JOC The Journal of Organic Chemistry

Article

Application of C-terminal 7-azabicyclo[2.2.1]heptane to stabilize # -strand-like extended conformation of a neighboring #-amino acid

Luhan Zhai, Siyuan Wang, Masayuki Nara, Koh Takeuchi, Ichio Shimada, Yuko Otani, and Tomohiko Ohwada

J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.8b01756 • Publication Date (Web): 04 Oct 2018

Just Accepted

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Application of C-terminal 7-azabicyclo[2.2.1]heptane to stabilize β−strand-like extended conformation of a neighboring α-amino acid

Luhan Zhai,[1] Siyuan Wang,[1] Masayuki Nara,[2] Koh Takeuchi,[3] Ichio Shimada,[3,4] Yuko Otani,[1,*] Tomohiko Ohwada [1,*]

[1] Laboratory of Organic and Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan

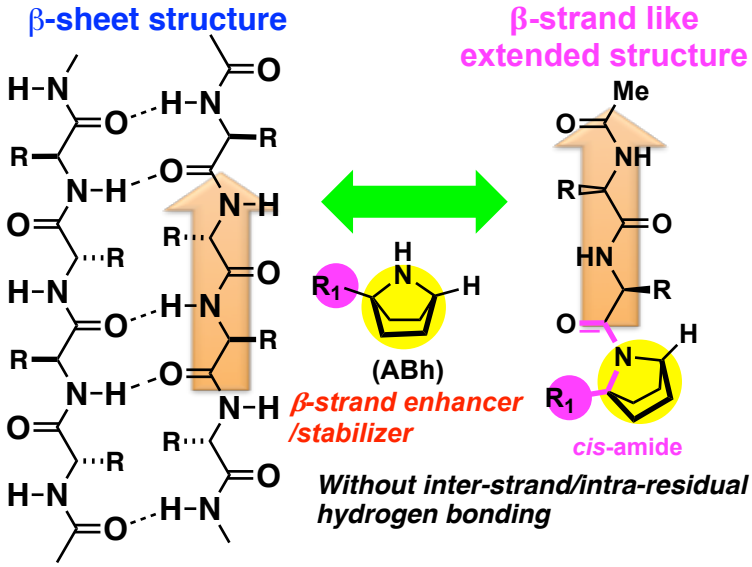
[2] Department of Chemistry, College of Liberal Arts and Sciences, Tokyo Medical and Dental University, Ichikawa, Chiba 272-0827, Japan

[3] Molecular Profiling Research Center for Drug Discovery, National Institute of Advanced Industrial Science and Technology (AIST), Aomi, Koto-ku, Tokyo 135-0064, Japan

[4] Laboratory of Physical Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan

E-mail: otani@mol.f.u-tokyo.ac.jp, ohwada@mol.f.u-tokyo.ac.jp

Graphical Abstract



Abstract

β-Strand is formed by extended linear peptide chains that are usually paired to form β-sheet structure through inter-strand hydrogen bonding. Linking a structured organic molecule with α-amino acid(s) can enforce or stabilize β-strand-like extended structures of the jointed amino acids. Spectroscopic and simulation studies indicated that the presence of a C-terminal 7-azabicyclo[2.2.1]heptane amine (Abh) favors a β–strand-like extended conformation of the adjacent α-amino acid on the N side. The bridgehead substitution of the Abh unit biases the amide *cis-trans* equilibrium of the adjacent α-amino acid residue to *cis* conformation. The proximity, specified by the presence of bond paths (such as H-H bond path) between the bridgehead proton of Abh and the α-proton of the α-amino acid provides a driving force favoring the extended conformation, which is independent of solvents. These results provide a basis for *de novo* design of β-strand-mimicking extended peptides by using β-strand enforcer/stabilizer even in the absence of the inter-strand hydrogen bonding.

Introduction

About 30% of protein secondary structure consists of β-strand/β-sheet structure, which is formed by extended linear peptide chains that are usually paired to form antiparallel, parallel or barrel β-sheet structure through inter-strand hydrogen bonding.[1-5] Distinctive phi (ϕ) and psi (ψ) dihedral torsion angles distinguish β-strands/β-sheets (e.g. antiparallel, ϕ=-139°, ψ=-135°; parallel, ϕ=-119°, ψ=113°) from α-helix (-58°, -47°) and β-turn (e.g. type I -60°, -30°) structures.[1] Amino acids that favor strand formation in proteins are branched residues such as Val, Ile, and Thr, as well as Tyr, Cys, Trp, and Phe.[1] There have been two strategies of molecular design to mimic β-strand structures and hydrogen-bonding β-sheet structures. One is the use of non-peptidic scaffolds to attain shape similarities to β-strand-like extended structures,[6-20] and the other is utilizing amino acids and amino acid surrogates to stabilize inter-strand hydrogen-bonding β-sheet structures, which includes macrocyclization[21] (backbone or side chain to side chain) and replacement of amino acids.[22-28] A major difference between these two strategies is the absence or presence of the ability to form inter-strand hydrogen bonding. Many examples of the former approach have been reported, using various non-amino acid units such as dibenzofurans,[6,7] oligoureas,[8-10] metallopeptides,[11-16] indolin-3-ones,[17] imidazolidin-2-ones,[18] epiindolines,[19] and oligothienylpyridines.[20] These mimics do not contain inter-strand hydrogen bonding sites. Therefore their general applicability to mimic protein-protein interaction via β-sheets formation is unclear. On the other hand, there are only a few examples of the latter approach, such as the use of stapling peptides (macrocyclization),[21] and inter-strand or intramolecular hydrogen bond-stabilizing scaffolds such as 1,2-dihydro-3(6H)-pyridinone,[22] the unnatural amino acid Hao (5-hydrazino-2-methoxybenzoic acid),[23-25] aminopyrazole,[26] N-difluoromethyl-triazole,[27]

and 4,4,4-trifluorothreonine.[28] In these examples, formation of intra- and/or inter-molecular hydrogen bonding stabilized paired β-sheet structures. Some important applications of these β-sheet peptide mimics include HIV-1 protease inhibitors and amyloid aggregation inhibitors.[21]

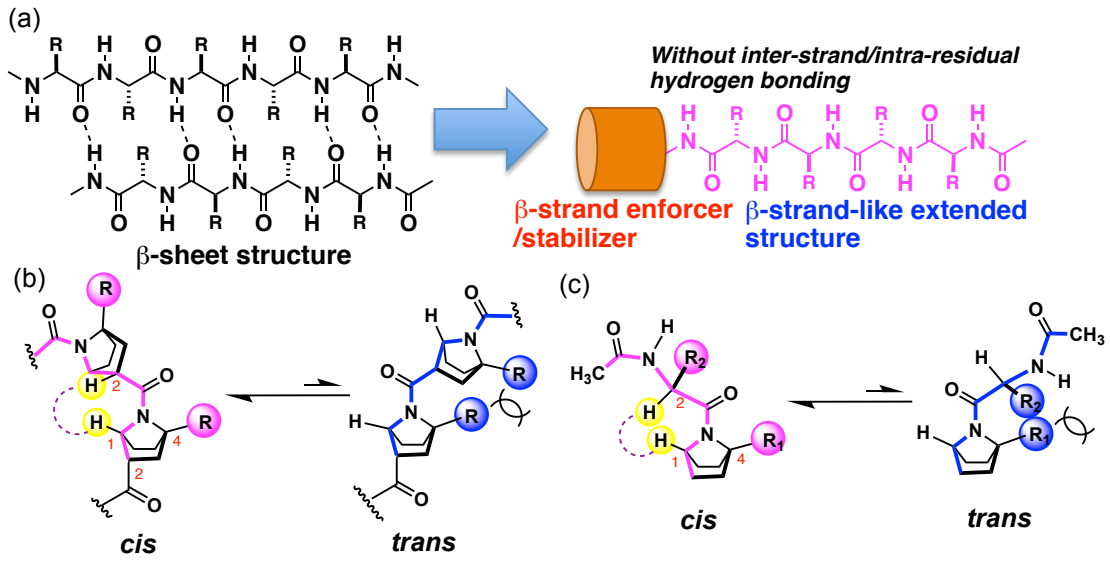


Figure 1. Inter-residue interactions to enforce β-strand extended structure by control of amide isomerization. (a) β-Strand enforcer/stabilizer (b) Amide equilibrium of homooligomers of bicyclic β-proline peptide surrogates. (c) C-Terminal 7-azabicyclo[2.2.1]heptane-linked α-amino acid. Heteropeptide dimers and bicyclic amides were used in this study.

As a hybrid strategy of the above two methods in terms of shape and hydrogen-bonding ability (Figure 1(a)), linking a structured organic molecule with α-amino acid(s) can enforce or stabilize β-strand-like extended structures of the jointed amino acids even in the absence of inter-strand hydrogen bond formation. Examination of tertiary amides of the bicyclic amine 7-azabicyclo[2.2.1]heptane (7-**aza**bicyclo[2.2.1]**h**eptane, **Abh**) has shown that this bicyclic system induces nitrogen pyramidalization.[29] Notably, the derivative bearing a 2-carboxylic acid functionality

can be regarded as a conformation-constrained β-proline surrogate (see Figure 1b).[30] Furthermore, in a bridgehead-substituted β-proline analogue (7-azabicyclo[2.2.1]-heptane-2-carboxylic acid) with an alkoxymethyl substituent installed at the C_4-bridgehead position, the bridgehead substituent completely biases the amide *cis-trans* equilibrium to the *cis* side, irrespective of the solvent (Figure 1b).[30] Homooligomers of a bridgehead-substituted bicyclic β-proline analogue were found to generate ordered structures such as helix, and spectroscopic analysis showed that only two units of 7-azabicyclo[2.2.1]heptane-2-carboxylic acid are sufficient to form a pre-organized structure.[30] The detection of ^{1}H-NOE between the C_1-bridgehead-proton and the α-H_2 proton of the N-side adjacent bicycle support the *cis* amide structure and the proximity of the bicyclic units (see Figure 1b). These observations led us to consider that this 7-azabicyclo[2.2.1]heptane unit would affect the conformation of the adjacent α-amino acid on the N-terminal side in a similar manner to that of the homo-oligomer of 7-azabicyclo[2.2.1]heptane-2-carboxylic acid through the interaction of the C_1-bridgehead hydrogen atom (Figure 1c).

In this work, we synthesized various kinds of hetero-peptides (Ac-X-Abh) and showed that they have complete *cis*-amide preference independently of the N-terminal α-amino acid and solvent (Figure 1c). Furthermore, ^{1}H NMR and Raman spectroscopic studies showed how the main-chain freedom of the neighboring α-amino acid is reduced by incorporation of the C-terminal-bicyclic amine (Abh), driving the neighboring α-amino acid to take a β-strand-like extended secondary structure. It has been shown that the dihedral angle ϕ (conformational preference) of α-amino acid dipeptides, i.e., Ac-X-NHMe (X = an amino acid) is similar to that of

short peptides.[31,32] Therefore, it is expected that the conformational effect of C-terminal Abh on the N-terminal side can be estimated by comparing conformational preferences of Ac-X-NHMe and Ac-X-Abh.

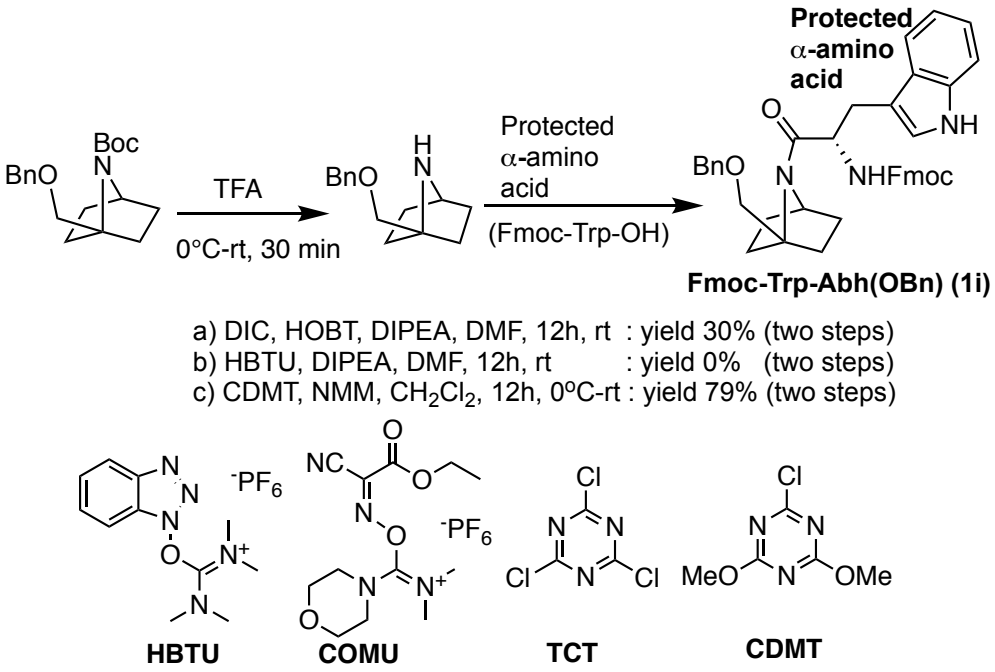
Results and Discussion

Synthesis and optimization of coupling reagents

First of all, coupling conditions for various protected α-amino acids and Abh were optimized (Scheme 1). It was expected that Abh would have low reactivity due to the presence of the bridgehead substituent as well as the secondary amine. Fifteen amino acids with either N-protected polar or non-protected non-polar side chains were connected to the nitrogen atom of Abh (**1a-1o**) (Table 1).

In the cases of amino acids with relatively small and hydrophobic side chains, conventional coupling conditions (i.e., DIC/HOBT (condition A)) were effective (Table 1). However, in the cases of polar amino acids with protecting groups on both the side chain and the N-terminal side, the yield was low or the product was not formed, probably due to the steric bulkiness of the protected α-amino acid and the low nucleophilic reactivity of the bicyclic amine (Abh). In the case of Fmoc-Trp, conventional coupling conditions (A) gave the product (Fmoc-Trp-Abh(OBn)) (**1i**) in 30% yield (Scheme 1). HTBU and COMU (condition B) also failed to give a good yield. Finally, we tried 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT)[33] (Scheme 1), which is a derivative of 2,4,6-trichloro-1,3,5-triazine (TCT) and causes less racemization (see Supporting Information), with N-methyl morpholine (NMM) as a base. The yield of Fmoc-Trp-Abh(OBn) was increased to 79%. We found that CDMT (condition C) can give good to moderate yields with amino acids for which other coupling reagents are unsuccessful (Table 1). The HPLC chromatograms of these

products were shown in Supporting Information.



Scheme 1. Synthesis of C-terminal Abh analogue of α-amino acid

Table 1 Synthesis of C-terminal Abh analogue of α-amino acid

Compound number	Amino acid (**AA**)	Product	coupling method	Yield(%)
1a	Boc-Gly	Boc-Gly-Abh(OBn)	A	85
			C	89
1b	Boc-Ala	Boc-Ala-Abh(OBn)	A	88
1c	Boc-Val	Boc-Val-Abh(OBn)	C	70
1d	Boc-Ile	Boc-Ile-Abh(OBn)	C	57
1e	Cbz-Leu	Cbz-Leu-Abh(OBn)	A	77
1f	Boc-Phe	Boc-Phe-Abh(OBn)	A	75
			B	38
			C	43
1g	Boc-β-Pro	Boc-β-Pro -Abh(OBn)	A	44
1h	Boc-(Trt)-Asn	Boc-(Trt)-Asn -Abh(OBn)	A	54
			C	72
1i	Fmoc-Trp	Fmoc-Trp-Abh(OBn)	A	30
			C	79
1j	Boc-(OBzl)-Tyr	Boc-(OBzl)-Tyr-Abh(OBn)	A	14
			B	16
			C	60
1k	Boc-(Trt)-His	Boc-(Trt)-His-Abh(OBn)	A	trace

			C	31
1l	Boc-(OBzl)-Asp	Boc-(OBzl)-Asp-Abh(OBn)	A	trace
			B	trace
			C	69
1m	Boc-(Cbz)-Lys	Boc-(Cbz)-Lys -Abh(OBn)	C	54
1n	Boc-(t-Bu)-Ser	Boc-(t-Bu)-Ser -Abh(OBn)	C	28
1o	Boc-(NO_2)-Arg	Boc-(NO_2)-Arg -Abh(OBn)	C	trace

Coupling conditions: **A**: **AA**-OH (1.5 eq.), DIC or EDCI (2 eq.), HOBt (2 eq.), iPr_2EtN (3.0 eq.), DMF, rt, 12 h. **B**: **AA**-OH (1.5 eq.), COMU (2 eq.), collidine (3.0 eq.), DCM, rt, 12 h. **C**: **AA**-OH (1.5 eq.), CDMT (2 eq.), NMM (3 eq.), DMAP (0.1 eq.), DCM, rt, 12 h.

Cis-amide preference of α-amino acid-Abh derivatives

The amide equilibrium of α-amino acid-Abh derivatives was studied by ^{1}H-NOESY spectroscopy. As shown in Figure 2, NOE can be detected between the bridgehead proton (H_1) and the α proton (H_2) of the side chain of the α-amino acid, which is consistent with *cis*-amide structure. The NMR results indicated that all the dimers (Table 1) take the *cis*-amide structure, and *trans*-amide structure was not observed.

In the case of alanine, the α-carbon has a methyl group, making it one of the simplest amino acids. N-Boc-alanine was coupled with the Abh to form a hetero-dimer (**1b**). The observation of NOE between the bridgehead proton (H_1) and methyl proton (Figure 2 (a)) is consistent with *cis*-amide structure.

Furthermore, deprotected H-Ala-Abh(OMe) (**H-1b(OMe)**) also takes *cis*-amide structure in D_2O (Figure 2 (b)). The NOE signal between the bridgehead proton H_1 and α proton (H_2) of Ala was detected even in water. This is consistent with *cis*-amide structure, and demonstrates stability in water.

Phenylalanine can be regarded as an amino acid in which a hydrogen of the methyl group of Ala is substituted with a phenyl group. The NOE signal between the bridgehead proton H_1 and the α proton (H_2) of Phe was detected in the NOESY

spectra of Boc-Phe-Abh(OBn) **1f** in CD_3OD, suggesting *cis*-amide structure (Figure 2 (c)). The *cis*-amide preference is also shown in other dimers (see Supporting Information).

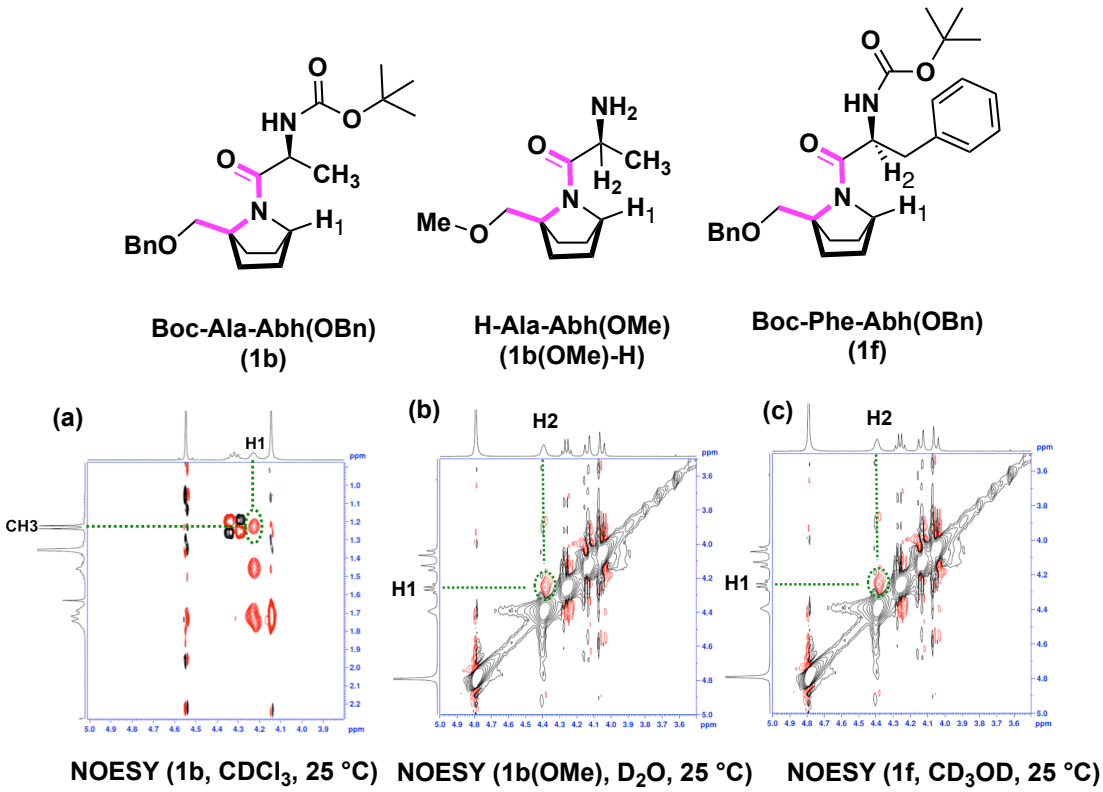


Figure 2. Various N-terminal of α-amino acids. **NOESY spectra of (a) Boc-Ala-Abh(OBn) (1b), (b) H-Ala-Abh(OMe) (H-1b(OMe)) and (c) Boc-Phe-Abh(OBn) (1f).**

Effect of the bridgehead group on amide isomerization

Various kinds of bridgehead substituents of different sizes, CH_2OBn, CH_2OMe, CH_2OH, CO_2Me, and CN, were chosen to investigate the effect of bridgehead substituents on the amide isomerization (Figure 3). Boc-alanine was coupled with the bicyclic amines.

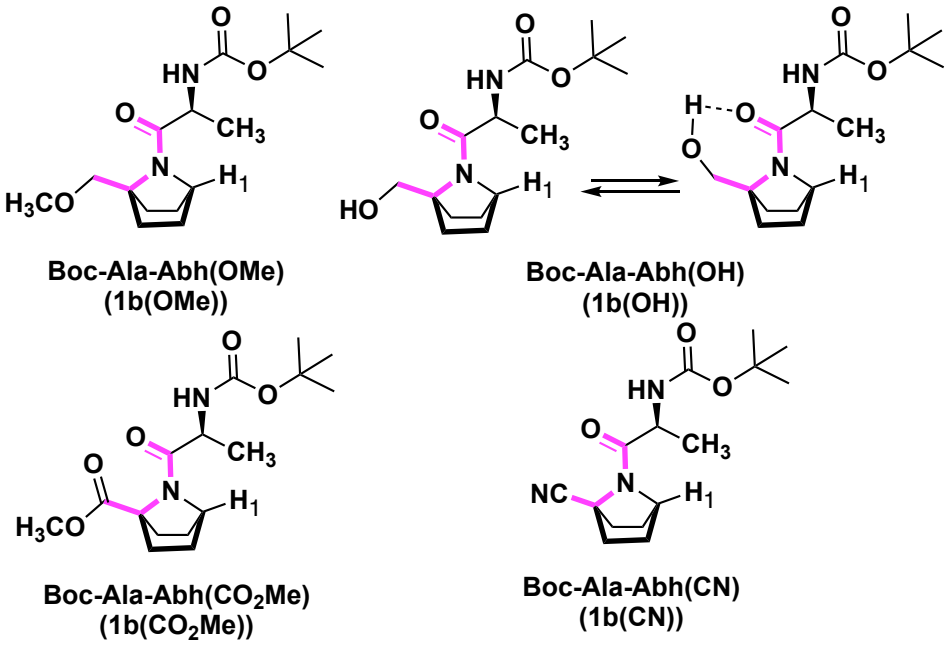


Figure 3. Effect of bridgehead substitution

In Boc-Ala-Abh(OMe) **1b(OMe)**, the bridgehead group was changed from Bn to smaller Me, but *cis*-amide conformation was retained, as indicated by the observation of NOE between the bridgehead proton H1 and the methyl group (Figure 3). The DFT calculation at 298K showed that the *cis* structure is 2.4 kcal/mol more stable than the *trans* one. The difference in calculated Gibbs free energy is about 2.9 kcal/mol, suggesting that the *cis* amide contribution is more than 98%.

In the cases of Boc-Ala-Abh(CO_2Me) **1b(CO_2Me)** and Boc-Ala-Abh(CN) **1b(CN)**, which have an sp^2 carbon and an sp carbon on the bridgehead, respectively (Figure 3), NOE was observed between the bridgehead proton H_1 and the α-proton H_2 in CD_3OD. This is consistent with *cis*-amide structure. We consider that the *cis*-amide conformation is preferred due to the rigid ester group and the linear nitrile group at the bridgehead position.

In the case of a hydroxy group at the bridgehead position in Boc-Ala-Abh(OH) **1b(OH)**, NOE between the bridgehead proton H_1 and α proton H_2 was detected in

$CDCl_3$ (Figure 3). This is consistent with *cis*-amide structure. Temperature coefficient studies also supported the existence of intramolecular hydrogen bonding between the amide carbonyl and the hydroxy group in Boc-Ala-Abh(OH) **1b(OH)** (Figure 3); small temperature coefficients for the OH resonance (-3.47 ppb/K) were obtained in DMSO-d_6.[34-36] This small value is consistent with intramolecular hydrogen bonding, which may contribute synergistically to stabilization of the *cis*-amide structure.

NMR study of the conformation of α-amino acid linked to C-terminal Abh

The temperature dependence of the $^3J(H^{\alpha}, H^{N})$ coupling constants of amide protons of Ac-α-amino acid-NHMe provides a good index of conformational stability.[37-39]

The following relationship between the $^3J(H^{\alpha}, H^{N})$ coupling constant and the dihedral angle φ of the respective amino acid residue was used in this study: [40]

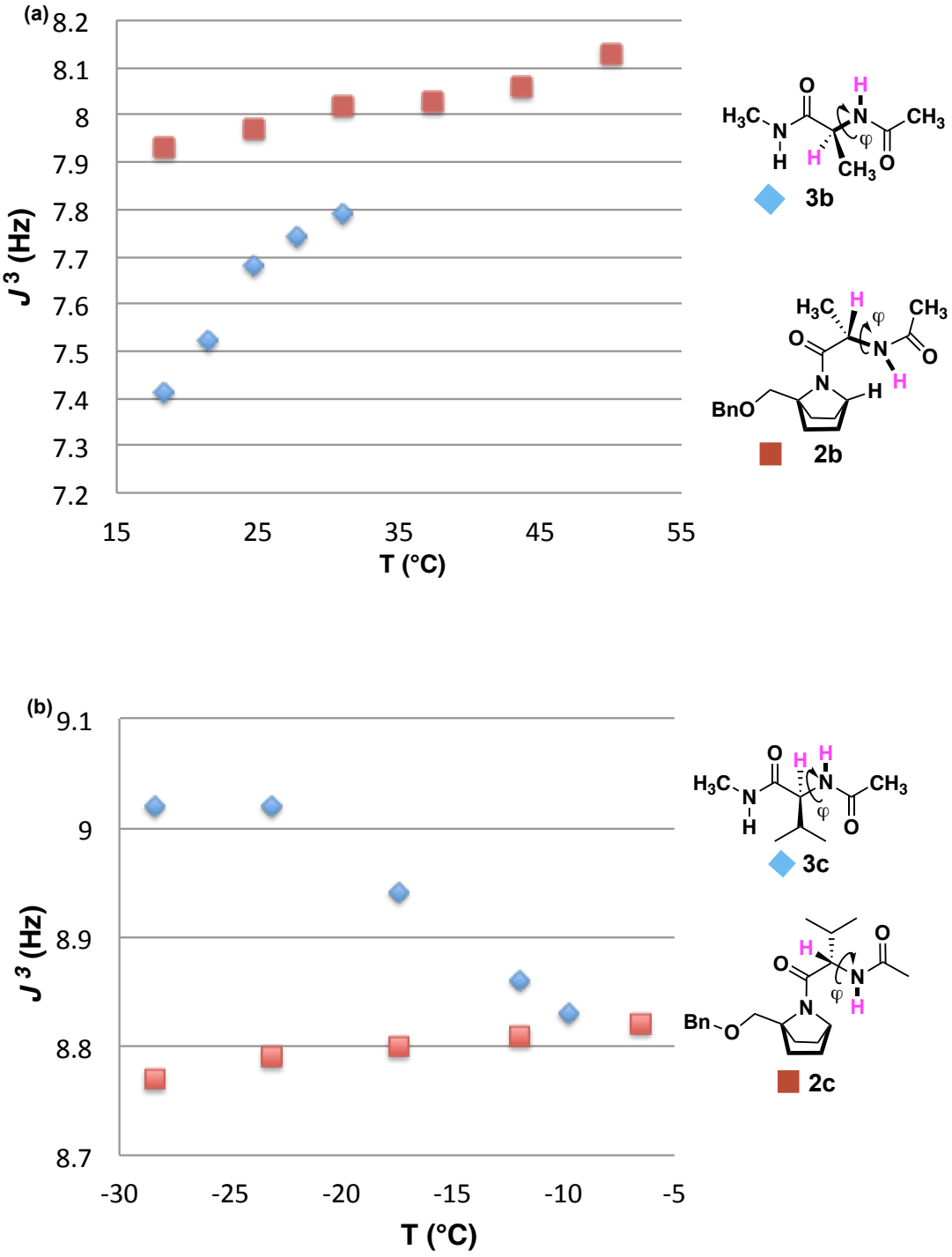
$J(\varphi) = A \cos^2(\varphi\text{-}60) + B \cos(\varphi\text{-}60) + C$

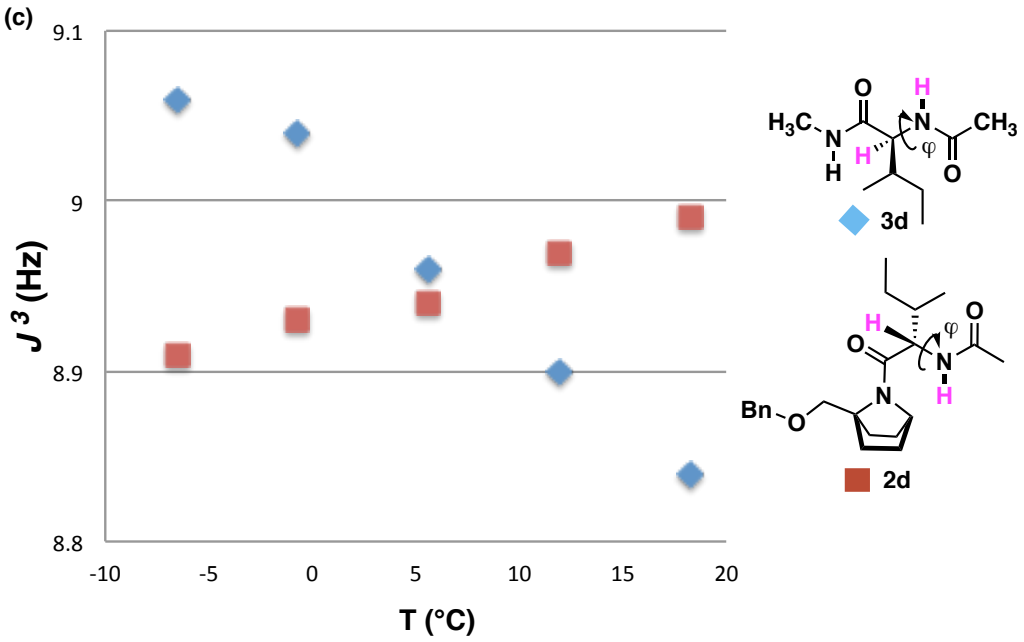
A = 6.51, B = -1.76 and C = 1.60

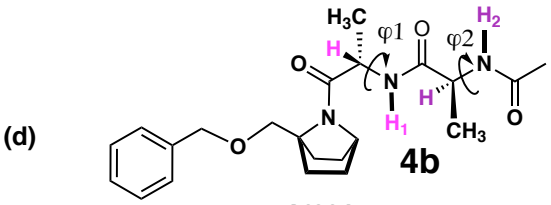
To obtain residue-level details of the peptide conformations, we performed ^{1}H NMR spectroscopy to study the conformational preferences of three kinds of α-amino acids (Ala, Val, and Ile). Amino acid dipeptides are in equilibrium mainly between α-helix (α), β-strand (β), and polyproline II (P_{II}) conformations. These three conformations are well populated for most amino acids: β (φ = -120°, ^3J =9.6±0.3Hz), P_{II} (φ = -75°, ^3J = 5.8± 0.4Hz), and α_R (φ = -60°, ^3J =3.8±0.5Hz).[41] Thus, the ^3J -value of β is considerably larger than those of P_{II} and α_R (right-handed α-helix).[41] Also, it is reported that alanine dipeptide (Ac-Ala-NHMe) **3b** takes predominantly a P_{II} conformation while valine dipeptide (Ac-Val-NHMe) **3c** and isoleucine dipeptide

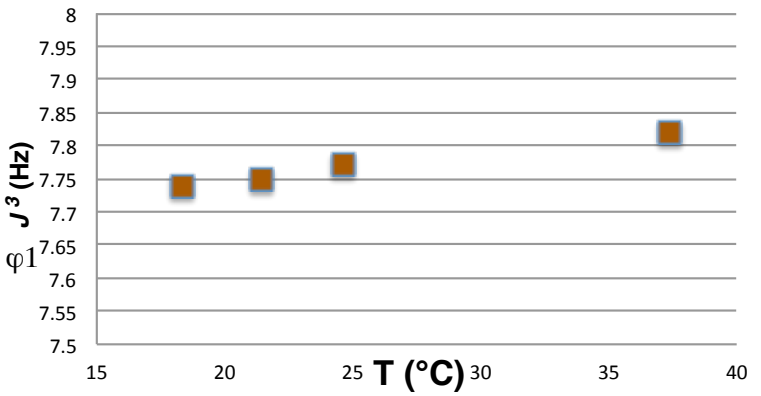
(Ac-Ile-NHMe) **3d** favor β conformations.[31,42] First, the conformational stability of Ac-Ala-Abh(OBn) **2b** was compared with that of alanine dipeptide (Ac-Ala-NHMe, **3b**). Then, ^{1}H-NMR spectra of Abh compounds with the same bicyclic skeleton but different α-amino-acid side chain bulkiness (valine and iso-leucine) were measured in organic solvent, and the values of $^{3}J(H^{\alpha}, H^{N})$ coupling constant were determined as a function of temperature. Here, the coupling constant reflects the φ-angle of the α-amino acid residue in the α-amino acid-Abh system (see Figure 4).

The conformation of alanine dipeptide in various solvents has been widely studied.[43] The values of $^{3}J(H^{\alpha}, H^{N})$ of the alanine dipeptide **3b** at 25°C in DMSO-d_6 and in water were 7.68 Hz and 6.03 Hz, respectively (Table 2), which are comparable to the reported values (7.60 Hz, and 6.02 Hz, respectively).[43] The values of the ^{3}J-coupling constant of Ac-Ala-Abh(OBn) **2b** in DMSO-d_6 and in water were 7.97 Hz and 6.89 Hz, respectively, being larger than those of Ac-Ala-NHMe **3b**; this suggests that Ac-Ala-Abh(OBn) **2b** has a higher population of β conformation in both solvents. The temperature dependency of $^{3}J(H^{\alpha}, H^{N})$ of Ac-Ala-Abh(OBn) **2b** was compared with that of alanine dipeptide (Ac-Ala-NHMe, **3b**) in DMSO-d_6 (Figure 4(a)). In the case of Ac-Ala-Abh(OBn) **2b**, the change of the coupling constants with temperature is small over a nearly 40-degree temperature range, whereas in the case of the alanine dipeptide **3b**, the change of the coupling constants with temperature is large over a 20-degree temperature range. The strong temperature dependency of the alanine dipeptide **3b** suggests that its conformation is very flexible. On the other hand, Ac-Ala-Abh(OBn) **2b** exhibits greater conformational rigidity and stability (Figure 4(a)).


(a)
8.2
8.1
8
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
J 3 (Hz)
15
25
35
45
55
T (°C)
3b
2b
(b)
9.1
9
8.9
8.8
8.7
J 3 (Hz)
-30
-25
-20
-15
-10
-5
T (°C)
3c
2c


(c)
9.1
9
8.9
8.8
J 3 (Hz)
-10
-5
0
5
10
15
20
T (°C)
3d
2d


(d)
4b


NH1
8
7.95
7.9
7.85
7.8
7.75
7.7
7.65
7.6
7.55
7.5
J 3 (Hz)
φ1
15
20
25
T (°C)
30
35
40

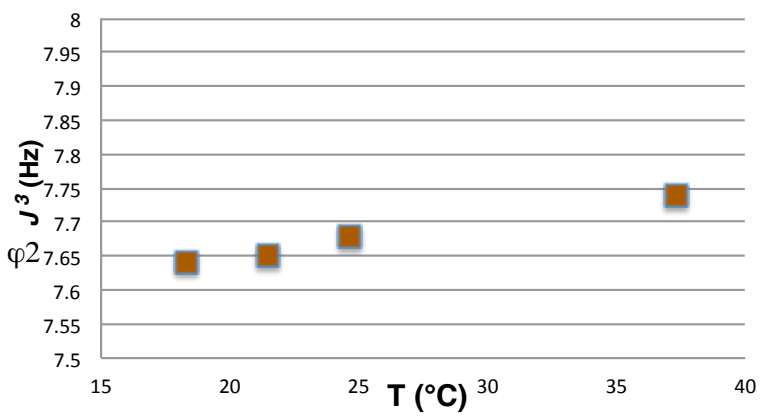

NH2
8
7.95
7.9
7.85
7.8
7.75
7.7
7.65
7.6
7.55
7.5
J 3 (Hz)
φ2
15
20
25
T (°C)
30
35
40

Figure 4 (a-c) Comparison of the temperature dependency of ^3J-coupling constants of α-amino acid moiety with different C-terminal groups (d) temperature-dependency of two dihedral angles of tripeptide Ac-Ala-Ala-Abh 4b.

Table 2 Values of ^3J-coupling constants at 25°C.

	Water (298 K)	DMSO-d_6 (298 K)
Ac-Ala-Abh(OBn) **2b**	6.89 Hz [a]	7.97 Hz
Ac-Ala-NHMe **3b**	6.03 Hz [b]	7.68 Hz

	Water (294 K)	$CDCl_3$ (291 K)
Ac-Ile-Abh(OBn) **2d**	7.87 Hz [c]	8.99 Hz
Ac-Ile-NHMe **3d**	7.20 Hz [d]	8.84 Hz

[a] 10% CD_3OD+90% H_2O, pH=2-3. [b] 10% D_2O+90% H_2O, pH=2-3.

[c] 14% CD_3OD+86% H_2O, pH=2-3. [d] 10% D_2O+90% H_2O, pH=2-3.

To confirm the reproducibility of the restrictiveness of the bicyclic scaffold, the conformations of Ac-Val-Abh(OBn) **2c** and Ac-Ile-Abh(OBn) **2d** were studied (Figure 4(b)(c)). Compared with alanine, valine has a bulkier side chain. Variable-temperature NMR spectra of Ac-Val-Abh(OBn) **2c** and valine dipeptide (Ac-Val-NHMe, **3c**) in $CDCl_3$ were measured and the values of the ^3J coupling constants were plotted (Figure 4(b)(c)). In the case of Ac-Val-Abh(OBn) **2c**, the change of the ^3J coupling constants upon change in temperature is small over a 25-degree temperature range, while in the case of the valine dipeptide (Ac-Val-NNMe, **3c**), the corresponding change is large in the same temperature range. The strong temperature dependency of the valine dipeptide **3c** suggests that it is conformationally flexible, whereas Ac-Val-Abh(OBn) **2c** shows conformational rigidity and stability (Figure 4(b)).

Compared with Val, Ile has a bulkier side chain. The corresponding variable-temperature NMR spectra of Ac-Ile-Abh(OBn) **2d** and the Ile dipeptide (Ac-Ile-

NHMe, **3d**) measured in $CDCl_3$ showed little change in the case of Ac-Ile-Abh(OBn) **2d** over a 25-degree temperature range, whereas the Ile dipeptide **3d** showed a marked change in the same temperature range (Figure 4(c)). The strong temperature dependency of the Ile dipeptide **3d** suggests that its conformation is flexible, whereas Ac-Ile-Abh(OBn) **2d** shows conformational rigidity and stability (Figure 4(c)). The values of $^3J(H^{\alpha}, H^{N})$ of the Ile dipeptide **3d** in $CDCl_3$ and in water were 8.84 Hz and 7.20 Hz, respectively (Table 2). The values of the ^3J-coupling constant of Ac-Ile-Abh(OBn) **2d** in $CDCl_3$ and in water were 8.99 Hz and 7.87 Hz respectively (Table 2), being larger than those of the Ile dipeptide **3d**; this result suggests that Ac-Ile-Abh(OBn) **2d** has a higher population of β-strand conformation than **3d** in both solvents.

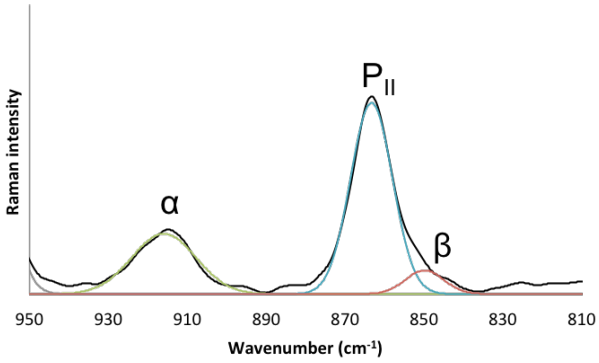
To know how far the restriction can be transferred, we elongated the main chain and studied the conformation of Ac-Ala-Ala-Abh(OBn) **4b**. Variable-temperature NMR spectra were measured in DMSO-d_6 and the values of the ^{3}J coupling constant of the two NHs were plotted against temperature (Figure 4(d)). For both NHs (NH1 and NH2), the change was rather flat over a 20-degree temperature range, suggesting that the whole molecule is conformationally restricted. Thus, the conformational restriction arising from the C-terminal bicyclic moiety appears to suppress rotation for at least two α-amino acid residues from the N-terminal (Figure 4(d)).

Raman spectroscopy

Raman spectroscopy is a powerful tool to detect the distribution of main-chain conformations of peptides.[31, 42] Here, we examined the skeletal vibrations (810-950 cm^{-1}) in the Raman spectrum of the alanine dipeptide (Ac-Ala-NHMe, **3b**). These

vibrations consist of one main-chain Cα-C stretching vibration, and two skeletal Cα-N stretching and N-Cα-C bending modes.[31] We measured the Raman spectrum of the alanine dipeptide (Ac-Ala-NHMe, **3b**) and Ac-Ala-Abh(OBn) **2b** (Figure 5) and observed three characteristic bands in the region between 810 and 950 cm^{-1}. In the case of the alanine dipeptide **3b**, the band frequencies for P_{II}, α_R and β are well separated and were assigned as 863 cm^{-1}, 915 cm^{-1}, and 849 cm^{-1}, respectively (Table 3), in accordance with reported data.[31] In the case of Ac-Ala-Abh(OBn) **2b**, the band frequencies for P_{II} and β are well separated, and were assigned as 879 cm^{-1}, 854 cm^{-1}, respectively, while α_R is lost (Figure 5b)). The conformational populations can be calculated from the band areas (Table 3). In the case of alanine dipeptide **3b**, the predominant conformation is P_{II} (calculated population: 63%), whereas the population of α_R conformation is 23%, and the population of β conformation is 14%. In the case of Ac-Ala-Abh(OBn) **2b**, the predominant conformation is β (66%), and the population of P_{II} conformation is 34%. Thus, the Abh scaffold alters the predominant conformation from P_{II} to β conformation in the Ala moiety.

(a)



(b)

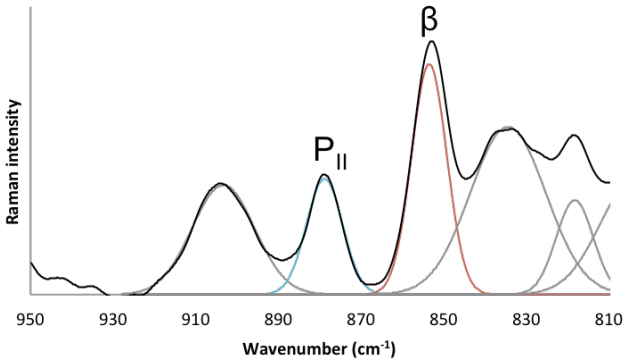


Figure 5. Comparison of Raman skeletal vibrations of C-NHMe and C-Abh amino acids (a) Raman spectrum of alanine dipeptide **3b** (b) Raman spectrum of Ac-Ala-Abh(OBn) **2b**. Color: black=observed spectrum, green= deconvolution spectrum of α_R, blue= deconvolution spectrum of P_{II}, red= deconvolution spectrum of β.

Table 3. Raman band frequencies of skeletal vibrations.

	β	α_R	P_{II}
Alanine dipeptide **3b** [a]	849 cm^{-1} (14%)	915 cm^{-1} (23%)	863 cm^{-1} (63%)
Ac-Ala-Abh(OBn) **2b** [a]	854 cm^{-1} (66%)	ND	879 cm^{-1} (34%)

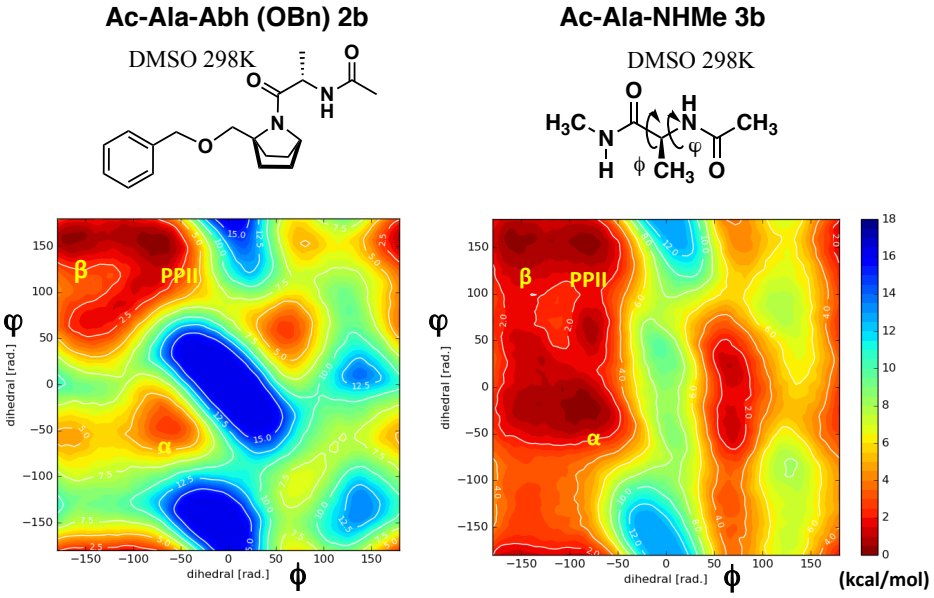
[a] The sample was measured in hexane dry film. ND= not detected.

Metadynamics simulation of the conformations of α-amino acid moiety bound to Abh

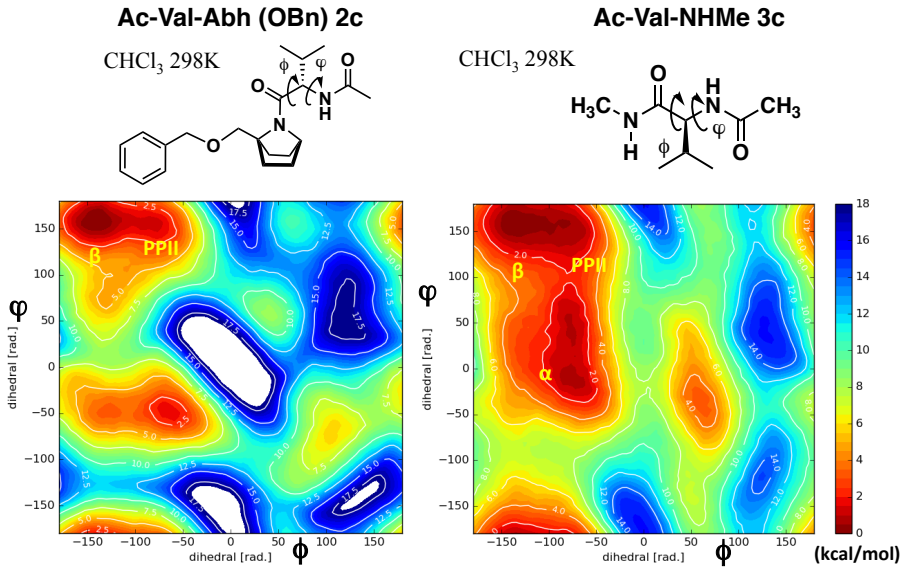
We carried out metadynamics simulation in explicit DMSO (in the cases of Ala derivatives) and in chloroform (in the cases of Val and Ile derivatives) to obtain the potential energy landscape of the conformations of the peptides (Figure 6).[44] The solvent corresponds to the NMR experiments, but water environment provided similar results. Metadynamics simulations at 298K in DMSO suggested that Ac-Ala-

Abh(OBn) **2b** favors mainly β and PPII conformations (Figure 6(a)), while the alanine dipeptide **3b** exhibits β, PPII, and α-helix conformations, and its low potential energy region is much broader than that of **2b**.

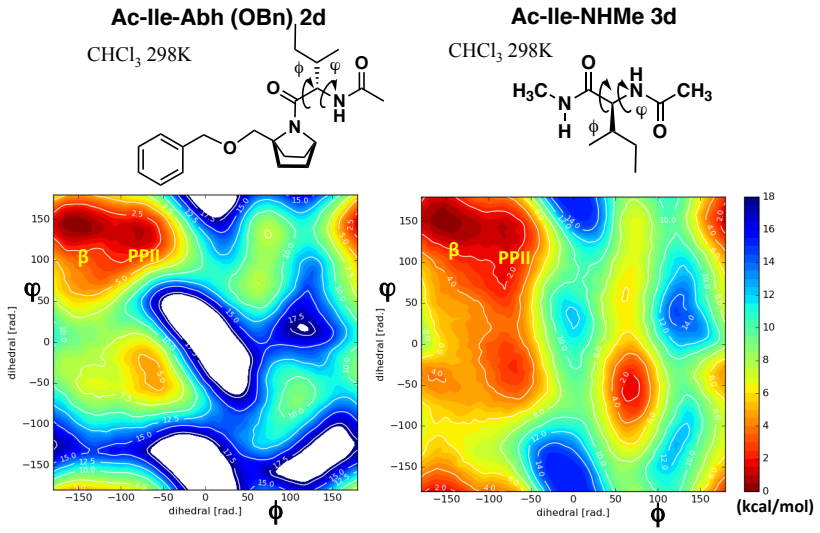
(a)



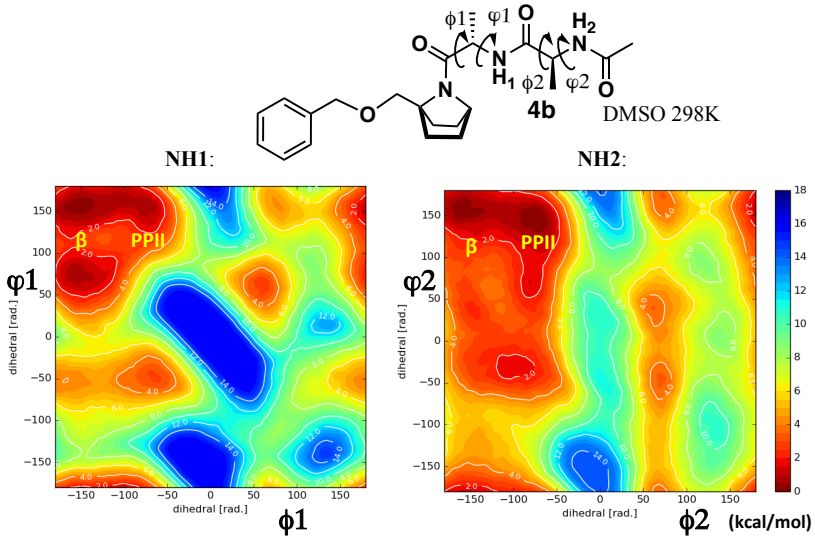
(b)



(c)


Ac-Ile-Abh (OBn) 2d
CHCl3 298K
Ac-Ile-NHMe 3d
CHCl3 298K
β
PPII
φ
dihedral [rad.]
ϕ
(kcal/mol)

(d)


4b
DMSO 298K
NH1:
NH2:
β
PPII
φ1
φ2
dihedral [rad.]
ϕ1
ϕ2
(kcal/mol)

(e)

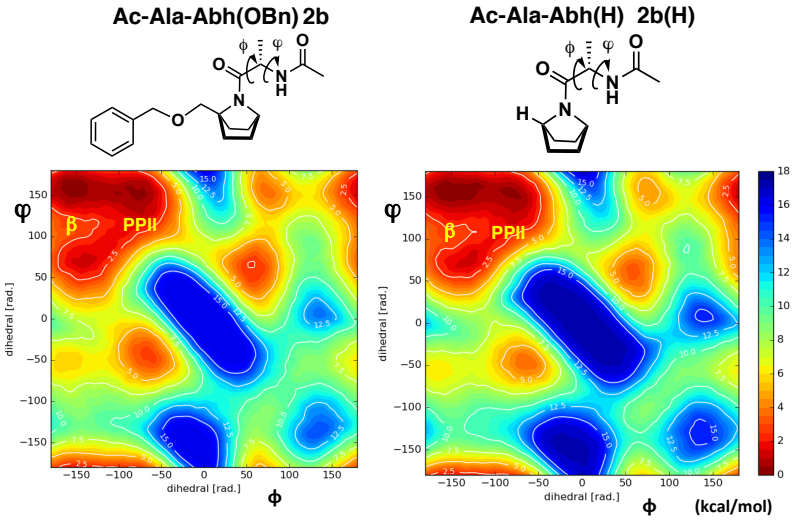


Figure 6. Energy landscapes of metadynamics simulations. (a) Ac-Ala-Abh(OBn) 2b and alanine dipeptide 3b in DMSO, (b) Ac-Val-Abh(OBn) 2c and valine dipeptide 3c in chloroform and (c) Ac-Ile-Abh(OBn) 2d and isoleucine dipeptide 3d in chloroform. (d) Ac-Ala-Ala-Abh(OBn) 4b and Ac-Ala-Ala-NHMe (Supporting Information) (e) alanine derivatives bearing bridgehead-substituted and unsubstituted Abh

Since Ac-Ala-Abh(OBn) **2b** shows markedly fewer accessible minimum conformations, it is more restricted in terms of the conformation of the Ala moiety (Figure 6(a)). The low potential energy region of Ac-Val-Abh(OBn) **2c** and Ac-Ile-Abh(OBn) **2d** is mainly localized in the β conformation region (Figure 6 (b)) and is narrower than those of the valine (**3c**) and isoleucine (**3d**) dipeptides, indicating a stronger restriction of the conformations of the Val and Ile moieties (Figure 6(b) and (c)).

Metadynamics simulation of the two alanine moieties of Ac-Ala-Ala-Abh(OBn) **4b** (Figure 6(e)) also showed the restriction as compared with Ac-Ala-Ala-NHMe **5b** (see Supporting Figure). This is consistent with the weak temperature dependency of

the $^{3}J(H^{\alpha}, H^{N})$ values of the two Ala NH protons of Ac-Ala-Ala-Abh(OBn) **4b** (Figure 4(d)).

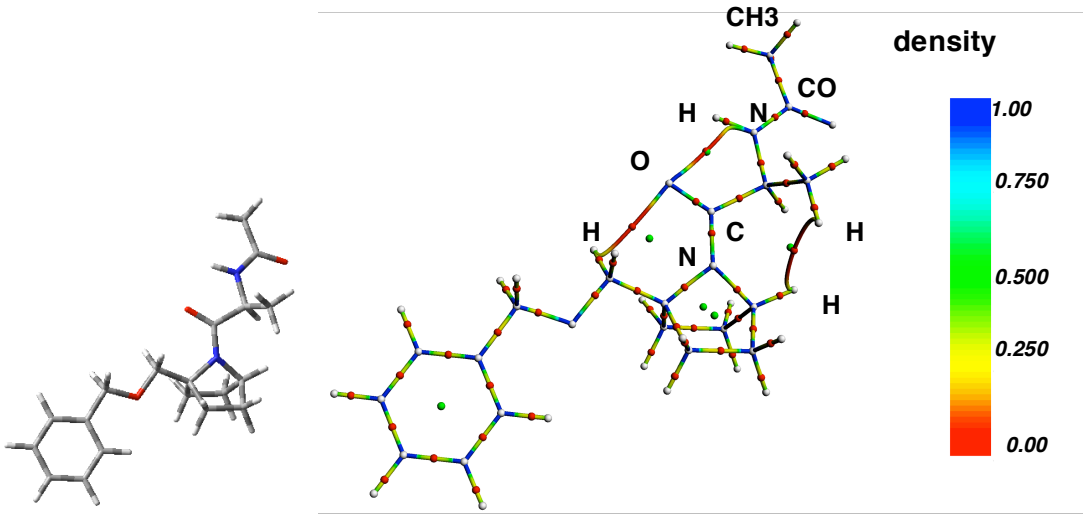
As described in the previous section, the NMR study indicated that the bridgehead proton is close to the α-proton of the α-amino acid, due to the characteristic upward-projecting direction of the bridgehead proton, and we postulated that this proximity would influence the main chain rotation of the adjacent α-amino acid. To test this idea, we compared the simulated potential energy landscapes of Ac-Ala-Abh(OBn) **2b** and Ac-Ala-Abh(H) **2b(H)** (Figure 6(e)). The energy maps were similar, suggesting similar conformational preferences. This implies that some interaction between the bridgehead proton of the Abh and the α-proton and the side chain of the α-amino acid both contribute to the restriction of the main chain rotation of the linked α-amino acid residue.

Interaction of the C-terminal Abh unit with the adjacent α-amino acid in β-strand conformation

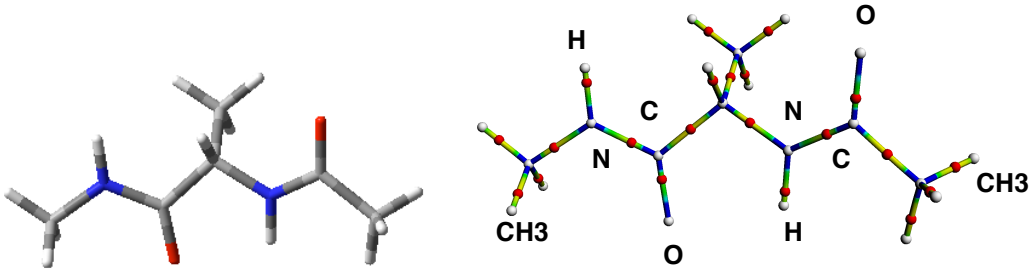
Proximity interaction of the Abh unit with the β-strand conformers of adjacent α-amino acids (**2b**, **2c** and **2d**) can be validated in terms of molecular graphs of quantum theory of atoms-in-molecules (QTAIM) analysis.[45] Figure 7 showed the presence of through-space weak accumulation of electron density (bond path, red line in Figure 7) between the Abh unit and the β-strand conformers of adjacent Ala, Val and Ile. In all cases ((a) Ala **2b**, (b) Val **2c** and (d) Ile **2d**) a bond path between the carbonyl oxygen atom and the methylene H atom (in the case of **2b** and **2d**) or the C atom (in the case of **2c**) of the bridgehead substituent, and several bond paths between the methylene H atom(s) in the amino acid side chain and the Abh bridgehead H atom and/or the Abh

bicyclic ethano-bridge H atom can be found (Figure 7 (a)(c)(d)). In the case of Ala (**2b**, Figure 7(a)), the intraresidual carbonyl O---amide N bond path was detected within the Ala moiety. This kind of interaction may be relevant to the putative weak intraresidual carbonyl O---amide H(N) C5 hydrogen bonding,[46] particularly important in β-sheet formation.[47] While there have been arguments of the presence and the interpretation of H—H bond path,[48-49] the present H—H interactions, detected in Figure 7, are at least consistent with the proximity of these relevant atoms and also indicative of interactions in the C-terminal Abh derivatives. In the non-Abh derivative, the NHMe C-terminal case (**3b**), no such weak interactions were detected in the Ala derivative (Figure 7(b) (Val and Ile derivative cases, see also Supporting Figures). Therefore these results indicated that such intramolecular weak atomic link arising from the Abh unit can contribute to enhancement of taking β-strand-like extended structures of the adjacent α-amino acid residue.

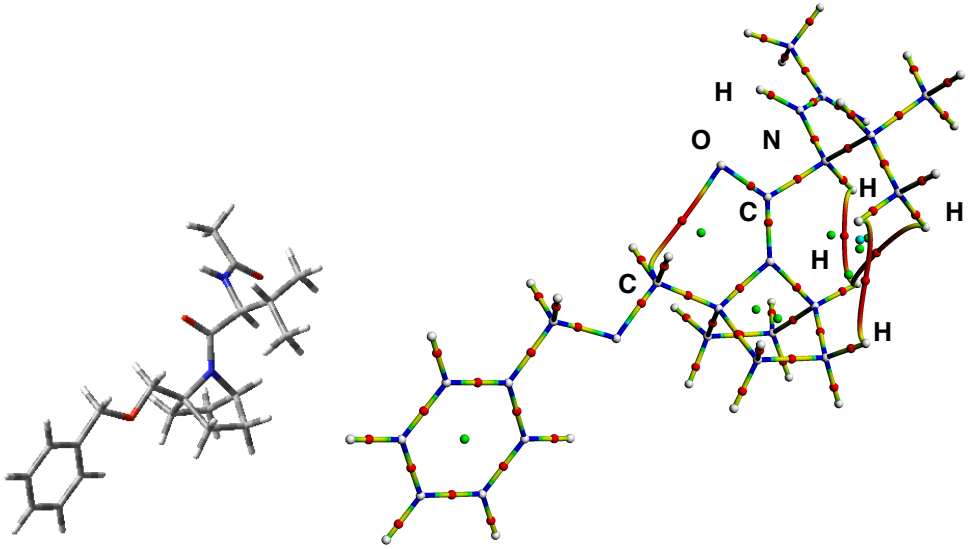
(a) Ac-Ala-Abh(OBn) **2b**



(b) Ac-Ala-NHMe **3b**



(c) Ac-Val-Abh(OBn) **2c**



(d) Ac-Ile-Abh(OBn) **2d**

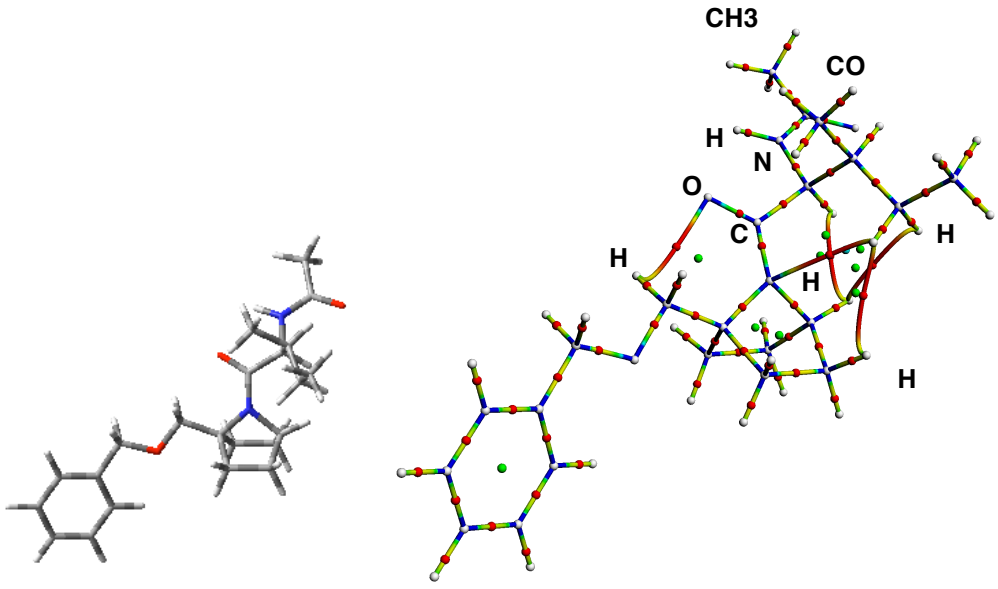


Figure 7. Molecular Graphs obtained in QTAIM, indicating proximity (red colored bond paths) of the C-terminal Abh unit in the β-strand conformation of α-amino acids (a, c, d). The β-strand conformer of NHMe C-terminal Ala derivative (b) is a reference compound.

Conclusion

In summary, introduction of the C-terminal bicyclic scaffold (Abh) restricted the overall conformation of the adjacent α-amino acid residue(s). The combination of ^{1}H-NMR and Raman spectroscopies and metadynamics simulations suggest that the present bicyclic amine (Abh) can work as an effective scaffold to constrain or enforce local peptide conformation. Both of the bridgehead groups, even the hydrogen atom (H-H bond path), have proximity interactions with the α-amino acid side chain and furthermore influence the adjacent α-amino acid to favor extended β-strand-like conformation. These results provide a new perspective for enforcing/stabilizing extended β-strand-like conformation through the proximity interactions and also provide a basis for *de novo* design of β-strand-mimicking peptides and small proteins by using β-strand enforcer/stabilizer even in the absence of the inter-strand hydrogen bonding.

Experimental Section

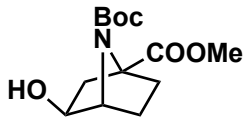
General methods

Open column chromatography was carried out on silica gel (silica gel 60N (100-210 μm), Kanto Chemicals, Japan). All the NMR experiments were recorded on a Bruker Avance 400 NMR spectrometer. ^{1}H-NMR and ^{13}C-NMR chemical shifts (δ) were calibrated with the solvent peak and are shown in ppm. Coupling constants are given in Hz. Mass spectra were recorded on a Bruker micrOTOF-05. All the melting points were measured with a Yanaco Micro Melting Point Apparatus without corrected. The combustion analyses were carried out in the microanalytical laboratory of this faculty.

HPLC data were obtained using a Hitachi instrument with Senshu Pak Pegasil Silica SP-100 (250 mm x 20 mm) for a normal phase column, and Mightysil RP-18 GP Aqua (250 mm x 10 mm) for a reverse phase column. Flow rate: 5.0 ml/min. The HPLC chromatograms of these products were shown in Supporting Information.

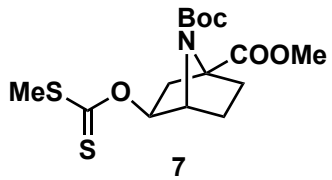
Synthesis

The synthesis scheme before compound **6** is shown in the supporting information.



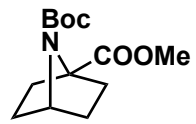
6

To a solution of **5** (6.2 g, 19.8 mmol) in MeOH (40 ml) was added DBU (5.9 ml 39.7 mmol). The reaction mixture was stirred for 20 h at room temperature. After solvent was evaporated, AcOEt was added to the residue and the organic phase was washed with saturated aqueous solution of NH_4Cl. The aqueous layer was combined and extracted with $CHCl_3$. The organic layers were combined, dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (hexane: AcOEt= 3:7) gave compound **6** (4.7 g, 88%) as colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 4.223 (1H, d, J=5.2 Hz), 3.953-3.915 (1H, m), 3.805 (3H, s), 2.183-2.133 (1H, m), 2.128-2.094 (1H, m), 2.044 (3H, s), 1.994-1.902(1H, m), 1.676-1.507 (1H, m), 1.428 (9H, s).



7

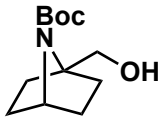
To a suspension of NaH in THF (80 ml) under argon were added CS_2 (6.8 mL, 113.0 mmol) and **6** (3.1 g, 11.3 mmol) in THF (20ml) at 0 °C. The reaction mixture was stirred for 2 h at 40-50 °C and then cooled to room temperature. MeI (3.5 ml, 56.5 mmol) was added at room temperature and the reaction mixture was stirred at 30-40 °C for 2 h. The reaction was quenched by addition of saturated aqueous solution of NH_4Cl (30 ml), extracted with AcOEt, and the organic layer was dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (hexane: AcOEt= 10:1-20:3) gave compound **7** (4.0 g, 97%) as a yellow oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 5.442-5.417 (1H, m), 4.538 (1H, brd, J = 5.6 Hz), 3.805 (3H, s), 2.543 (3H, s), 2.381-2.175 (2H, m), 2.124-2.105 (1H, m), 2.019-1.932 (1H, m), 1.691-1.521 (1H, m), 1.509-1.487 (1H, m), 1.425 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): 215.5, 170.3, 155.7, 85.1, 81.3, 67.9, 63.2, 52.5, 41.7, 32.8, 28.2, 24.1, 19.4. HRMS (ESI^+, $[M+Na]^+$): Calcd. for $C_{15}H_{23}NNaO_5S_2^+$, 384.0910. Found: 384.0906.



8

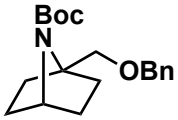
To a solution of **7** (4.0 g, 10.9 mmol) in toluene (150 ml) under argon were added

Tris(trimethylsilyl)silane (TTMSS, 5.03 ml, 16.4 mmol) and AIBN (179 mg, 1.1 mmol) at room temperature. The mixture was heated at reflux for 30min and the solvent was evaporated. Column chromatography (hexane: AcOEt= 10:1) gave compound **8** (2.6 g, 92%) as a colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 4.327-4.303 (1H, m), 3.799 (3H, s), 2.217-2.134 (2H, m), 1.976-1.883 (2H, m), 1.777-1.714 (2H, m), 1.523-1.461 (2H, m), 1.416 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): 171.8, 156.6, 80.8, 68.8, 59.8, 52.2, 33.5, 29.4, 28.2. HRMS (ESI$^+$, $[M+Na]^+$): Calcd. for $C_{13}H_{21}NNaO_4^+$, 278.1363. Found: 278.1366.



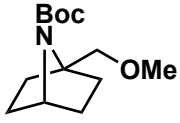
9

To a solution of **8** (2.7 g, 10.7 mmol) in EtOH/THF (3:2, 50 ml) were added $CaCl_2$ (2.4 g, 21.4 mmol) and $NaBH_4$ (1.6 g, 42.8 mmol) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then stirred for 3 h at room temperature. The reaction was quenched by adding cold saturated aqueous solution of NH_4Cl (30 ml). The water phase was extracted with AcOEt, dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (hexane: AcOEt= 3:1) gave compound **9** (2.3 g, 94%) as a colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 4.894 (1H, brs), 4.231-4.208 (1H, m), 3.882 (2H, brd, *J* = 6.8 Hz), 1.875-1.705 (4H, m), 1.478-1.319 (13H, m, at 1.428 ppm (^{t}Bu group, 9H, s)). ^{13}C-NMR (100 MHz, $CDCl_3$): 155.3, 80.2, 69.2, 62.1, 58.5, 31.9, 29.4, 28.5. HRMS (ESI$^+$, $[M+Na]^+$): Calcd. for $C_{12}H_{21}NNaO_3^+$, 250.1414. Found: 250.1423.



10

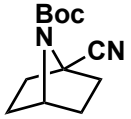
To a solution of NaH (420 mg, 10.5 mmol) in THF (20 ml), **9** (796 mg, 3.5 mmol) in THF (10 ml) was added dropwise at 0°C under argon. The solution was stirred for 30min at 0 °C. Then BnBr (0.83 ml, 7 mmol) was added dropwise. The solution was warmed to room temperature and stirred at 50°C overnight. The reaction was quenched by adding water (20 ml), extracted with Et_2O, and the organic layer was dried over Na_2SO_4 and evaporated the solvent. Column chromatography (hexane: EtOAc = 10:1) gave compound **10** (949 mg, 85%) as a colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 7.345-7.244 (5H, m), 4.612 (2H, s), 4.279-4.260 (1H, m), 4.068 (2H, s), 1.787-1.425 (8H, m), 1.425 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): 155.6, 139.0, 128.4, 127.7, 127.5, 79.6, 73.5, 72.7, 67.3, 58.9, 33.9, 29.0, 28.5. HRMS (ESI$^+$, $[M+Na]^+$): Calcd. for $C_{19}H_{27}NNaO_3^+$, 340.1883. Found: 340.1884.



11

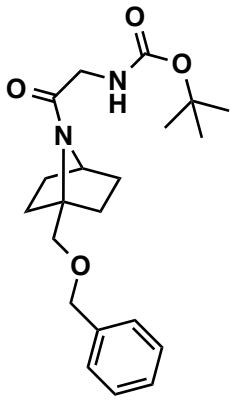
To a solution of NaH (117.6 mg, 2.94 mmol) in THF (10 ml), **9** (223 mg, 0.98 mmol) in THF (4 ml) was added dropwise at 0°C under argon. The solution was stirred for 30 min at 0°C. Then MeI (0.122 ml, 1.96 mmol) was added dropwise. The solution

was warmed to room temperature and stirred for 3 h. The reaction was quenched by adding water (20 ml) and extracted with Et_2O. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (hexane: EtOAc = 4:1) gave compound **11** (194 mg, 82%) as a colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 4.265-4.242 (1H, m), 3.970 (2H, s), 3.415 (3H, s), 1.798-1.251 (17H, m, at 1.437 ppm (t-Bu group, 9H, s)). ^{13}C-NMR (100 MHz, $CDCl_3$): 155.6, 79.5, 74.9, 67.3, 59.5, 58.8, 33.6, 28.9, 28.5. HRMS (ESI$^+$, $[M+Na]^+$): Calcd. for $C_{13}H_{23}NNaO_3^+$, 264.1570. Found: 264.1568.



12

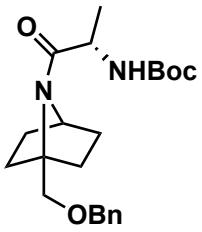
To a solution of **9** (220 mg, 1 mmol) in MeCN-H_2O (9:1, 3 ml), TEMPO (7.8 mg, 5 mol%), NH_4OAc (308 mg, 4 mmol), and $PhI(OAc)_2$ (708 mg, 2.2 mmol) were successively added. The suspension was stirred at room temperature overnight. The reaction was quenched by adding water and the whole was extracted with Et_2O. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (hexane: EtOAc = 6:1) gave compound **12** (92 mg, 43%) as a colorless oil and **9** (110 mg, 50% recovery).
^{1}H-NMR (400 MHz, $CDCl_3$): 4.325-4.302 (1H, m), 2.217-2.133 (2H, m,), 1.956-1.854 (4H, m), 1.536-1.472 (2H, m), 1.486 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): 155.6, 118.8, 82.1, 58.5, 56.1, 36.0, 29.0, 28.2. HRMS (ESI$^+$, $[M+Na]^+$): Calcd. for $C_{12}H_{18}N_2NaO_2^+$, 245.1260. Found: 245.1263.



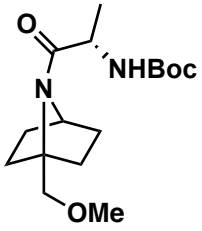
Boc-Gly-Abh(OBn) (1a)

To a solution of **10** (102 mg, 0.32 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) were added Boc-Gly-OH (84 mg, 0.48 mmol), CDMT (112 mg, 0.64 mmol), DMAP (5 mg, 0.04 mmol) and NMM (70 µl, 0.64 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic

layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/Acetone = 4/1) to afford **Boc-Gly-Abh(OBn)** (**1a**) (107 mg, 89%), as a colorless oil.
^{1}H NMR (CD_3OD, 400MHz) δ 7.365-7.242 (5H, m), 4.585 (2H, s), 4.376 (1H, s), 4.161 (2H, m), 3.839 (2H, s), 1.822-1.444 (8H, m), 1.444 (9H, s) ^{13}C-NMR (100 MHz, $CDCl_3$): 168.1, 158.3, 139.8, 129.3, 128.9, 128.6, 80.5, 74.4, 72.9, 69.8, 58.6, 43.6, 34.2, 30.1, 28.7. HRMS (ESI$^+$): Calcd. for $C_{21}H_{30}N_2NaO_4^+$ ([M+Na]$^+$): 397.2098. Found: 397.2099. HPLC (Hexane: EtOAc = 1:1, 265 nm): t_R 21.47min, 98% purity.

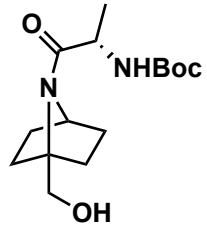


Boc-Ala-Abh(OBn) (1b)
To a solution of compound **10** (194 mg, 0.61 mmol) in CH_2Cl_2 (3 ml) was added TFA (2 ml) at 0°C. The solution was stirred for 40 min at room temperature. The whole was washed with 10% aqueous solution of Na_2CO_3 (20 ml), and the aqueous layer was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated. The crude amine was obtained and was used to the next step directly. To a solution of crude amine in DMF (5 ml), Boc-L-Ala (174 mg, 0.92 mmol), DIC (0.188 ml, 1.22 mmol), HOBT (164.85 mg, 1.22 mmol) and DIPEA (0.168 ml, 1.83 mmol) were added. The solution was stirred overnight at room temperature. The reaction was quenched by adding water (10 ml) and then the whole was extracted with CH_2Cl_2. The organic phase was washed with 5% aqueous solution of citric acid and saturated aqueous solution of $NaHCO_3$, dried over Na_2SO_4 and evaporated the solvent. Column chromatography (hexane: EtOAc = 3:1) gave compound **Boc-Ala-Abh(OBn)** (**1b**) (209 mg, 88%) as a colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 7.338-7.230 (5H, m), 5.439 (1H, d, J = 7.60 Hz), 4.646-4.583 (2H, m), 4.405-4.369 (1H, m), 4.296 (1H, s), 4.214 (2H, s), 1.832-1.423 (8 H, m), 1.423 (9H, s), 1.299 (3H, d, J = 6.8 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): 169.4, 155.3, 139.0, 128.6, 127.8, 127.7, 79.6, 73.7, 72.3, 68.5, 58.3, 48.5, 34.0, 33.3, 30.2, 29.5, 28.7, 20.3. HRMS (ESI, [M+Na]$^+$): Calcd. for $C_{22}H_{32}N_2NaO_4^+$, 411.2254. Found: 411.2254.

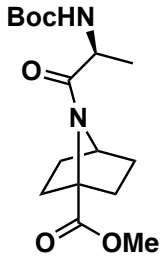


Boc-Ala-Abh(OMe) (1b(OMe))
To a solution of compound **11** (194 mg, 0.82 mmol) in CH_2Cl_2 (3 ml) was added TFA (2 ml) at 0 °C. The solution was stirred for 40 min at room temperature. The solution was washed with 10% Na_2CO_3 (20 ml), and the aqueous phase was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated. The crude amine was obtained and was used to the next step directly. To a solution of crude amine in DMF (5 ml), Boc-L-Ala (227 mg, 1.2 mmol), DIC (0.246 ml, 1.6 mmol), HOBT (216

mg, 1.6 mmol) and DIPEA (0.22 ml, 2.4 mmol) were added. The mixture was stirred overnight at room temperature. The reaction was quenched by adding water (10 ml) and the whole was extracted with CH_2Cl_2. The organic phase was washed with 5% aqueous solution of citric acid and saturated aqueous solution of $NaHCO_3$, dried over Na_2SO_4 and evaporated. Column chromatography (hexane: EtOAc = 3:1) gave compound **Boc-Ala-Abh(OMe)** (50 mg, 20%) as a colorless solid.
^{1}H-NMR (400 MHz, $CDCl_3$): 5.444 (1H, d, J = 7.60 Hz), 4.397-4.344 (1H, m), 4.292 (1H, m), 4.109 (2H, s), 3.422 (3H, s), 1.827-1.419 (17H, m, at 1.419 ppm (t-Bu group, 9H, s)), 1.302 (3H, d, J = 6.8 Hz) ^{13}C-NMR (100 MHz, $CDCl_3$): 169.2, 155.2, 79.5, 74.3, 68.3, 59.5, 58.0, 48.4, 33.6, 32.8, 30.0, 29.4, 28.5, 20.2. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{16}H_{28}N_2NaO_4^+$, 335.1947. Found: 335.1948. Anal. Calcd for $C_{16}H_{28}N_2O_4$: C, 61.51; H, 9.03; N, 8.97. Found: C, 61.45; H, 8.97; N, 8.97.

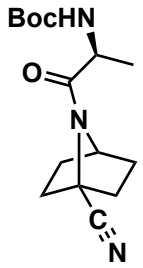


Boc-Ala-Abh(OH) (1b(OH))
To a solution of **Boc-Ala-Abh(OBn)** (38 mg, 0.1 mmol) in EtOH (5 ml), 5% Pd/C (15 mg) was added. The reaction was stirred for 4 h under H_2 atmosphere. The reaction mixture was passed through the Celite pad, washed with EtOH and concentrated. Compound **Boc-Ala-Abh(OH)** (26.5 mg, 91%) was afforded, as a colorless solid.
^{1}H-NMR (400 MHz, $CDCl_3$): 5.413 (1H, d, J = 7.6 Hz), 4.995-4.958 (1H, m), 4.451-4.414 (1H, m), 4.310-4.299 (1H, m), 3.951-3.856 (2H, m), 1.969-1.429 (8H, m), 1.429 (9H, s), 1.307 (3H, d, J = 6.8 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): 168.5, 155.1, 79.8, 71.1, 61.5, 58.3, 48.3, 31.8, 30.8, 30.0, 29.6, 28.5, 19.9. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{15}H_{26}N_2NaO_4^+$, 321.1785. Found: 321.1785.



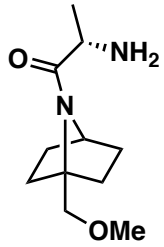
Boc-Ala-Abh(CO_2Me) (1b(CO_2Me))
To a solution of compound **8** (110 mg, 0.43 mmol) in CH_2Cl_2 (3 ml) was added TFA (2 ml) at 0°C. The solution was stirred for 40 min at room temperature. The mixture was washed with 10% aqueous solution of Na_2CO_3 (20 ml), and the aqueous phase was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated. The crude amine was obtained and was used to the next step directly. To a solution of crude amine in DMF (5 ml), Boc-L-Ala (122 mg, 0.65 mmol), DIC (0.13 ml, 0.86 mmol), HOBT (116 mg, 0.86 mmol) and DIPEA (0.12 ml, 1.3 mmol) were added. The mixture was stirred overnight at room temperature. The reaction was quenched by adding water (10 ml) and the whole was extracted with CH_2Cl_2. The organic phase was washed with 5% aqueous solution of citric acid and saturated aqueous solution of

$NaHCO_3$, dried over Na_2SO_4 and evaporated. Column chromatography (hexane: EtOAc = 3:1) gave compound **Boc-Ala-Abh(CO_2Me)** (51 mg, 36%) as a colorless oil. ^{1}H-NMR (400 MHz, CD_3OD): 4.580-4.556 (1H, m), 4.419-4.367 (1H, m), 3.742 (3H, s), 2.179-1.615 (8H, m), 1.429 (9H, s), 1.277 (3H, brd, J = 7.2 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.6, 170.9, 155.2, 79.8, 68.3, 67.3, 59.1, 52.6, 48.0, 33.8, 31.0, 30.3, 28.5, 20.1. HRMS (ESI$^+$, $[M+Na]^+$): Calcd. for $C_{16}H_{26}N_2NaO_5^+$, 349.1734. Found: 349.1732.



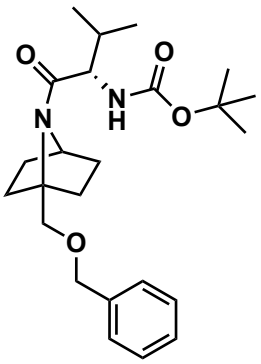
Boc-Ala-Abh(CN) (1b(CN))

To a solution of compound **12** (88 mg, 0.39 mmol) in CH_2Cl_2 (3 ml) was added TFA (2 ml) at 0°C. The solution was stirred for 40 min at room temperature. The solution was washed with 10% aqueous solution of Na_2CO_3 (20 ml), and the aqueous phase was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The crude amine was obtained and was used to the next step directly. To a solution of crude amine in DMF (5 ml), Boc-L-Ala (112 mg, 0.59 mmol), DIC (0.12 ml, 0.79 mmol), HOBT (107 mg, 0.79 mmol) and DIPEA (0.11 ml, 1.2 mmol) were added. The mixture was stirred overnight at room temperature. The reaction was quenched by adding water (10 ml) and the whole was extracted with CH_2Cl_2. The organic phase was washed with 5% aqueous solution of citric acid and saturated aqueous solution of $NaHCO_3$, dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (hexane: EtOAc = 3:1) gave compound **Boc-Ala-Abh(CN)** (80 mg, 70%) as a colorless oil.
^{1}H-NMR (400 MHz, CD_3OD): 4.647-4.625 (1H, m), 4.436-4.400 (1H, m), 2.172-1.682 (8H, m), 1.455 (9H, s), 1.330 (3H, brd, J = 7.2 Hz). ^{13}C-NMR (100 MHz, CD_3OD): 175.2, 157.6, 119.3, 80.5, 59.5, 55.8, 36.7, 35.3, 31.1, 28.7, 18.4. HRMS (ESI$^+$, $[M+Na]^+$): Calcd. for $C_{15}H_{23}N_3NaO_3^+$, 316.1632. Found: 316.1620.



H-Ala-Abh(OMe) (H-1b(OMe))
To a solution of **Boc-Ala-Abh(OMe)** (17mg, 0.05mmol) in CH_2Cl_2 (1mL) was added TFA (0.3mL) at 0 °C. The solution was stirred for four hours at room temperature. The solvent was then evaporated. Column chromatography ($CHCl_3$: MeOH: Et3N = 9:1:0.1) gave compound **H-Ala-Abh(OMe)** (18mg, containing Et_3N salt) as a colorless oil.

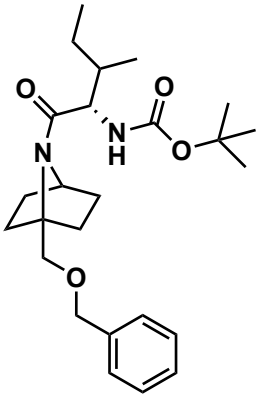
^{1}H-NMR (400 MHz, D_2O): 4.392 (1H, s), 4.288-4.235 (1H, m), 4.152-4.038 (2H, dd), 3.445 (3H, s), 1.881-1.667 (8H, m), 1.481 (3H, d, J = 6.8 Hz). HRMS (ESI, $[M+H]^+$): Calcd. for $C_{11}H_{21}N_2O_2^+$, 213.1598. Found: 213.1603. Reverse-phase HPLC (CH_3CN, 215 nm): t_R 4.56 min, 92%.



Boc-Val-Abh(OBn) (1c)

To a solution of **10** (60 mg, 0.19 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) were added Boc-Val-OH (63mg, 0.29 mmol), CDMT (67 mg, 0.38 mmol), DMAP (3mg, 0.02 mmol) and NMM (63 µl, 0.57 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/Acetone = 4/1) to afford dimer **Boc-Val-Abh(OBn)** (55mg, 70%), as a colorless oil.

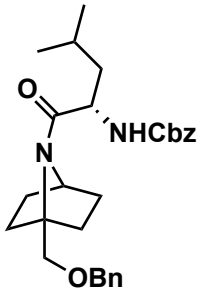
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.337-7.246 (5H, m), 5.231 (1H, brd, J=8.8 Hz), 4.651-4.570 (2H, m), 4.353 (1H, s), 4.241-4.187 (3H, m), 1.917-1.422 (18H, m, at 1.422 ppm (t-Bu group, 9H, s)), 0.958-0.866 (6H, dd, J=6.8Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): 168.9, 155.9, 138.9, 128.4, 127.6, 127.5, 79.4, 73.5, 72.4, 68.2, 58.7, 57.6, 34.1, 32.7, 32.1, 30.6, 29.1, 28.5, 19.8, 17.4. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{24}H_{36}N_2NaO_4^+$, 439.2567. Found: 439.2587.



Boc-Ile-Abh(OBn) (1d)

To a solution of **10** (97 mg, 0.31 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at

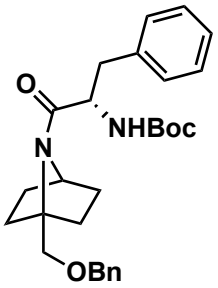
0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) were added Boc-Ile-OH (109 mg, 0.47 mmol), CDMT (109 mg, 0.62 mmol), DMAP (4 mg, 0.03 mmol) and NMM (102 μl, 0.93 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/Acetone = 4/1) to afford dimer Boc-Ile-Abh(OBn) (75mg, 57%), as a colorless oil.
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.335-7.237 (5H, m), 5.179 (1H, brd, J=4.2Hz), 4.649-4.568 (2H, m), 4.383 (1H, m), 4.246-4.201 (3H, m), 1.855-1.417 (18H, m, at 1.417 ppm (t-Bu group, 9H, s)), 1.105-0.910 (8H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): 169.0, 155.9, 138.9, 128.4, 127.6, 127.5, 79.5, 73.4, 72.5, 68.2, 58.8, 57.2, 39.0, 38.5, 34.1, 32.7, 30.6, 29.1, 28.5, 26.6, 24.2, 16.0, 14.1, 12.1, 11.7. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{25}H_{38}N_2NaO_4^+$, 453.2724. Found: 453.2727.



Cbz-Leu-Abh(OBn) (1e)

To a solution of compound **10** (202 mg, 0.64 mmol) in CH_2Cl_2 (3 ml) was added TFA (2 ml) at 0°C. The solution was stirred for 40 min at room temperature. The solution was washed with 10% aqueous solution of Na_2CO_3 (20 ml), and the aqueous phase was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The crude amine was obtained and was used to the next step directly. To a solution of crude amine in DMF (5 ml), CBZ-L-Leu (204 mg, 0.77 mmol), DIC (0.197 ml, 1.28 mmol), HOBT (173 mg, 1.28 mmol) and DIPEA (0.176 ml, 1.92 mmol) were added. The mixture was stirred overnight at room temperature. The reaction was quenched by adding water (10 ml) and then extracted with CH_2Cl_2. The organic phase was washed with 5% aqueous solution of citric acid and saturated aqueous solution of $NaHCO_3$, dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (hexane: EtOAc = 3:1) gave compound **Cbz-Leu-Abh(OBn)** (228 mg, 77%) as a colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 7.347-7.254 (10H, m), 5.436 (1H, d, J = 9.20 Hz), 5.086-5.046(2H, m), 4.643-4.574 (2H, m), 4.518-4.461 (1H, m), 4.346 (1H, s), 4.249-4.196 (2H, m), 1.830-1.398 (11H, m), 0.990 (3H, d, J = 6.4 Hz), 0.915 (3H, d, J = 6.4 Hz) ^{13}C-NMR (100 MHz, $CDCl_3$): 169.2, 156.2, 138.9, 136.6, 128.6, 128.4, 128.2, 128.1, 127.7, 127.6, 73.5, 72.3, 68.3, 66.9, 58.4, 51.6, 43.5, 33.9, 32.9, 30.4, 29.3,

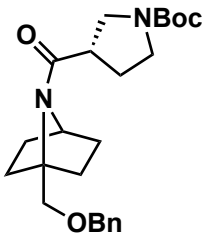
24.8, 23.4, 22.3 HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{28}H_{36}N_2NaO_4^+$, 487.2567. Found: 487.2566.



Boc-Phe-Abh(OBn) (1f)

To a solution of compound **10** (147 mg, 0.46 mmol) in CH_2Cl_2 (3 ml) was added TFA (2 ml) at 0°C. The solution was stirred for 40 min at room temperature. The solution was washed with 10% aqueous solution of Na_2CO_3 (20 ml), and the aqueous solution was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The crude amine was obtained and was used to the next step directly. To a solution of crude amine in DMF (5 ml), Boc-L-Phe (148 mg, 0.56 mmol), DIC (0.143 ml, 0.93 mmol), HOBT (125 mg, 0.93 mmol) and DIPEA (0.127 ml, 1.39 mmol) were added. The mixture was stirred overnight at room temperature. The reaction was quenched by adding water (10 ml) and then extracted with CH_2Cl_2. The organic phase was washed with 5% aqueous solution of citric acid and saturated aqueous solution of $NaHCO_3$, dried over Na_2SO_4 and evaporated the solvent. Column chromatography (hexane: EtOAc = 3:1) gave compound **Boc-Phe-Abh(OBn)** (160 mg, 75%) as a colorless oil.

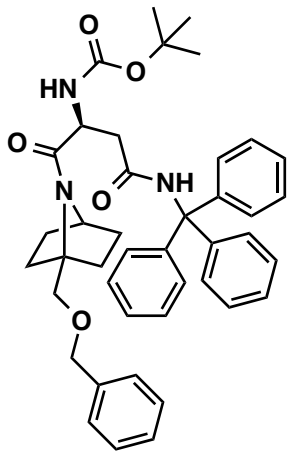
^{1}H-NMR (400 MHz, CD_3OD): 7.352-7.202 (10H, m), 4.579 (2H, s), 4.511 (1H, brs), 4.259 (1H, s), 4.137 (2H, brs), 2.939-2.826 (2H, m), 1.823-1.229 (8H, m), 1.400 (9H, s). ^{13}C-NMR (100 MHz, CD_3OD): 169.6, 157.3, 140.0, 138.4, 130.6, 129.4, 129.3, 128.7, 128.5, 127.8, 80.5, 74.3, 73.2, 69.4, 60.1, 55.6, 40.0, 34.4, 33.5, 30.8, 29.2, 28.7. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{28}H_{36}N_2NaO_4^+$, 487.2567. Found: 487.2562.



Boc-β-Pro-Abh(OBn) (1g)

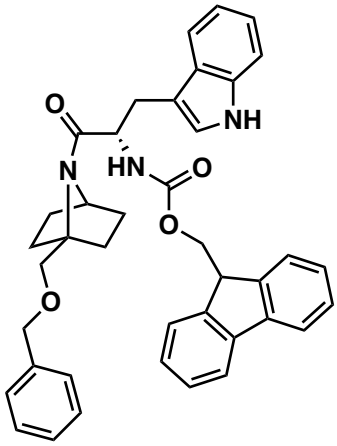
To a solution of compound **10** (80 mg, 0.25 mmol) in CH_2Cl_2 (3 ml) was added TFA (2 ml) at 0°C. The solution was stirred for 40 min at room temperature. The solution was washed with 10% aqueous solution of Na_2CO_3 (20 ml), and the organic phase was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The crude amine was obtained and was used to the next step directly. To a solution of crude amine in DMF (5 ml), Boc-β-proline (65 mg, 0.3 mmol), HOBT (68 mg, 0.5 mg) and DIPEA (0.69 μl, 0.75 mmol) were added. After the mixture was stirred for 5min, EDCI (96 mg, 0.5 mmol) was added. The mixture was stirred overnight at room temperature. The reaction was quenched by adding water (10 ml) and the whole was extracted with CH_2Cl_2. The organic phase was washed with 5% aqueous solution of citric acid and saturated aqueous solution of $NaHCO_3$,

dried over Na_2SO_4 and evaporated. Column chromatography (hexane: EtOAc = 3:1) gave compound **Boc-β-Pro-Abh(OBn)** (45 mg, 44%) as a colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 7.335-7.324 (5H, m), 4.607 (2H, s), 4.263 (1H, br s), 4.224 (2H, br s), 3.662-3.268 (4H, m), 3.064-3.044 (1H, m), 2.225-1.447 (19H, m, at 1.447 ppm (t-Bu group, 9H, s)) ^{13}C-NMR (100 MHz, $CDCl_3$): 169.5, 154.5, 138.8, 128.4, 127.7, 127.5, 79.4, 73.5, 68.0, 58.3, 48.8, 45.7, 45.5, 33.5, 29.9, 29.1, 28.6. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{24}H_{34}N_2NaO_4^+$, 437.2411. Found: 437.2410.



Boc-Asn(Trt)-Abh(OBn) (1h)
To a solution of **10** (107 mg, 0.34 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) were added Boc-Asn(Trt)-OH (240 mg, 0.51 mmol), CDMT (119 mg, 0.68 mmol), DMAP (5 mg, 0.04 mmol) and NMM (75 μl, 0.68 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/Acetone = 4/1) to afford **Boc-Asn(OBzl)-Abh(OBn)** (163 mg, 72%), as a colorless oil.

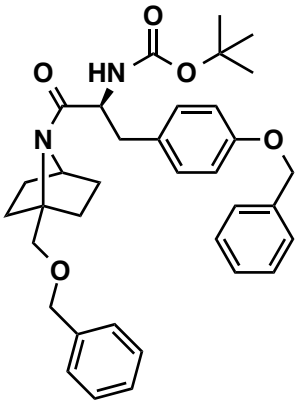
^{1}H NMR ($CDCl_3$, 400MHz) δ 7.414-7.204 (21H, m), 5.442 (1H, brd, J=6.8Hz) 4.685-4.668 (1H, m), 4.579 (2H, s), 4.341 (1H, s), 4.200-4.129 (2H, m), 2.720-2.575 (2H, m), 1.744-1.256 (17H, m, at 1.397 ppm (t-Bu group, 9H, s)), ^{13}C-NMR (100 MHz, $CDCl_3$): 168.8, 167.4, 155.2, 144.8, 138.9, 128.9, 128.7, 128.5, 128.3, 128.0, 127.6, 127.0, 80.2, 73.5, 72.3, 70.6, 68.6, 58.4, 50.3, 41.4, 33.5, 33.4, 29.8, 29.4, 28.4. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{42}H_{47}N_3NaO_5^+$, 696.3408. Found: 696.3408. HPLC (Hexane: EtOAc = 1:1, 265 nm): t_R 17.96 min, 98% purity.



Fmoc-Trp-Abh(OBn) (1i)

To a solution of **10** (108 mg, 0.34 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) were added Fmoc-Trp-OH (217 mg, 0.51 mmol), CDMT (119 mg, 0.68 mmol), DMAP (5 mg, 0.04 mmol) and NMM (56 μl, 0.51 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/Acetone = 4/1) to afford **Fmoc-Trp-Abh(OBn)** (173.1 mg, 79%), as a yellow solid.

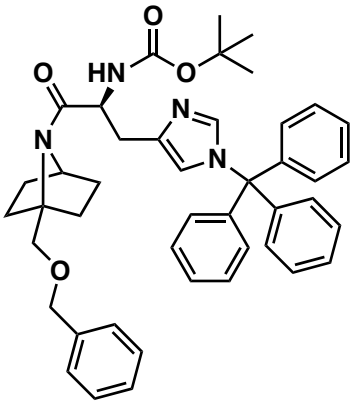
^{1}H-NMR (400 MHz, $CDCl_3$): 7.781-6.929 (20H, m), 4.633-4.595 (1H, m), 4.540 (2H, s), 4.385-4.294 (2H, m), 4.213-4.134 (2H, m), 4.055-3.996 (2H, m), 3.161-3.062 (2H, m), 1.752-1.287 (8H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.0, 158.1, 145.3, 142.6, 140.1, 138.0, 129.3, 128.8, 128.5, 128.2, 126.3, 126.2, 124.8, 124.5, 120.9, 120.0, 119.4, 112.3, 110.9, 74.4, 73.6, 70.6, 69.2, 67.9, 60.3, 56.0, 55.7, 34.4, 33.4, 29.5, 28.7. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{40}H_{39}N_3NaO_4^+$, 648.2833. Found: 648.2815. HPLC (Hexane: EtOAc = 1:1, 280 nm): t_R 24.56 min, 98% purity.



Boc-Tyr(OBzl)-Abh(OBn) (1j)

To a solution of **10** (108 mg, 0.34 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) were added Boc-Tyr(OBzl)-OH (189 mg, 0.51 mmol), CDMT (119 mg, 0.68 mmol), DMAP (5 mg, 0.04 mmol) and NMM (56 μl, 0.51 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/Acetone = 4/1) to afford **Boc-Tyr(OBzl)-Abh(OBn)** (116 mg, 60%), as a colorless oil.

^{1}H NMR (CD_3OD, 400MHz) δ 7.381-7.273 (10H, m), 7.081 (2H, d, J = 8.4 Hz), 6.820 (2H, d, J = 8.4 Hz), 5.035 (2H, s), 4.567 (2H, s) 4.432 (1H, s), 4.215 (1H, s), 4.139-4.085 (2H, m), 2.858-2.755 (2H, m), 1.815-1.093 (17H, m, at 1.402 ppm (t-Bu group, 9H, s)) ^{13}C-NMR (100 MHz, $CDCl_3$): 159.1, 157.3, 140.1, 138.9, 131.7, 130.5, 129.5, 129.3, 128.8, 128.8, 128.6, 128.4, 116.0, 80.5, 74.4, 70.8, 69.4, 60.2, 55.8, 39.1, 34.4, 33.5, 29.2, 28.7. HRMS (ESI^+): Calcd. for $C_{35}H_{42}N_2NaO_5^+$ ($[M+Na]^+$): 593.2986. Found: 593.2994. HPLC (Hexane: EtOAc = 1:1, 265 nm): t_R 14.24 min, 97% purity.

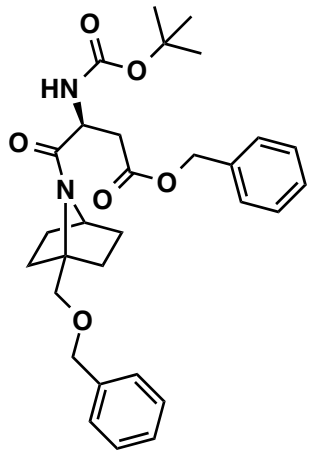


Boc-His-Abh(OBn) (1k)

To a solution of **10** (90 mg, 0.28 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) were added Boc-His(Trt)-OH (169 mg, 0.34 mmol), CDMT (98 mg, 0.56 mmol), DMAP (4 mg, 0.03 mmol) and NMM (92 μl, 0.84 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was

dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/Acetone = 4/1) to afford **Boc-His(Trt)-Abh(OBn)** (65 mg, 33%), as white powder.

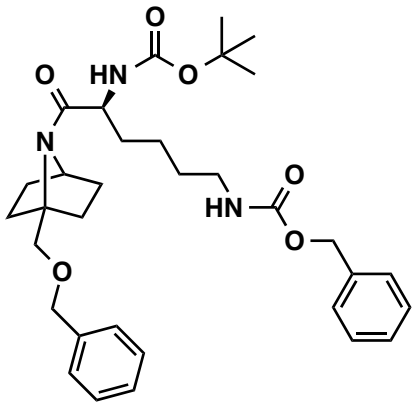
^{1}H-NMR (400 MHz, $CDCl_3$): 7.431-7.079 (22H, m), 5.439 (1H, d, J=8.8Hz), 4.717-4.662 (1H, m), 4.573 (2H, s), 4.501 (1H, m), 4.247-4.123 (2H, m), 3.006-2.763 (2H, m), 1.763-1.390 (8H, m), 1.360 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): 167.9, 155.2, 142.0, 138.9, 129.9, 128.4, 128.3, 128.1, 128.0, 127.6, 127.5, 127.4, 119.7, 79.5, 77.4, 73.4, 68.4, 58.5, 52.4, 34.0, 33.1, 30.2, 29.4, 28.5. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{44}H_{48}N_4NaO_4^+$, 719.3568. Found: 719.3560. Reverse-phase HPLC (CH_3CN, 265 nm): t_R 9.49 min, 93% purity.



Boc-Asp(OBzl)-Abh(OBn) (1l)

To a solution of **10** (65 mg, 0.20 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) were added Boc-Asp(OBzl)-OH (97 mg, 0.30 mmol), CDMT (70 mg, 0.40 mmol), DMAP (3 mg, 0.02 mmol) and NMM (33 μl, 0.30 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/Acetone = 4/1) to afford **Boc-Asp(OBzl)-Abh(OBn)** (72 mg, 69%), as a colorless oil.

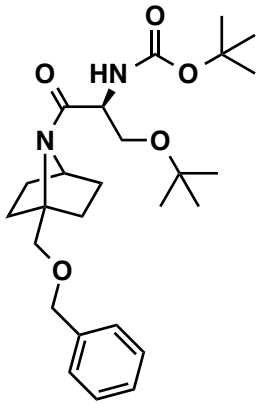
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.337-7.240 (10H, m), 5.388 (1H, brd, J=9.6Hz), 5.138-5.059 (2H, s), 4.816-4.761 (1H, m), 4.599 (2H, s), 4.501 (1H, s), 4.235-4.154 (2H, m), 2.813 (1H, dd, J=15.6, 7.0Hz), 2.619 (1H, dd, J=14.4, 6Hz), 1.808-1.422 (17H, m, at 1.422 ppm (t-Bu group, 9H, s)). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.7, 167.0, 155.0, 138.9, 135.8, 128.6, 128.4, 128.4, 128.4, 127.6, 127.6, 80.1, 73.4, 72.2, 68.5, 66.7, 58.5, 49.7, 38.0, 33.6, 33.3, 29.9, 29.4, 28.4. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{30}H_{38}N_2NaO_6^+$, 545.2622. Found: 545.2626. HPLC (Hexane: EtOAc = 1:1, 265 nm): t_R 13.45 min, 98% purity.



Boc-Lys(Cbz)-Abh(OBn) (1m)

To a solution of **10** (115 mg, 0.36 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) were added Boc-Lys(Cbz)-OH (205 mg, 0.54 mmol), CDMT (126 mg, 0.72 mmol), DMAP (5 mg, 0.04 mmol) and NMM (59 μl, 0.54 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/Acetone = 4/1) to afford **Boc-Lys(Cbz)-Abh(OBn)** (112 mg, 54%), as a colorless oil.

Colorless oil.^{1}H NMR (CD_3OD, 400MHz) δ 7.340-7.248 (10H, m), 5.052 (2H, s), 4.572 (2H, s), 4.471 (1H, s), 4.288-4.273 (1H, m) 4.165 (2H, brs), 3.085 (2H, brs), 1.866-1.263 (23H, m, at 1.423 ppm (t-Bu group, 9H, s)), ^{13}C-NMR (100 MHz, $CDCl_3$): 173.6, 170.4, 157.6, 139.9, 138.6, 130.4, 129.4, 129.3, 128.8, 128.6, 127.7, 80.7, 74.4, 73.0, 69.7, 59.7, 57.2, 39.1, 34.4, 33.9, 30.6, 30.0, 28.6, 18.7. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{33}H_{45}N_3NaO_6^+$, 602.3201. Found: 602.3226. HPLC (Hexane: EtOAc = 1:1, 265 nm): t_R 24.68 min, 94% purity.

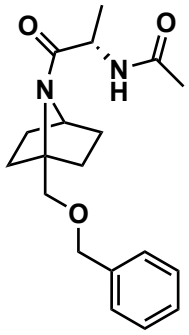


Boc-Ser(t-Bu)-Abh(OBn) (1n)

To a solution of **10** (110 mg, 0.35 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred

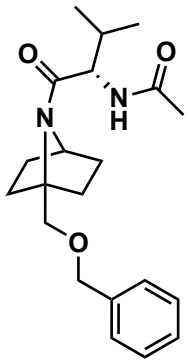
for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) were added Boc-Ser-(t-Bu)-OH (138 mg, 0.53 mmol), CDMT (123 mg, 0.70 mmol), DMAP (5 mg, 0.04 mmol) and NMM (58 µl, 0.53 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/Acetone = 4/1) to afford **Boc-Ser(t-Bu)-Abh(OBn)** (44 mg, 28%), as a colorless oil.

^{1}H NMR (CD_3OD, 400 MHz) δ 7.484-7.233 (5H, m), 4.586 (2H, s), 4.503 (1H, s), 4.435-4.420 (1H, m), 4.218-4.158 (2H, m), 3.529-3.430 (2H, m), 1.902-1.149 (17H, m, at 1.432 ppm (t-Bu, 9H, s)), 1.149 (t-Bu, 9H, s) ^{13}C-NMR (100 MHz, $CDCl_3$): 169.2, 157.5, 140.0, 129.3, 128.8, 128.6, 80.6, 74.6, 74.4, 73.1, 69.7, 59.9, 54.5, 34.5, 33.9, 30.5, 30.0, 28.7,27.7. HRMS (ESI^+): Calcd. for $C_{26}H_{40}N_2NaO_5^+$ ($[M+Na]^+$): 483.2829. Found: 483.2827. HPLC (Hexane: EtOAc = 1:1, 265 nm): t_R 13.76 min, 98% purity.



Ac-Ala-Abh(OBn) (2b)

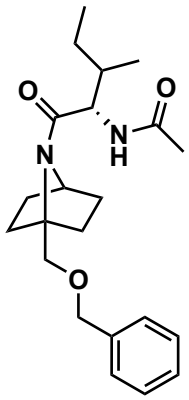
To a solution of Boc-Ala-Abh (OBn) (30 mg, 0.08mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) was added Ac_2O (38µl, 0.4mmol) and Et_3N (56 µl, 0.4mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for overnight. Saturated aqueous solution of $NaHCO_3$ was added, extracted with EtOAc (3 times), and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/EtOAc = 1/2) to afford **Ac-Ala-Abh (2b)** (26 mg, 98%) as a colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 7.345-7.260 (5H, m), 6.534 (1H, brs), 4.660-4.625 (3H, m), 4.306 (1H, s), 4.195 (2H, s), 1.973 (3H, s), 1.825-1.526 (8H, m), 1.321 (3H, d, J=6.4Hz).^{13}C-NMR (100 MHz, $CDCl_3$): 169.3, 168.7, 138.7, 128.5, 127.7, 127.6, 73.5, 72.0, 68.5, 58.1, 47.4, 33.8, 33.1, 30.0, 29.3, 23.5, 20.0. HRMS (ESI^+): Calcd. for $C_{19}H_{26}N_2NaO_3^+$ ($[M+Na]^+$): 353.1836. Found: 353.1835. HPLC (Hexane: iPrOH = 1:2, 265 nm): t_R, 14.68 min, 99% purity.



Ac-Val-Abh(OBn) (2c)

To a solution of Boc-Val-Abh (OBn) (42 mg, 0.10 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (2 mL) was added Ac_2O (62 µl, 0.50 mmol) and Et_3N (42 µl, 0.30mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for overnight. Saturated aqueous solution of $NaHCO_3$ was added, extracted with EtOAc (3 times), and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/EtOAc = 1/2) to afford **Ac-Val-Abh(OBn) (2c)** (30 mg, 83%), as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.338-7.244 (5H, m), 6.193 (1H, brd, J=9.2Hz), 4.651-4.563 (3H, m), 4.383 (1H, s), 4.258-4.189 (2H, m), 2.001 (3H, s), 1.968-1.919 (1H, m) 1.902-1.496 (8H, m), 0.957-0.9869 (6H, dd, J=6.8Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.0, 168.3, 138.8, 128.4, 127.6, 127.6, 73.5, 68.4, 56.0, 34.0, 32.6, 32.2, 30.6, 29.1, 23.6, 19.7, 17.6. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{21}H_{30}N_2NaO_3^+$, 381.2149. Found: 381.2153. Reverse-phase HPLC (CH_3CN, 265 nm): t_R 4.82 min, 97% purity.

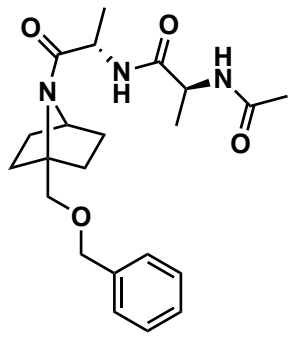


Ac-Ile-Abh(OBn) (2d)

To a solution of Boc-Ile-Abh (OBn) (38 mg, 0.09 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (2 mL) was added Ac_2O (56 µl, 0.45 mmol) and Et_3N (38 µl, 0.27 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature,

and stirred for overnight. Saturated aqueous solution of $NaHCO_3$ was added, extracted with EtOAc (3 times), and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/EtOAc = 1/2) to afford **Ac-Ile-Abh(OBn) (2d)** (21mg, 64%), as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.338-7.7.275 (5H, m), 6.117 (1H, brd, J=4.2 Hz), 4.652-4.579 (3H, m), 4.405 (1H, brs), 4.242-4.184 (2H, m), 1.992 (3H, s), 1.840-1.426 (8H, m), 0.939-0.836 (9H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): 169.9, 168.4, 138.9, 128.4, 127.6, 127.6, 73.5, 72.4, 68.3, 59.0, 55.8, 38.6, 32.7, 29.2, 24.4, 23.6, 19.1, 17.4, 15.6, 11.7. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{22}H_{32}N_2NaO_3^+$, 395.2305. Found: 395.2305. Reverse-phase HPLC (CH_3CN, 265 nm): t_R 4.65 min, 100% purity.



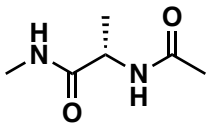
Ac-Ala-Ala-Abh(OBn) (4b)

To a solution of Boc-Ala-Abh(OBn) (35 mg, 0.09 mmol) in CH_2Cl_2 (2 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (2 mL) were added Boc-Ala (26 mg, 0.14 mmol), CDMT (32 mg, 0.18 mmol), DMAP (3mg, 0.1eq) and NMM (30 µl, 0.27 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/EtOAc = 1/1) to afford Boc-Ala-Ala-Abh (39 mg, 94%), as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$): 7.343-7.269 (5H, m), 6.905 (1H, brd, J=6.0Hz), 5.016 (1H, brs), 4.619 (3H, s), 4.294 (1H, m), 4.198-4.127 (3H, m), 1.825-1.544 (8H, s), 1.434 (9H, s), 1.352-1.314 (6H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): 171.7, 168.3, 155.3, 138.8, 128.5, 127.7, 127.6, 80.1, 73.5, 72.1, 68.5, 58.1, 50.4, 47.4, 33.9, 33.1, 30.0, 29.4, 28.5, 19.7, 19.0. HRMS (ESI^+): Calcd. for $C_{25}H_{38}N_3O_5^+$ ($[M+H]^+$): 460.2806. Found: 460.2807.

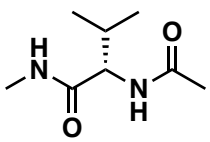
To a solution of Boc-Ala-Ala-Abh (OBn) (39 mg, 0.08mmol) in CH_2Cl_2 (2 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (5 mL) was added Ac_2O (38µl, 0.4mmol) and Et_3N (111 µl,

0.8 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for overnight. Saturated aqueous solution of $NaHCO_3$ was added, extracted with EtOAc (3 times), and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with HPLC to afford **Ac-Ala-Ala-Abh** (24mg, 71%), as a colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): 7.344-7.271 (5H, m), 6.854 (1H, brd, J=6.8Hz), 6.185 (1H, brd, J=6.8Hz), 4.621-4.569 (3H, m), 4.480-4.410 (1H, m), 4.285 (1H, s), 4.202 (2H, s), 2.002 (3H, s), 1.825-1.528 (8H, m), 1.366-1.322 (6H, m).^{13}C-NMR (100 MHz, $CDCl_3$): 171.4, 169.7, 168.0, 138.7, 128.5, 127.7, 127.7, 73.5, 72.0, 68.5, 58.1, 49.1, 47.6, 33.9, 33.0, 30.0, 29.4, 23.4, 19.7, 19.2. HRMS (ESI$^+$): Calcd. for $C_{22}H_{31}N_3NaO_4^+$ ([M+Na]$^+$): 424.2207. Found: 424.2209. HPLC (iPrOH/Hexane=6:1, 265 nm): t_R 25.52 min, 91% purity.



Ac-Ala-NHMe (3b)[50]
To a solution of Boc-Ala-OH (300 mg, 1.6 mmol) in CH_2Cl_2 (8 mL) was added $MeNH_2$·HCl (130 mg, 1.92 mmol), EDCI.HCl (613 mg, 3.2 mmol) and Et_3N (669 µl, 4.8 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for overnight. Saturated aqueous solution of $NaHCO_3$ was added, extracted with EtOAc (3 times), and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/EtOAc = 1/2) to afford Boc-Ala-NHMe (160 mg, 50%), as white powder.

To a solution of Boc-Ala-NHMe (59 mg, 0.29 mmol) in CH_2Cl_2 (2 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (3 mL) was added Ac_2O (181µl, 1.45mmol) and Et_3N (404 µl, 2.9 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for overnight. The solution was concentrated, and the residue was purified with column chromatography ($CHCl_3$/MeOH = 9/1) to afford **Ac-Ala-NHMe (3b)** (38mg, 90%), as white powder.
^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.823 (1H, brs), 6.716 (1H, brd), 4.530-4.458 (1H, m), 2.785 (3H, s), 1.995-1.982 (3H, m), 1.348 (3H, d, J=6.8Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): 173.3, 170.4, 48.9, 26.3, 23.2, 18.5. HRMS (ESI, [M+Na]$^+$): Calcd. for $C_6H_{12}N_2NaO_2^+$, 167.0791. Found: 167.0790. Reverse-phase HPLC (CH_3CN, 215 nm): t_R 7.56 min, 93% purity.

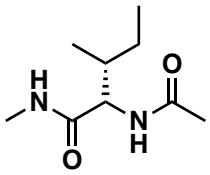


Ac-Val-NHMe (3c) [50]
To a solution of Boc-Val-OH (500 mg, 2.3 mmol) in CH_2Cl_2 (10 mL) were added $MeNH_2$·HCl (236 mg, 3.5 mmol), CDMT (808 mg, 4.6 mmol), DMAP (28 mg, 0.2 mmol) and NMM (0.76 mL, 6.9 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 12 h. The reaction mixture was diluted

with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated and affords Boc-Val-NHMe (525 mg, 99%), as white powder, which was used in the next step without further purification.

To a solution of Boc-Val-NHMe (100 mg, 0.43 mmol) in CH_2Cl_2 (2 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (3 mL) was added Ac_2O (268 µl, 2.2 mmol) and Et_3N (599 µl, 4.3 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for overnight. The solution was concentrated, and the residue was purified with column chromatography ($CHCl_3$/MeOH = 9/1) to afford **Ac-Val-NHMe (3c)** (63 mg, 85%), as white powder.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.030 (1H, brd, J=8.4Hz), 5.770 (1H, brs), 4.147-4.107 (1H, m), 2.827 (3H, d), 2.085-2.009 (4H, m), 0.954-0.933 (6H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.0, 170.3, 59.0, 31.1, 26.3, 23.4, 19.4, 18.6. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_8H_{16}N_2NaO_2^+$, 195.1104. Found: 195.1102. HPLC (iPrOH, 215 nm): t_R 19.30 min, 96% purity.



Ac-Ile-NHMe (3d) [50]

To a solution of Boc-Ile-OH (200 mg, 0.86 mmol) in CH_2Cl_2 (8 mL) were added $MeNH_2$·HCl (88 mg, 1.3 mmol), CDMT (298 mg, 1.7 mmol), DMAP (11 mg, 0.09 mmol) and NMM (286 µL, 2.6 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 12 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated and affords Boc-Ile-NHMe (178 mg, 84%), as white powder, which was used in the next step without further purification.

To a solution of Boc-Ile-NHMe (70 mg, 0.29 mmol) in CH_2Cl_2 (2 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (3 mL) was added Ac_2O (181 µl, 1.45 mmol) and Et_3N (121 µl, 0.87 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for overnight. The solution was concentrated, and the residue was purified with column chromatography ($CHCl_3$/MeOH = 9/1) to afford **Ac-Ile-NHMe (3d)** (36mg, 67%), as white powder.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.115 (1H, brd, J=8.4Hz), 6.043 (1H, brs), 4.315-4.171 (1H, m), 2.814 (3H, d), 2.009 (3H, s), 1.857-1.789 (1H, m), 1.565-1.486 (1H, m), 1.161-1.091 (1H, m), 0.920-0.883 (6H, m).^{13}C-NMR (100 MHz, $CDCl_3$): 172.1, 170.3, 58.0, 37.3, 26.3, 25.2, 23.4, 15.5, 11.. HRMS (ESI, $[M+Na]^+$): Calcd. for

$C_9H_{18}N_2NaO_2^+$, 209.1260. Found: 209.1264. Reverse-phase HPLC (CH_3CN, 215 nm): t_R 4.86 min, 99% purity.

NMR measurements

Dry samples were dissolved in organic solvents and aqueous solvents and the final concentrations were 1-2 mg in 700µl NMR solvents. In the cases of aqueous solvents, the compounds were dissolved in either 90% H_2O/10% D_2O (in the cases of Ac-X-NHMe) or 90% H_2O/10% CD_3OD (in the cases of Ac-X-Abh) (X=Ala, Ile or Val), and the pH was adjusted to 2-3 with citric acid.

NMR experiments were performed on a Bruker Avance 400MHz NMR spectrometer. The temperatures were calibrated with ethylene glycol and methanol as references by using a standard method.[51] The NMR data were processed with Bruker TopSpin 2.1. The $^3J(H_N,H_\alpha)$ coupling constants were determined with the amide proton doublets by deconvolution of the peaks by fitting a Lorentz function to the peaks or by iterative matching of simulated spectra with the experimental spectra with the Dynamic NMR(DNMR) program. About Lorentz fitting, a representative fit for Ac-Ala-NHMe (**3b**) in DMSO at 298K is shown in Figure S14. This process was repeated for samples: Ac-Ala-Abh(OBn) (**2b**) in DMSO, Ac-Val-NHMe (**3c**) in $CDCl_3$, Ac-Val-Abh(OBn) (**2c**) in $CDCl_3$, Ac-Ile-NHMe (**3d**) in $CDCl_3$, Ac-Ile-Abh(OBn) (**2d**) in $CDCl_3$ and Ac-Ala-Ala-Abh(OBn) (**4b**) in $CDCl_3$. A representative DNMR fit for Ac-Ala-NHMe in H_2O at 298K is shown in Figure S15. This method was also used for samples: Ac-Ala-Abh(OBn) in H_2O, Ac-Ile-NHMe in H_2O, and Ac-Ile-Abh(OBn) in H_2O. NOESY spectra were obtained with a mixing time of 300 ms.

Raman spectroscopic measurements

0.7 mg of sample powder was dissolved in 40 µl hexane. A sample in dried film was prepared as follows: about 0.01 mL of hexane solution was spread in a Low E microscope Slide (Czitek) and spontaneously evaporated at room temperature. Raman spectra were collected at room temperature (25 ^oC) with the Raman microscope (Kaiser HoloLab 5000 of Kaiser Optical System Inc.) using 785 nm semiconductor laser (12 mW at the sample surface), holographic transmission grating, and charge-coupled-device (CCD). The spectral resolution in the present system is approximately 4.8 cm^{-1}. We obtained the Raman spectrum for the spot (about 2 mm in diameter) with two accumulations of 30 seconds each. The Raman skeletal vibrations were fitted by the sum of bands with Guassian shape. Guassian curve fitting was performed with the software named GRAMS.

Metadynamics Simulations

Metadynamics calculations were performed with Desmond using OPSL3 force field (Schrodinger Inc., U.S.A.). The simulation conditions are as follows: Temperature=300.0 K, Pressure=1.01325 bar, Ensemble=NPT, Solvent=DMSO or $CHCl_3$.

QTAIM Calculations

Bond path analysis with Atoms in molecular theory was performed at TZP level with ADF (SCM, Netherlands). The structures were optimized with M062X/6-31G(d) in bulk solvation effects (self-consistent reaction field, SCRF) simulated by the SMD

method in DMSO (**2b** and **3b**) and chloroform (**2c** and **2d**) with the Gaussian 09 suites of programs (Gaussian, Inc., U. S. A.).[52]

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

NMR spectra, additional data of synthesis, HPLC chromatograms of compounds, synthesis scheme of the key intermediate **10**, and calculation details (PDF).

Acknowledgements

The computations were performed at the Research Center for Computational Science, Okazaki, Japan. Some computations were performed as part of the "High Performance Computing Infrastructure (HPCI)" project (hp150177 and hp170146), and some were done on the TSUBAME2.5 and TSUBAME3.0 supercomputers at Tokyo Institute of Technology as a part of the TSUBAME encouragement program for female users (16IJ0003). This work was supported by JSPS KAKENHI Grant Numbers JP 26104508 and 16K08157 (YO) and 26293002 and 18H02552 (TO).

Competing interests

There is no conflict of interest.

References

(1) Hill, T. A.; Shepherd, N. E.; Diness, F.; Fairlie, D. P. Constraining Cyclic Peptides to Mimic Protein Structure Motifs. *Angew. Chem. Int. Ed.* **2014**, *53*, 13020–13041.

(2) Nowick, J. S. Chemical Models of Protein β-Sheets. *Acc. Chem. Res.* **1999**, *32*, 287–296.

(3) Moriuchi, T.; Hirao, T. Highly Ordered Structures of Peptides by Using Molecular Scaffolds. *Chem. Soc. Rev.* **2004**, *33*, 294–301.

(4) Nowick, J. S. Exploring Beta-Sheet Structure and Interactions with Chemical Model Systems. *Acc. Chem. Res.* **2008**, *41*, 1319–1330.

(5) Cheng, P. N.; Pham, J. D.; Nowick, J. S. The Supramolecular Chemistry of β-Sheets. *J. Am. Chem. Soc.* **2013**, *135*, 5477–5492.

(6) Díaz, H.; Espina, J. R.; Kelly, J. W. A Dibenzofuran-Based Amino Acid Designed To Nucleate Antiparallel β-Sheet Structure: Evidence for Intramolecular Hydrogen-Bond Formation. *J. Am. Chem. Soc.* **1992**, *114*, 8316–8318.

(7) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W. Hydrophobic Cluster Formation Is Necessary for Dibenzofuran-Based Amino Acids to Function as β-Sheet Nucleators. *J. Am. Chem. Soc.* **1994**, *116*, 3988–4005.

(8) Nowick, J. S.; Powell, N. A.; Martinez, E. J.; Smith, E. M.; Noronha, G. Molecular Scaffolds. I. Intramolecular Hydrogen Bonding in a Family of Di- and Triureas. *J. Org. Chem.* **1992**, *57*, 3763–3765.

(9) Nowick, J. S.; Abdi, M.; Bellamo, K. A.; Love, J. A.; Martinez, E. J.; Noronha, G.; Smith, E. M.; Ziller, J. W. Molecular Scaffolds. 2. Intramolecular Hydrogen Bonding in 1,2-Diaminoethane Diureas. *J. Am. Chem. Soc.* **1995**, *117*, 89–99.

(10) Nowick, J. S.; Smith, E. M.; Noronha, G. Molecular Scaffolds. 3. An Artificial Parallel β-Sheet. *J. Org. Chem.* **1995**, *60*, 7386–7387.

(11) Schneider, J. P.; Kelly, J. W. Synthesis and Efficacy of Square Planar Copper Complexes Designed to Nucleate /3-Sheet Structure. *J. Am. Chem. Soc.* **1995**, *117*, 2533–2546.

(12) Barooah, N.; Sarma, R. J.; Baruah, J. B. Metal Directed Assemblies of a Dipeptide: Formation of β-Pleated Sheets. *Eur. J. Inorg. Chem.* **2006**, *9471*, 2942–2946.

(13) Ogawa, M. Y.; Gretchikhine, A. B.; Soni, S.-D.; Davis, S. M. Synthesis and Characterization of a Water Soluble Metallopeptide That Resembles a Parallel .Beta.-Pleated Sheet. *Inorg. Chem.* **1995**, *34*, 6423–6424.

(14) Chowdhury, S.; Schatte, G.; Kraatz, H. B. How Useful Is Ferrocene as a Scaffold for the Design of β-Sheet Foldamers? *Angew. Chem. Int. Ed.* **2008**, *47*, 7056–7059.

(15) Kruppa, M.; Bonauer, C.; Michlová, V.; König, B. Enhanced Peptide β-Sheet Affinity by Metal to Ligand Coordination. *J. Org. Chem.* **2005**, *70*, 5305–5308.

(16) Laungani, A. C.; Slattery, J. M.; Krossing, I.; Breit, B. Supramolecular Bidentate Ligands by Metal-Directed in Situ Formation of Antiparallel β-Sheet Structures and Application in Asymmetric Catalysis. *Chem. Eur. J.* **2008**, *14*, 4488–4502.

(17) Phillips, S. T.; Rezac, M.; Abel, U.; Kossenjans, M.; Bartlett, P. A. "@-Tides": The 1,2-Dihydro-3(6h)-Pyridinone Unit as a β-Strand Mimic. *J. Am. Chem. Soc.* **2002**, *124*, 58–66.

(18) Wyrembak, P. N.; Hamilton, A. D. Alkyne-Linked 2,2-Disubstituted-Indolin-3-One Oligomers as Extended β-Strand Mimetics. *J. Am. Chem. Soc.* **2009**, *131*, 4566–4567.

(19) Sutherell, C. L.; Thompson, S.; Scott, R. T. W.; Hamilton, A. D. Aryl-Linked Imidazolidin-2-Ones as Non-Peptidic β-Strand Mimetics. *Chem. Commun.* **2012**, *48*, 9834.

(20) Xin, D.; Perez, L. M.; Ioerger, T. R.; Burgess, K. A Multifaceted Secondary

Structure Mimic Based on Piperidine-Piperidinones. *Angew. Chem. Int. Ed.* **2014**, *53*, 3594–3598.

(21) Jouanne, M.; Voisin-Chiret, A. S.; Legay, R.; Coufourier, S.; Rault, S.; Sopkova-de Oliveira Santos, J. β-Strand Mimicry: Exploring Oligothienylpyridine Foldamers. *European J. Org. Chem.* **2016**, *2016*, 5686–5696.

(22) Glenn, M. P.; Pattenden, L. K.; Reid, R. C.; Tyssen, D. P.; Tyndall, J. D. A.; Birch, C. J.; Fairlie, D. P. β-Strand Mimicking Macrocyclic Amino Acids: Templates for Protease Inhibitors with Antiviral Activity. *J. Med. Chem.* **2002**, *45*, 371–381.

(23) Nowick, J. S.; Holmes, D. L.; Mackin, G.; Noronha, G.; Shaka, A. J.; Smith, E. M. An Artificial β-Sheet Comprising a Molecular Scaffold, a β-Strand Mimic, and a Peptide Strand. *J. Am. Chem. Soc.* **1996**, *118*, 2764–2765.

(24) Zeng, H.; Yang, X.; Flowers, R. A.; Gong, B. A Noncovalent Approach to Antiparallel β-Sheet Formation. *J. Am. Chem. Soc.* **2002**, *124*, 2903–2910.

(25) Khasanova,T.V.; Khakshoor, O.; Nowick, J. S. Functionalized Analogues of an Unnatural Amino Acid that Mimic a Tripeptide β-Strand. *Org. Lett.* **2008**, *10*, 5293-5296.

(26) Rzepecki, P.; Schrader, T. β-Sheet Ligands in Action: KLVFF Recognition by Aminopyrazole Hybrid Receptors in Water. *J. Am. Chem. Soc.* **2005**, *127*, 3016–3025.

(27) Bag, S. S.; Jana, S.; Yashmeen, A.; De, S. Triazolo-β-Aza-ε-Amino Acid and Its Aromatic Analogue as Novel Scaffolds for β-Turn Peptidomimetics. *Chem. Commun.* **2015**, *51*, 5242–5245.

(28) Xu, Y.; Correia, I.; Ha-Duong, T.; Kihal, N.; Soulier, J. L.; Kaffy, J.; Crousse,

B.; Lequin, O.; Ongeri, S. The Use of 4,4,4-Trifluorothreonine to Stabilize Extended Peptide Structures and Mimic β-Strands. *Beilstein J. Org. Chem.* **2017**, *13*, 2842–2853.

(29) Otani, Y.; Nagae, O.; Naruse, Y.; Inagaki, S.; Ohno, M.; Yamaguchi, K.; Yamamoto, G.; Uchiyama, M.; Ohwada, T. An Evaluation of Amide Group Planarity in 7-Azabicyclo[2.2.1]Heptane Amides. Low Amide Bond Rotation Barrier in Solution. *J. Am. Chem. Soc.* **2003**, *125*, 15191–15199.

(30) Hosoya, M.; Otani, Y.; Kawahata, M.; Yamaguchi, K.; Ohwada, T. Water-Stable Helical Structure of Tertiary Amides of Bicyclic β -Amino Acid Bearing 7-Azabicyclo[2.2.1]Heptane. Full Control of Amide Cis-Trans Equilibrium by Bridgehead Substitution. *J. Am. Chem. Soc.* **2010**, *132*, 14780–14789.

(31) Grdadolnik, J.; Grdadolnik, S. G.; Avbelj, F. Determination of Conformational Preferences of Dipeptides Using Vibrational Spectroscopy. *J. Phys. Chem. B* **2008**, *112*, 2712–2718.

(32) Beck, D. A. C.; Alonso, D. O. V.; Inoyama, D.; Daggett, V. The Intrinsic Conformational Propensities of the 20 Naturally Occurring Amino Acids and Reflection of These Propensities in Proteins. *Proc. Natl. Acad. Sci.* **2008**, *105*, 12259–12264.

(33) Montalbetti, C. A. G. N.; Falque, V. Amide Bond Formation and Peptide Coupling. *Tetrahedron* **2005**, *61*, 10827–10852.

(34) Wang, S.; Taniguchi, T.; Monde, K.; Kawahata, M.; Yamaguchi, K.; Otani, Y.; Ohwada, T. Hydrogen Bonding to Carbonyl Oxygen of Nitrogen-Pyramidalized Amide – Detection of Pyramidalization Direction Preference by Vibrational Circular Dichroism Spectroscopy. *Chem. Commun.* **2016**, *52*,

4018–4021.

(35) Bernet, B.; Vasella, A. 1H-NMR Analysis of Intra- and Intermolecular H-Bonds of Alcohols in DMSO: Chemical Shift of Hydroxy Groups and Aspects of Conformational Analysis of Selected Monosaccharides, Inositols, and Ginkgolides. *Helv. Chim. Acta* **2000**, *83*, 995–1021.

(36) Cierpicki, T.; Otlewski, J. Amide Proton Temperature Coefficients as Hydrogen Bond Indicators in Proteins. *J. Biomol. NMR* **2001**, *21*, 249–261.

(37) Toal, S.; Amidi, O.; Schweitzer-Stenner, R. Conformational Changes of Trialanine Induced by Direct Interactions between Alanine Residues and Alcohols in Binary Mixtures of Water with Glycerol and Ethanol. *J. Am. Chem. Soc.* **2011**, *133*, 12728–12739.

(38) Shi, Z.; Olson, C. A.; Rose, G. D.; Baldwin, R. L.; Kallenbach, N. R. Polyproline II Structure in a Sequence of Seven Alanine Residues. *Proc. Natl. Acad. Sci.* **2002**, *99*, 9190–9195.

(39) Bundi, A.; Wüthrich, K. 1H‐NMR Parameters of the Common Amino Acid Residues Measured in Aqueous Solutions of the Linear Tetrapeptides H‐Gly‐Gly‐X‐L‐Ala‐OH. *Biopolymers* **1979**, *18*, 285–297.

(40) Vuister, G. W.; Bax, A. Quantitative J Correlation - a New Approach for Measuring Homonuclear 3-Bond J(H(N)H(Alpha) Coupling-Constants in N-15-Enriched Proteins. *J. Am. Chem. Soc.***1993**, *115*, 7772–7777.

(41) Avbelj, F.; Grdadolnik, S. G.; Grdadolnik, J.; Baldwin, R. L. Intrinsic Backbone Preferences Are Fully Present in Blocked Amino Acids. *Proc. Natl. Acad. Sci.* **2006**, *103*, 1272–1277.

(42) Grdadolnik, J.; Mohacek-Grosev, V.; Baldwin, R. L.; Avbelj, F. Populations of the Three Major Backbone Conformations in 19 Amino Acid Dipeptides. *Proc.*

Natl. Acad. Sci. **2011**, *108*, 1794–1798.

(43) Madison, V.; Kopple, K. D. Solvent-dependent conformational distributions of some dipeptides. *J. Am. Chem. Soc.* **1980**, *102*, 4855–4863.

(44) Ensing, B.; De Vivo, M.; Liu, Z.; Moore, P.; Klein, M. L. Metadynamics as a Tool for Exploring Free Energy Landscapes of Chemical Reactions. *Acc. Chem. Res.* **2006**, *39*, 73–81.

(45) Bader, R. F. W. *Atoms in Molecules: A Quantum Theory*. **1990**, International Series of Monographs on Chemistry 22 (Oxford Science Publications: Oxford).

(46) Newberry, R. W.; Raines, R. T. A prevalent interaresidue hydrogen bond stabilized proteins. *Nature, Chem. Biol.* **2016**, *12*, 1084-1089.

(47) Matta, C. F.; Gernandez-Trujillo, J.; Tang, T.-H.; Bader, R. F. W. Hydrogen-Hydrogen Bonding: A Stabilizing Intersctions in Molecules and Crystals. *Chem. Eur. J.* **2003**, *9*, 1940-1951.

(48) Wick, C. R.; Clark, T. On bond-critical points in QTAIM and weak interactions. *J Mol Modeling*, **2018**, *24*, 142.

(49) Pendas, A. M.; Francisco, E.; Blanco, M. A.; Gatti, C. Bond Paths as Privileged Exchange Channels, *Chem. Eur. J.* **2007**, *13*, 9362-9371.

(50) Sijpkes, A. H.; Somsen, G. Enthalpic Interaction Coefficients of some Dipeptides Dissolved in N,N-Dimethylformamide. *J. Chem.* Soc., *Faraday Trans. I*, **1989**, *85* (8)**,** 2563-2573.

(51) Van Geet, A. L. Calibration of Methanol Nuclear Magnetic Resonance Thermometer at Low Temperature. *Anal. Chem.* **1970**, *42* (6), 679–680.

(52) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino,

J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, revision D.01; Gaussian, Inc.: Wallingford, CT, **2013**.