometries of the double bonds of **9** and **11** were unambiguously confirmed by nuclear Overhauser effect (NOE) experiments.

After screening coupling conditions for the C(sp^2)-N bond formation, the Buchwald reagent system [CuI, *N*,*N*′-dimethylethylenediamine (**36**), Cs_2CO_3][20] was successfully employed for construction of the three enamides (Scheme 3). However, amounts of the reagents, solvent, and concentration had to be carefully tuned to obtain high-yielding transformations. When N-*t*-butoxycarbonyl (N-Boc) glycinamide **10** and *E*-alkenyl iodide **11** (1.2 equiv) were treated with CuI (30 mol %), **36** (2 equiv), and Cs_2CO_3 (1.2 equiv) in dioxane (1 M) at 70 °C, the hindered C–N bond was stereoselectively formed to afford *E*-enamide **37** in 96% yield. Dioxane was used in this reaction instead of more common solvents (e.g., toluene, THF) in order to realize high concentrations of the substrates, because dilution resulted in significantly lower yields. Further application of these optimized conditions enabled the coupling reactions of the primary amides proximal to the sterically encumbered tetrasubstituted *β*-carbons of **8** and **15**.[21] The Cu catalyst and Cs_2CO_3 promoted stereoselective substitution of iodine of *Z*-alkenyl iodide **9** with the primary amides of **8** and **15**, giving rise to the corresponding *Z*-enamides **39** (87% yield) and **41** (88% yield), respectively. These stereoselective constructions of the three hindered tetrasubstituted olefins **37**, **39**, and **41** under mild conditions demonstrated the versatility of the present protocol.

The bis-Boc protected dipeptide fragments **4**, **3**, and **5** were synthesized from **37**, **39**, and **41**, respectively, by applying the same six-step sequence (Scheme 3). Enamide **37**, **39**, or **41** was treated with TBAF to provide the corresponding allylic alcohol, which was oxidized to carboxylic acid **38**, **40**, or **42** by sequential reactions using SO_3·pyridine/DMSO or Dess–Martin reagent[22] and $NaClO_2$.[23] Next, the additional Boc group was introduced to the secondary amide of **38**, **40**, or **42** by the standard protective group manipulations. Allyl ester formation (allylbromide and Cs_2CO_3) from **38**, **40**, or **42**, chemoselective attachment of the Boc group at the enamide nitrogen (Boc_2O, Et_3N, and DMAP) and removal of the allyl group [$Pd(PPh_3)_4$, morpholine and 2-methyl-2-butene][24] gave rise to the requisite compound **4**, **3**, or **5**.

Scheme 3. Stereoselective Syntheses of *E*- and *Z*-Dehydroisoleucine Moieties[a]

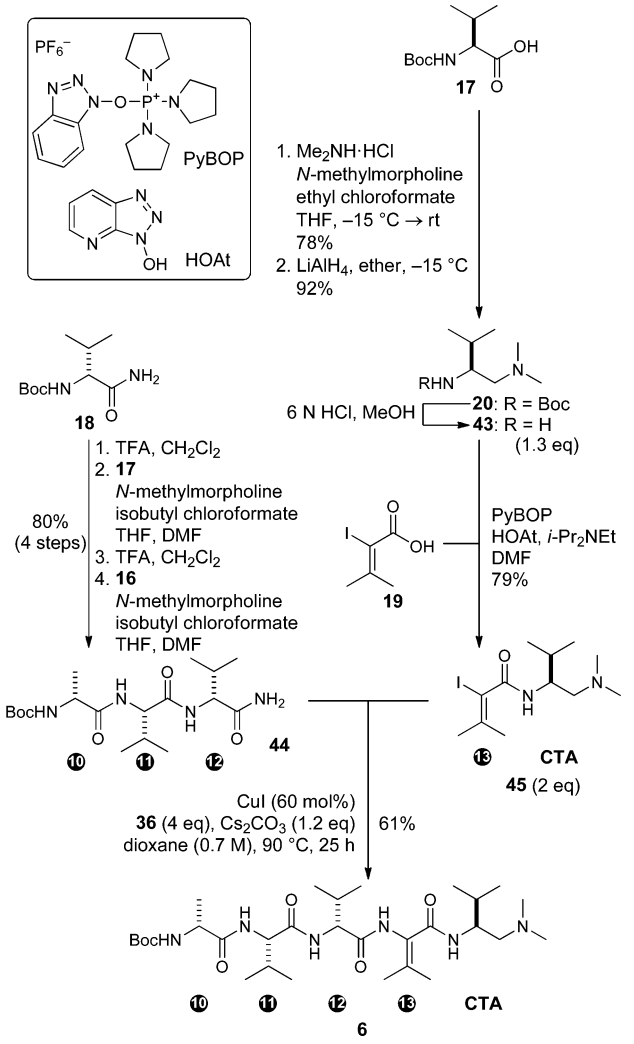


[a]TBAF = tetra-*n*-butylammonium fluoride; *t*-Bu = *t*-butyl; Alloc = allyloxycarbonyl; Ph = phenyl; DMF = *N*,*N*-dimethylformamide; Py = pyridine.

dx.doi.org/10.1021/ja401457h | *J. Am. Chem. Soc.* XXXX, XXX, XXX–XXX

Having synthesized the three geometrically pure dipeptides **4**, **3**, and **5**, the next task was to assemble the C-terminal tetrapeptide **6** through incorporation of the α,β-dehydrovaline residue (Scheme 4). This was efficiently realized by the Cu-mediated

Scheme 4. Synthesis of C-terminal Tetrapeptide[a]

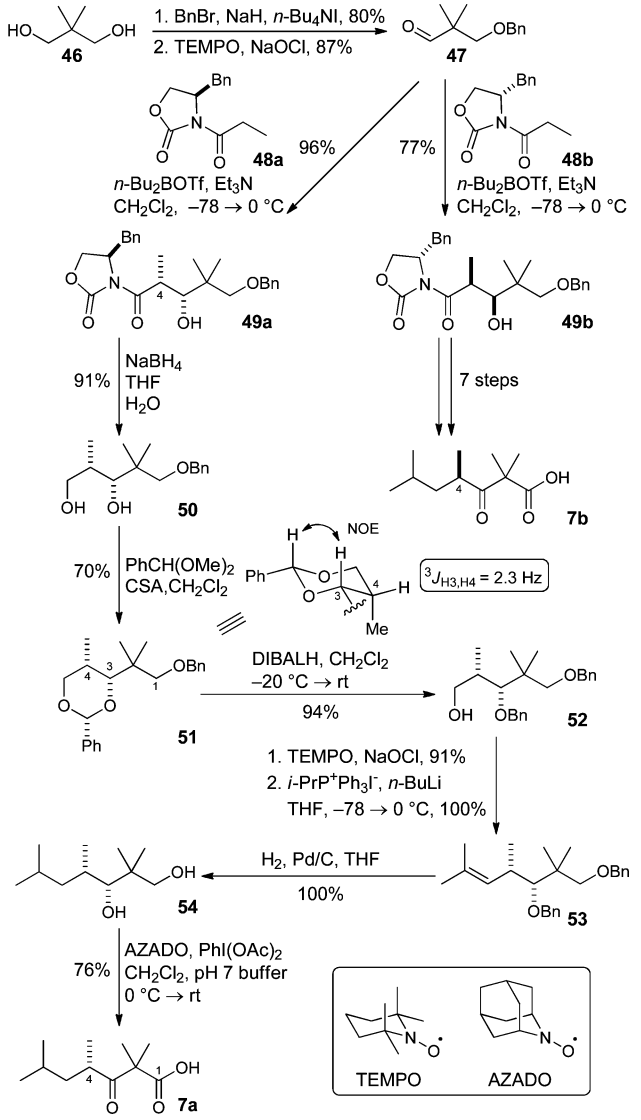


[a]TFA = trifluoroacetic acid; HOAt = 1-hydroxy-7-azabenzotriazole; PyBOP = benzotriazol-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate.

coupling reaction between **44** and **45**, which were prepared from the valine derivatives **18** and **17**, respectively. First, Boc-deprotection of N-Boc-D-valinamide **18** and subsequent condensation with N-Boc-L-valine **17** provided the dipeptide, which was further elongated via deprotection and subsequent attachment of Boc-D-alanine **16** to produce tripeptide **44**. Introduction of the dimethyl amine moiety and subsequent $LiAlH_4$ reduction converted N-Boc-L-valine **17** into the Boc-protected CTA **20**.[25] After HCl-mediated deprotection of **20**, amidation between amine **43** and alkenyl iodide **19**[26] using PyBOP,[27] HOAt,[28] and *i*-Pr_2NEt resulted in the adduct **45**. When **44** and **45** (2 equiv), thus obtained, were exposed to the Cu-catalyzed cross-coupling conditions [CuI (60 mol %), **36** (4 equiv), Cs_2CO_3 (1.2 equiv)] in dioxane at 90 °C, peptide **6** was smoothly obtained in 61% yield. This particular intermolecular C(sp^2)−N formation clearly showed the high applicability of this method to the convergent synthesis of the complex peptide sequence.

The remaining fragment that required synthetic preparation was NTA **7a**/**7b** (Scheme 5). The C4-stereochemistries of

Scheme 5. Synthesis of Two Enantiomeric NTAs[a]



[a]TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxyl; CSA = camphorsulfonic acid; AZADO = 2-azaadamantane *N*-oxyl.

S-isomer **7a** and *R*-isomer **7b** were installed using the Evans asymmetric aldol reaction.[29] Aldehyde **47** used for the aldol reaction was prepared from diol **46** through monobenzylation and following TEMPO/NaOCl-promoted oxidation.[30] The boron enolates derived from **48a** and **48b** were reacted with **47**, leading to the *syn*-aldol adducts **49a** and **49b**, respectively, as the sole products. The next seven transformations from **49a** and **49b** produced enantiomeric NTAs **7a** and **7b**, respectively. Only the route to **7a** is detailed in Scheme 5. Reductive cleavage of the chiral auxiliary by $NaBH_4$ from **49a**,[31] followed by the protection of 1,3-diol **50**, afforded benzylidene acetal **51**. The NMR data of cyclic **51** confirmed the configurations of the newly generated C3- and C4-stereocenters in the aldol addition.[32] DIBALH-promoted acetal cleavage[33] of **51** gave primary alcohol **52**, which underwent oxidation (TEMPO, NaOCl) and subsequent Wittig reaction to generate the trisubstituted olefin **53**. Treatment of **53** with H_2 in the presence

dx.doi.org/10.1021/ja401457h | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

Table 1. ^{1}H (δ_H) and ^{13}C NMR (δ_C) Chemical Shifts (ppm) of the NTA Moieties of Natural 1 and Synthetic 1a and 1b

	natural (1)[a]		1a[b]		1b[b]	
pos	δ_H	δ_C	δ_H ($\Delta\delta_H$)[c]	δ_C ($\Delta\delta_C$)[c]	δ_H ($\Delta\delta_H$)[c]	δ_C ($\Delta\delta_C$)[c]
1		172.3		172.4 (−0.1)		172.7 (−0.4)
2		55.7		55.8 (−0.1)		56.1 (−0.4)
3		212.8		212.9 (−0.1)		213.4 (−0.6)
4	2.84	38.6	2.84 (0.00)	38.8 (−0.2)	2.84 (0.00)	38.9 (−0.3)
5	1.13	42.5	1.13 (0.00)	42.5 (0.0)	1.13 (0.00)	42.4 (0.1)
	1.28		1.28 (0.00)		1.32 (−0.04)	
6	1.52	24.5	1.52 (0.00)	24.5 (0.0)	1.48 (0.04)	24.4 (0.1)
7	0.80	21.1	0.79 (0.01)	21.2 (−0.1)	0.78 (0.02)	21.3 (−0.2)
8	0.84	23.1	0.83 (0.01)	23.3 (−0.2)	0.83 (0.01)	23.1 (0.0)
9	0.93	17.3	0.92 (0.01)	17.2 (0.1)	0.94 (−0.01)	17.4 (−0.1)
10	1.28	21.6	1.28 (0.00)	21.6 (0.0)	1.32 (−0.04)	21.4 (0.2)
11	1.36	21.2	1.36 (0.00)	21.1 (0.0)	1.34 (0.02)	21.3 (−0.1)

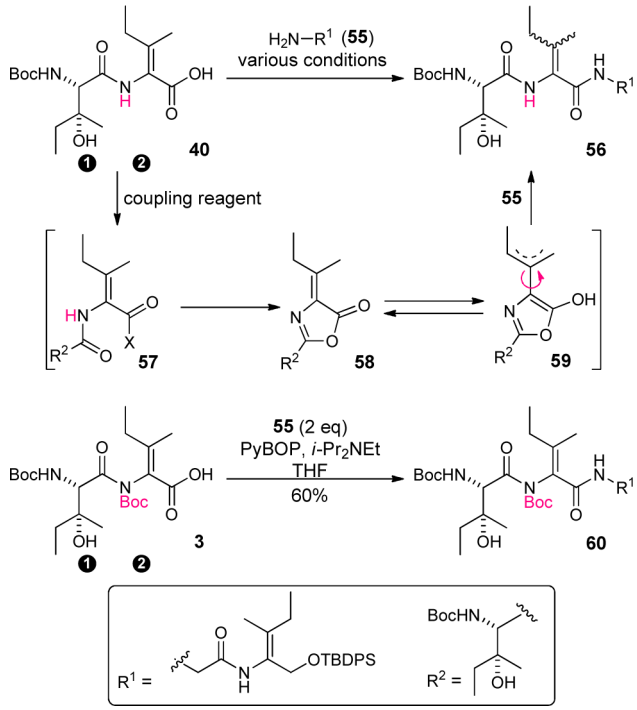
[a]600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR. [b]500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR. [c]$\Delta\delta = \delta_{nat} - \delta_{syn}$.

of Pd/C resulted in formation of saturated diol **54** through hydrogenation of the olefin and hydrogenolysis of the benzyl group. Finally, one-step oxidation of 1,3-diol **54** to β-keto acid **7a** was achieved by the action of AZADO[34] and $PhI(OAc)_2$ in the presence of water.[35] It is noteworthy that these mild conditions eliminated the risk of both C4-epimerization and C1-decarboxylation.

With all the necessary fragments in hand, multiple amide bond formations would complete the total synthesis. The major obstacle in doing so was the isomerizable nature of the *E*- or *Z*-dehydroisoleucine acid during amidation. As shown in Scheme 6, a model study uncovered the importance of the Boc-group at the secondary amine to prevent generation of the geometrical mixture. While coupling between N-unsubstituted acid **40** and amine **55**[36] under various conditions led to a 1:1 mixture of stereoisomers **56** presumably through intermediacy of **57**−**59**, amidation of N-substituted acid **3** with **55** using PyBOP afforded the adduct **60** as a sole isomer.[37] These results led us to utilize the Boc-protected fragments **4**, **3**, and **5** instead of the nonsubstituted counterparts **38**, **40**, and **42** for the total synthesis (see Scheme 3).

Assembly of the two possible C4-epimers of **1a** and **1b** was accomplished through repeating the seven Boc-removal/condensation procedures from **6** (Scheme 7). The removal of the Boc group of tetrapeptide **6** resulted in formation of amine **61**, which was then coupled with acid **5** using PyBOP in the presence of HOAt and *i*-Pr_2NEt to provide hexapeptide **62** without geometrical isomerization. Transformations of **62** to heptapeptide **64**, **64** to octapeptide **66**, and **66** to nonapeptide **68** were realized using TFA-mediated deprotection and amidation with the amino acid monomers **14**,[21] **13**, and **12**, respectively, using (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU)[38] and 2,4,6-collidine. Importantly, application of COMU efficiently inhibited the C_α-epimerization of the sterically hindered amino acids **12**−**14**. Nonapeptide **69** was in turn deprotected and coupled by the action of PyBOP and HOAt to the carboxylic acid of *E*-dehydroisoleucine derivative **4** to yield undecapeptide **70**, which underwent deprotection and condensation with *Z*-dehydroisoleucine analogue **3** to deliver tridecapeptide **72**. Finally, treatment of **72** with TFA liberated the corresponding amine **73**, and then the COMU-promoted introductions of the C4-epimeric NTA fragments **7a** and **7b** gave rise to the two possible structures of yaku'amide A (**1a** and **1b**, respectively) as the stereochemically pure forms after reversed-phase HPLC purification.

Scheme 6. Model Study for Isomerization-Free Amidation of α,β-Dehydroisoleucine



Comparison of the NMR spectra between synthetic **1a** and **1b** and natural yaku'amide A **1** revealed that **1** possessed the C4*S*-stereochemistry of **1a**.[39] Although the diastereomers **1a** and **1b** gave similar NMR spectra, the differences in the peaks corresponding to the NTA region of **1a** and **1b** were obvious. Accordingly, the ^{1}H and ^{13}C NMR chemical shifts corresponding to the NTA of **1a** and **1b** were compared with those of the natural product (Table 1) and proved that **1a** was identical with natural **1**. Thus, the complete structure of **1** was defined as depicted in **1a** for the first time.

A preliminary toxicity study of the naturally occurring **1**, the synthetic yaku'amide **1a** and C4-epimeric **1b** was carried out using mouse leukemia P388 cells. Intriguingly, both **1a** and **1b** displayed IC_{50} values (IC_{50} = 24 and 83 nM, respectively) comparable to that of the natural product **1** (IC_{50} = 46 nM). These data indicated that the effect of the C4-stereocenters on the potent toxicity of **1** was small.

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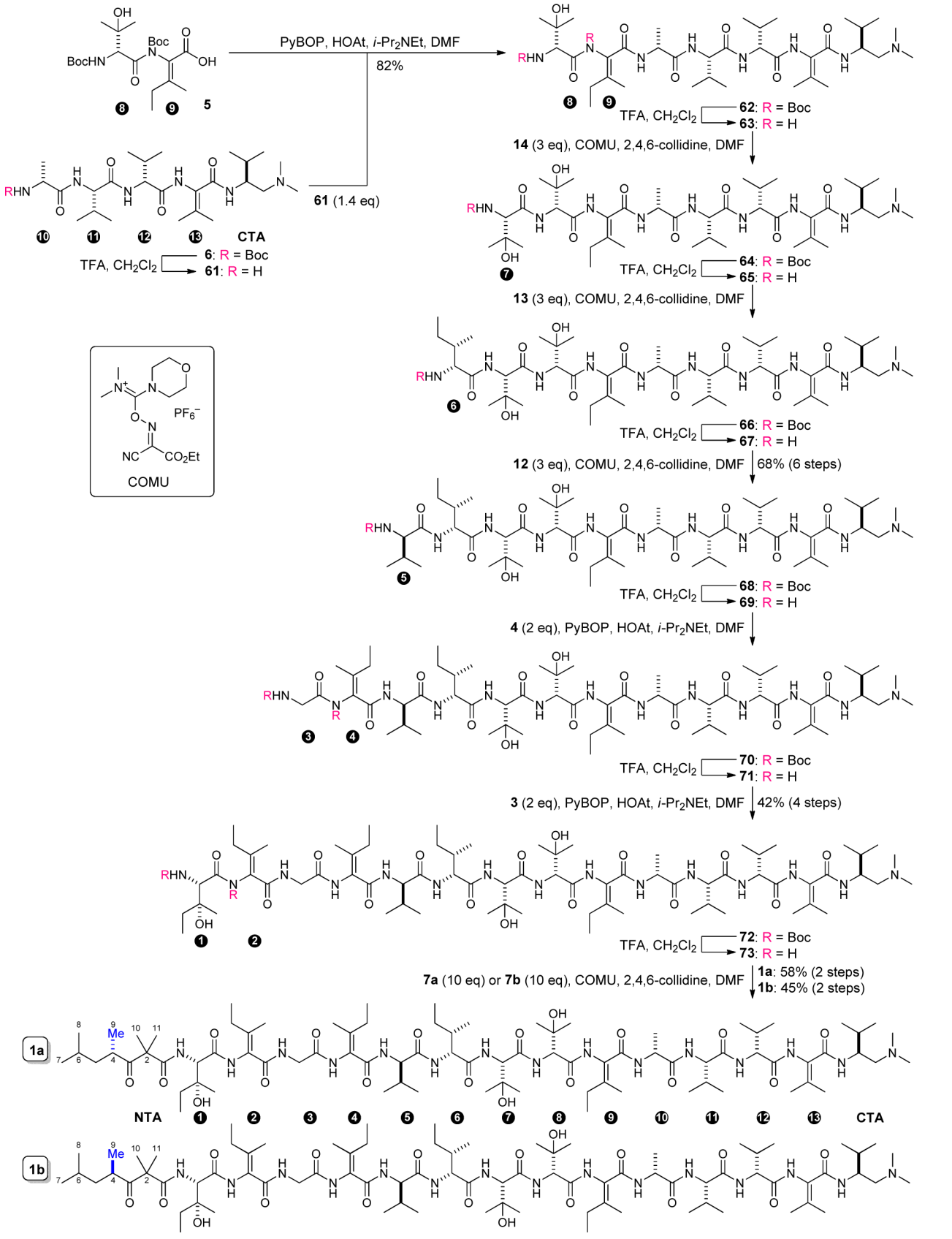
Scheme 7. Total Syntheses of Two Possible Isomers of 1[a]



[a]COMU = (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate.

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CONCLUSIONS

In summary, the total syntheses of the two possible C4-isomers of **1a** and **1b** were achieved. The key reactions in the present synthesis include application of the Cu-mediated cross-coupling reactions for *E*/*Z*-selective syntheses of the three dehydroisoleucine derivatives **37**, **39**, and **41** as well as convergent synthesis of tetrapeptides **6** with the dehydrovaline residue and isomerization-free condensations of **4**, **3**, and **5** upon elongation to the targeted compounds. The syntheses of stereochemically pure **1a** and **1b** enabled us to determine the complete stereochemical structure of natural **1** to be **1a** with the C4*S*-stereochemisty. Our versatile strategy should be useful for synthesizing various analogues to obtain insights into the structural and biological roles of dehydro amino acids. Future studies will include more detailed investigations on the structure–activity relationships and elucidation of the molecular mode of action of yaku'amides.

ASSOCIATED CONTENT

Supporting Information

Characterization data for all new compounds and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was financially supported by the Funding Program for Next Generation World-Leading Researchers (JSPS) to M.I. and a Grant-in-Aid for Young Scientists (B) (JSPS) to T.K. We thank Prof. Shigeki Matsunaga and Ms. Emi Takanashi (The University of Tokyo) for bioactivity evaluation.

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