(*c* 1.10, MeOH); ^{1}H NMR (CD_3OD) δ 3.38 (dd, 1H, J_{3-4} =J_{4-5}=9.4 Hz, H-4), 3.53 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}=10.0 Hz, H-2), 3.60 (ddd, 1H, J_{4-5}=9.4 Hz, J_{5-6a}=5.0 Hz, J_{5-6b} =2.4 Hz, H-5), 3.66 (dd, 1H, J_{5-6a}=5.0 Hz, J_{6a-6b}=11.8 Hz, H-6a), 3.72 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.80 (dd, 1H, J_{2-3}=J_{3-4}=9.4 Hz, H-3), 5.39 (d, 1H, J=3.6 Hz, H-1), 6.99 (d, 2H, J=9.2 Hz, -OC_6H_4NH-), 7.05 (d, 2 H, J=9.2 Hz, -OC_6H_4NH-), 7.79 (d, 2H, J=8.4 Hz, -$SO_2C_6H_4CF_3$), 7.87 (d, 2H, J=8.4 Hz, -$SO_2C_6H_4CF_3$), MS: 478 (M−H)$^-$.

4-(4-Methylphenylsulfonylamino)phenyl α-D-glucopyranoside (10). According to method E, compound **10** was prepared from **20** (0.64 g, 1.1 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford a quantitative yield (0.50 g) of **10**: [α]D +13.0° (*c* 1.98, MeOH); ^{1}H NMR (CD_3OD) δ 2.37 (s, 3H, -CH_3), 3.39 (dd, 1H, J_{3-4}=9.0 Hz, J_{4-5}=9.8 Hz, H-4), 3.53 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}=9.6 Hz, H-2), 3.60 (ddd, 1H, J_{4-5}=9.8 Hz, J_{5-6a}=5.2 Hz, J_{5-6b}=2.4 Hz, H-5), 3.66 (dd, 1H, J_{5-6a}=5.2 Hz, J_{6a-6b}=12.0 Hz, H-6a), 3.72 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.80 (dd, 1H, J_{2-3}=J_{3-4}=9.2 Hz, H-3), 5.37 (d, 1H, J=3.6 Hz, H-1), 6.97 (d, 2H, J=9.6 Hz, -OC_6H_4NH-), 7.02 (d, 2H, J=9.2 Hz, -OC_6H_4NH-), 7.26 (d, 2H, J=8.4 Hz, -$SO_2C_6H_4CH_3$), 7.57 (d, 2H, J=8.4 Hz, -$SO_2C_6H_4CH_3$), MS: 424 (M−H)$^-$.

4-(4-*tert*-Butylphenylsulfonylamino)phenyl α-D-glucopyranoside (11). According to method E, compound **11** was prepared from **21** (0.55 g, 0.9 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.40 g (99.7%) of **7**: [α]D +11.8° (*c* 1.47, MeOH); ^{1}H NMR (CD_3OD) δ 1.31 (s, 9H, -C (CH_3)$_3$), 3.39 (dd, 1H, J_{3-4}=9.0 Hz, J_{4-5}=9.8 Hz, H-4), 3.53 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}=10.0 Hz, H-2), 3.60 (ddd, 1H, J_{4-5}=9.8 Hz, J_{5-6a}=4.8 Hz, J_{5-6b}=2.4 Hz, H-5), 3.66 (dd, 1H, J_{5-6a}=4.8 Hz, J_{6a-6b}=12.0 Hz, H-6a), 3.72 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.80 (dd, 1H, J_{2-3}=J_{3-4} =9.4 Hz, H-3), 5.37 (d, 1H, J=3.6 Hz, H-1), 6.99 (d, 2H, J=9.2 Hz, -OC_6H_4NH-), 7.03 (d, 2H, J=9.6 Hz, -OC_6H_4NH-), 7.50 (d, 2H, J=8.4 Hz, -$SO_2C_6H_4C(CH_3)_3$), 7.62 (d, 2H, J =8.8 Hz, -$SO_2C_6H_4C$ (CH_3)$_3$), MS: 466 (M−H)$^-$.

4-(2-Naphthalenephenylsulfonylamino)phenyl α-D-glucopyranoside (12). According to method E, compound **12** was prepared from **22** (0.53 g, 0.9 mmol). The product was recrystallized from hot EtOH to afford a quantitative yield 0.40 g of **12**: [α]D +12.4° (*c* 1.24, MeOH); ^{1}H NMR (CD_3OD) δ 3.37 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=9.6 Hz, H-4), 3.50 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}=10.0 Hz, H-2), 3.56 (ddd, 1H, J_{4-5}=9.6 Hz, J_{5-6a}=4.6 Hz, J_{5-6b}=2.4 Hz, H-5), 3.63 (dd, 1H, J_{5-6a}=4.6 Hz, J_{6a-6b}=11.8 Hz, H-6a), 3.67 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=11.8 Hz, H-6b), 3.77 (dd, 1H, J_{2-3}=J_{3-4}=9.2 Hz, H-3), 5.33 (d, 1H, J=3.6 Hz, H-1), 7.00 (s, 4H, -OC_6H_4NH-), 7.56–7.72, 7.91–7.96, 8.23 (m, 7H, -$SO_2C_{10}H_7$), MS: 460 (M−H)$^-$.

Biological assays. The α-glucosidase inhibition assays were performed using *p*-nitrophenyl α-D-glucopyranoside (Aldrich) as a substrate and were assayed using previously reported methods.[13] The DNA breakage activity was investigated using previously reported methods.[16-18] Inhibition assays at the cellular level were performed by previously reported methods.[11]

RESULTS AND DISCUSSION

Synthesis of sulfonate and sulfonamide derivatives.

The synthesis of the sulfonate derivatives **1–6** that were used in the present study is presented in Fig. 3. α-Arbutin **13** was used as a starting material for the synthesis of compounds **1–6**. Compound **13** was sulfonated with 4-nitrobenzenesulfonyl chloride, 4-chlorobenzenesulfonyl chloride, 4-trifluoromethylbenzenesulfonyl chloride, 4-methylbenzenesulfonyl chloride, 4-*t*-butylbenzenesulfonyl chloride, and 2-naphthalenesulfonyl chloride in acetone to give compounds **1–6**, respectively.

The synthesis of the sulfonamide derivatives **7–12** that were used in the present study is presented in Fig. 4. *p*-Nitrophenyl α-D-glucopyranoside **14** was used as a starting material for the synthesis of compounds **7–12**. Compound **14** was acetylated with acetic anhydride in pyridine to give per-acetylated glucopyranoside **15**. Compound **15** was hydrogenated under H_2 with 20% palladium hydroxide on carbon to give the free-base **16**. Compound **16** was sulfonated with 4-nitrobenzenesulfonyl chloride, 4-chloro-

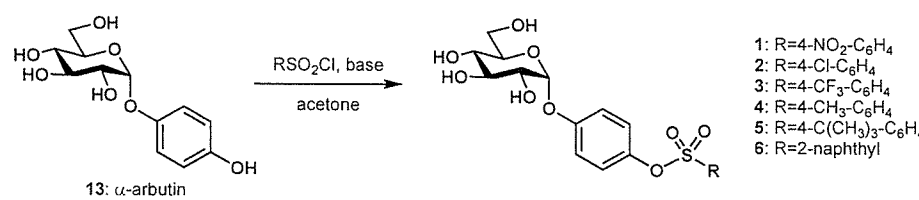

Fig. 3. Synthesis of compounds **1–6**.

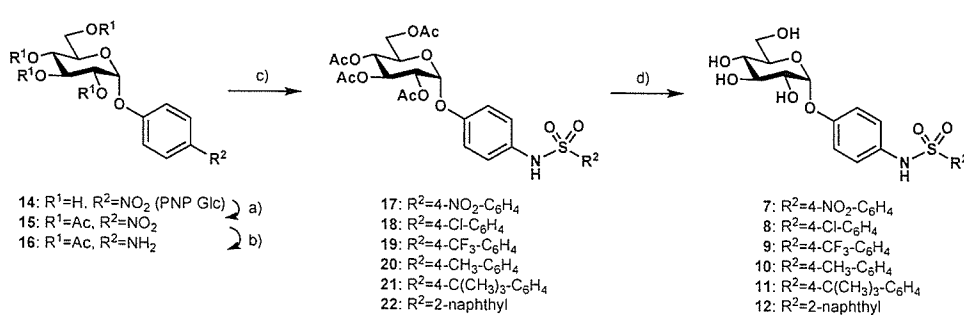

Fig. 4. Synthesis of compounds **7–12**.

benzenesulfonyl chloride, 4-trifluoromethylbenzenesulfonyl chloride, 4-methylbenzenesulfonyl chloride, 4-*t*-butylbenzenesulfonyl chloride, and 2-naphthalenesulfonyl chloride in pyridine to give **17**–**22** in good yields, respectively. Treatment of the resulting sulfonamides **17**–**22** with base gave compounds **7**–**12** in good yields, respectively. To the best of our knowledge, there have been no previous reports on the synthesis of compounds **1**–**6**, **8**–**9** or **11**. Data for NMR and MS spectra and optical rotation of all compounds **1**–**12** have not been reported.

Inhibition of α-glucosidases.

Inhibition studies on compounds **1**–**12** towards *Saccharomyces cerevisiae, Bacillus stearothermophilus* and rice α-glucosidases, and the results are listed in Table 1. Compounds **6** and **12**, with a terminal 2-naphthyl group, indicated inhibitions of α-glucosidases from *S. cerevisiae* (IC_{50}=51.7 μM and IC_{50}=74.1 μM) and *B. stearothermophilus* (IC_{50}=60.1 μM and IC_{50}=89.1 μM). Compounds **1**–**5** and **7**–**11** showed no significant inhibitory properties for *S. cerevisiae* or *B. stearothermophilus* α-glucosidases. No compounds inhibited rice α-glucosidase. Additionally, all α-glucosidases hydrolyzed compounds **1**–**12**. These results indicated that compounds **1**–**12** have properties of both substrate and inhibitor against *S. cerevisiae* α-glucosidase. Compounds **1**–**5** were substrate for *B. stearothermophilus* enzyme. However, compounds **6**–**12** were substrate and inhibitor for *B. stearothermophilus* enzyme. All compounds were substrate for rice enzyme. From these results if *p*-benzoquinone or *p*-benzoquinone imine are released during the liberation of the aglycon of compounds **1**–**12**, the huge differences in enzyme inhibitory activity among three kinds of enzymes will not result. This speculation is in conflict with those expected from the theory shown in Fig. 2. Therefore, the enzymatic hydrolysis reaction of compound **4** in the presence of *S. cerevisiae* α-glucosidase was analyzed using the LC/MS system as a model case. It was found that the major product of the hydrolysis reaction was aphenol compound corresponding to the aglycon moiety of compound **4** (data not shown). α-Glucosidase inhibition of compounds **1**–**12** was considered to be due to the enzymatic formation of phenol derivatives from compounds **1**–**12**, illustrated in Fig. 5, and/or compounds **1**–**12** themselves.

Table 1. Inhibitory activities of compounds **1**–**12** against α-glucosidases.

Compound	IC_{50} (μM)		
	S. cerevisaise	*B. stearothermophilus*	Rice
1	499	>500	>500
2	437	>500	>500
3	407	>500	>500
4	499	>500	>500
5	391	>500	>500
6	51.7	60.1	>500
7	239	218	>500
8	200	254	>500
9	146	244	>500
10	231	325	>500
11	136	237	>500
12	74.1	89.1	>500

The *S. cerevisiae* and *B. stearothermophilus* α-glucosidase inhibition assays were performed by using 1 mM PNP Glc as substrate. The assay conditions were potassium phosphate buffer (pH 7.0) at 37°C, 20 min. The rice α-glucosidase inhibition assay was performed by using 1 mM PNP Glc as substrate. The assay conditions were sodium acetate buffer (pH 4.0) at 37°C, 60 min. All α-glucosidases hydrolyzed compounds **1**–**12**.

DNA Cleavage activities.

Fukuhara *et al.* have reported that a phenol compound, resveratrol, induced Cu(II)-dependent DNA-strand scission under neutral conditions.[17] This DNA cleavage process occurs in the presence of Cu(II) and O_2. The ability of compounds **1**–**12** to induce DNA cleaving activity was examined using pBR322, a supercoiled, covalently closed circular DNA (Form I), and analyzed by agarose gel electrophoresis (Table 2). α-Glucosidase-triggered radical-mediated DNA breakage was very effectively observed for sulfonamide derivatives **7**–**12**. Consistent with the fact that Cu(II) and enzyme are required for potent DNA cleaving activity of compounds **7**–**12**, these compounds induced DNA cleavage only when the reaction was carried out in the presence of Cu(II) and enzyme; in the absence of Cu(II) and enzyme, no DNA cleavage was observed. On the other hand, sulfonate derivatives **1**–**6** caused only slight damage under the same conditions. The decrease in the DNA cleaving ability of compounds **1**–**6** compared to that of compounds **7**–**12** also indicated the importance of the sulfonamide structure, which might be

Table 2. Enzyme-triggered DNA-cleaving activities of compounds **1**–**12**.

Compound		Residual ratio of Form I plasmid (%)				
	$CuCl_2$	+	+	−	−	+
	NADH	+	−	+	−	+
	Enzyme	+	+	+	+	−
1		86	100	100	100	100
2		87	100	100	100	100
3		91	72	100	100	100
4		93	100	100	100	100
5		85	100	100	100	100
6		76	64	100	100	100
7		<1	<1	100	100	100
8		<1	<1	100	100	100
9		<1	23	100	100	100
10		<1	17	100	100	100
11		<1	6	100	100	100
12		<1	22	100	100	100

Analysis of DNA strand breaks generated in pBR322DNA with compounds **1**–**12**. Assays were performed by using 1 mM of compound, 100 μM $CuCl_2$, 500 μM NADH, and *S. cerevisiae* α-glucosidase. The assay conditions were sodium phosphate buffer (pH 7.0) containing pBR322DNA at 37 °C, for 20 h.

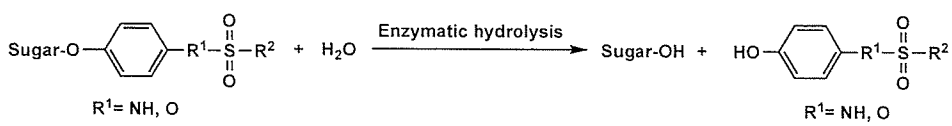

Fig. 5. Schematic diagram of enzymatic liberation of phenol derivatives.

effective not only for DNA binding for the conformation of the overall structure but also for the stability of the phenoxy radical. No effect of DNA cleaving activity of compounds **1**–**12** was observed in spite of the presence of NADH. It would appear that the enzymatic liberation of quinone derivative shown in Fig. 2 does not occur, since quinone derivatives showed DNA cleaving activity in the presence of NADH.[15] These findings can be explained by the fact that enzymatic liberation of the aglycon from compounds **1**–**12** was followed by the ejection of phenol derivatives, shown in Fig. 5.

Cellular level assays.

Compounds **1**–**12** were assayed with regard to their ability to inhibit ER glucosidase at the cellular level. Vesicular stomatitis virus glycoprotein (VSV G) was prepared from VSV-infected and probe-treated baby hamster kidney (BHK) cells. Analyses of the *N*-glycan structure of obtained VSV G using endo H, which is known to have hydrolytic activity against high-mannose type *N*-glycan, failed to confirm that these compounds inhibited ER glucosidases (data not shown).

We have shown that dual functional small molecules having both the α-glucosidase inhibitory activity and DNA breakage activity at the enzyme level can be designed, using our mechanism-based approach. We plan in the near future to study the structure-activity relationship and to extend the same strategies to more complicated cellular systems. We think that ER-targeted small molecule apoptosis inducers are necessary for the development of new and potent antitumor agents.

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DNA 切断活性を有する α-グルコシダーゼ阻害剤の設計と合成

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グルコース飢餓，ウイルス感染，低酸素状態などの小胞体ストレスは小胞体に高次構造異常タンパク質を蓄積させる。細胞は分子シャペロンを転写レベルで誘導する unfolded protein response(UPR)を誘起するなどして小胞体ストレスに抵抗するが，強い小胞体ストレスは細胞をアポトーシスへと誘導する。よって小胞体ストレスに起因するアポトーシスをがん細胞で誘導する化合物は抗がん剤となりうる。現在，新規な抗腫瘍薬の一つとして，がん細胞におけるアポトーシス誘導を標的とした抗腫瘍薬の開発が行われている。そこで我々は，*N*-結合型糖鎖プロセッシング酵素を阻害することにより小胞体に高次構造異常タンパク質を蓄積させ，それによる小胞体ストレスによって誘起される UPR を阻害することによりアポトーシスを誘導する化合物(小分子アポトーシス誘導化合物)としてスルホンエステル誘導体(**1**–**6**)とスルホンアミド誘導体(**7**–**12**)を設計し(Fig. 1)合成を行った(Fig. 3–4)。合成した化合物(**1**–**12**)の α-グルコシダーゼ阻害活性(Table 1)と DNA 切断活性(Table 2)について検討を行った。更に，細胞レベルでの *N*-結合型糖鎖プロセッシング酵素阻害活性についても検討した。その結果，ナフチル基を有する化合物 **6** と **12** が，*S. cerevisiae* 由来 α-グルコシダーゼに対し IC_{50}=51.7 μM と 74.1 μM，*B. stearothermophilus* 由来 α-グルコシダーゼに対し IC_{50}=60.1 μM と 89.1 μM の阻害活性を示し，化合物 **12** が最も強く DNA 切断活性を示した。しかし，すべての化合物が細胞レベルにおいて，酵素阻害活性を示さなかった。以上，酵素レベルにおいて α-グルコシダーゼ阻害活性と DNA 切断活性を有する小分子を見いだした。今後は，細胞レベルにおいても有効な化合物設計を行う予定である。

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Computational Study on Conformation of Oligopeptides Containing Chiral Cyclic α,α-Disubstituted α-Amino Acids

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Computational simulation using conformational search calculations (the Monte Carlo method of MacroModel) could predict the herical screw sense of oligopeptides containing chiral cyclic α,α-disubstituted α-amino acids. The global minimum energy conformation of peptide 2 was a 3_{10}-helix, which was in agreement with its X-ray structure.

Keywords: α,α-disubstituted α-amino acid, computational study, conformational search, α-helix, 3_{10}-helix

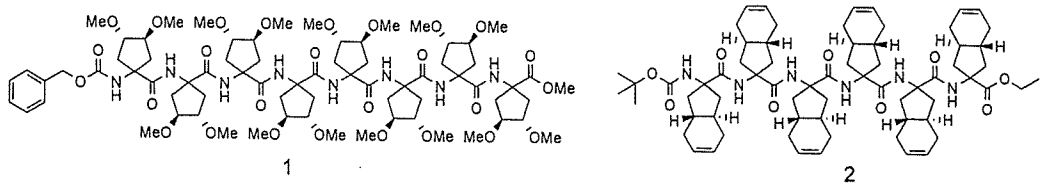
Introduction

We have studied the design of drug and functionalized molecules using computational methods [1]. Computational simulation of the conformation of oligopeptides presents an interesting challenge to predict the conformation for the design of functionalized and bioactive molecules. We have shown that the Monte Carlo conformational search using *MacroModel* is useful for conformational studies of oligopeptides prepared from α, α-disubstituted α-amino acids. Moreover, we performed conformational analysis of oligopeptides containing chiral ααdisubstituted α-amino acids to predict the helical screw sense of helical structures.

Results and Discussion

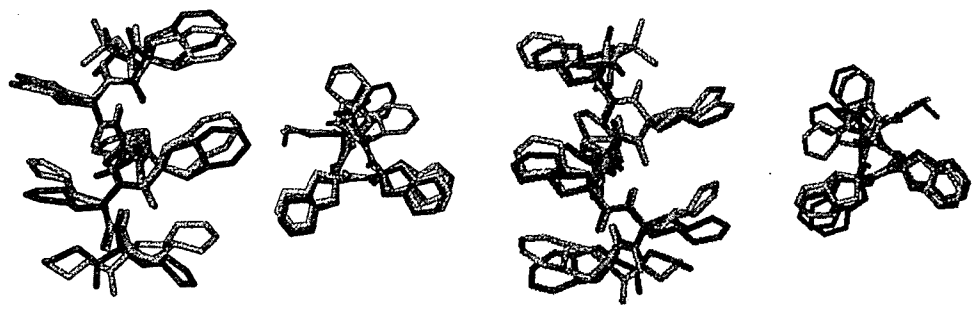
We have previously performed conformational search of oligopeptide **1** using the Monte Carlo method of *MacroModel* (ver. 8.1, Schrodinger, Inc). When AMBER* was used as the force field, the global minimum energy conformation of peptide 1 was a left-handed α-helix, which was more stable than a left-handed 3_{10}-helix by 4.2 kcal/mol. The results were in agreement with its X-ray structure, which showed a left-handed α-helix [2,3]. Conformational search of oligopeptide **2** was performed using the Monte Carlo method of *MacroModel*. When AMBER* was used as the force field, the global minimum energy conformation of peptide **2** was a right-handed

3_{10}-helix, which was more stable than a left-handed 3_{10}-helix by 1.6 kcal/mol. The difference of energies was small. There were both right- and left-handed 3_{10}-helices in the solid state [4]. These results indicated computational simulation using conformational search calculations could predict the helical screw sense of oligopeptides containing chiral α,α-disubstituted amino acids.



1 2

Fig. 1. Structures of peptides



(P)-3_{10}-helix *(M)*-3_{10}-helix

Fig. 2. Modeled structure(light) and X-ray structure (dark) of peptide 2.

Acknowledgements

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Sequence-specific recognition of double-stranded DNA by cooperative strand invasion

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ABSTRACT

A remarkable feature of peptide nucleic acid (PNA) is its ability to recognize some sequences within duplex DNA by strand invasion. In order to improve binding properties of PNA triplex invasion, we tested the effect of cooperativity on the sequence specificity. A PNA targeting six bases within duplex DNA stringently recognized 12 base-pair homopurine site at a single base level.

INTRODUCTION

Sequence-specific recognition of double helical DNA is crucial to genome study, disease diagnosis, and human gene therapy. Among the numerous methods for DNA recognition of particular interest is strand invasion by peptide nucleic acid (PNA). PNA is one of the most successful analogues of oligonucleotides with potential applications in antisense and antigene strategy.[1] Strand invasion can occur via several distinct mechanisms: triplex invasion,[1] double-duplex invasion,[2] and duplex invasion.[3,4] Among them most studies have focused on triplex invasion by using homopyrimidine PNAs, because a number of oligonucleotide-dependent enzymatic reactions are inhibited by PNA, including transcription, reverse transcription, translation, and restriction enzyme cleavage. Recognition of a unique site in the human genome requires discrimination of a specific sequence of 15-16 base pairs from all other possible sequences. However, the affinity of relatively short bis-PNAs (8-10 bases) to their target sites is so high that PNA binding to correct and even to mismatched sites is virtually irreversible. In this regard, sequence-specificity of PNA triplex invasion is limited and this limitation hinders the application in living cells.

The specificity of DNA recognition can be improved by cooperative binding of two ligands to a target site. Cooperative interactions between DNA binding ligands are critical to their affinity and specificity. Many DNA-binding proteins rely on dimerization of DNA recognition elements that each occupy 4-6 base pairs and target unique contiguous sites in genomic DNA. Previously, we demonstrated that a short PNA cooperatively binds to duplex DNA to afford stable invasion complexes.[5] We here report an extension of this idea to highly specific recognition of duplex DNA (Figure 1).

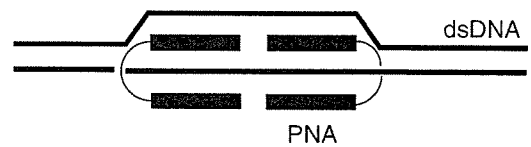

Figure 1. Schematic representation of cooperative strand invasion of dsDNA by PNA.

MATERIALS AND METHODS

Oligonucleotides.
DNA oligonucleotides were purchased from Operon Biotechnologies, Inc. Sequences are listed in Figure 2.
PNA.
PNA **1**(Figure 2) was synthesized by manual method. PNA monomers were purchased from Applied Biosystems.
Electrophoretic mobility shift assay (EMSA).
PNA **1** was incubated at 25 °C with DNAs, 30 mM NaCl, 0.1% IGEPAL CA 630, 1 mM EDTA, and 10 mM sodium phosphate at pH 6.73. The reaction proceeded for 24 h and analyzed by polyacrylamide gel electrophoresis, followed by staining with ethidium bromide. Binding efficiency was quantitated by CCD-based densitometry of the individual bands.

RESULTS AND DISCUSSION

The purine target sequence 5'-$GA_{10}G$-3' can be considered as two contiguous target sites, 5'-GA_5-3' and 5'-A_5G-3' (Figure 2). A hexameric bis-PNA, specific for the adjacent target sites, was used to test the effect of cooperative binding interaction on the sequence specificity. Figure 3 shows the results of the gel mobility shift assay. PNA **1** incubated with D^M generated no detectable band, indicating that this short pyrimidine PNA **1** has low binding affinity for its isolated target site. The PNA molecules are rapidly displaced by the internal competing complementary DNA strand during electrophoresis. In contrast, when **1** was incubated with D^D (0), clear bands corresponding to triplex

PNA 1

H-Cys(SBu^t)-eg-TTTTTC-Lys$_3$-eg$_3$-CTTTTT-eg-Cys(SBu^t)Lys-CONH$_2$

DNA D^M

5' ——— CTTTTTACATGCTA ——— 3'
3' ——— GAAAAATGTACGAT ——— 5'

DNA D^D(0)

5' ——— CTTTTTTTTTTC ——— 3'
3' ——— GAAAAAAAAAAG ——— 5'

DNA D^{D}mis

5' ——— CTTTTTTTATTC ——— 3'
3' ——— GAAAAAAATAAG ——— 5'

Figure 2. The sequences of PNA and DNA used in this study.

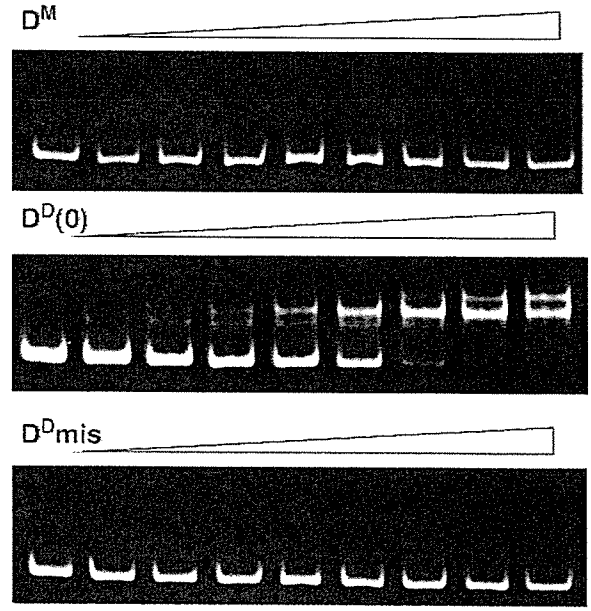

Figure 3. Polyacrylamide gel mobility shift assay showing the titration with increasing amounts of PNA **1** to dsDNA D^M, D^D(0), D^{D}mis. PNA concentrations were 0, 0.1, 0.19, 0.34, 0.63, 1.2, 2.2, 4.1, and 7.5 μM, respectively, from left to right. Conditions: 10 mM sodium phosphate buffer, 1 mM EDTA (pH 6.73), 30 mM NaCl, 0.1% IGEPAL CA 630, 25°C.

invasion complexes were observed. This marked improvement of the binding efficiency indicates a positive binding interaction between contiguous PNAs, aligned head to head in the invasion complex.

The sequence specificity of **1** was examined by comparing its affinity for fully matched 12 base pair target to that for a sequence containing a single-base mismatch (5'-GAAAAA AATAAG-3'), D^{D}mis. PNA **1** displayed excellent specificity in an all-or-none manner. Since each PNA molecule is bound to a short DNA target, a single mismatched base pair destabilizes such a complex to a large extent, thus destabilizing the whole invasion complexes. Consequently, high specificity was realized.

Because the stability of triplex invasion complexes formed with decameric bis-PNA oligomers is extraordinary high, such complexes are usually not at equilibrium in typical experiment. In this system, however, equilibrium is indeed reached. Thus, it may be possible to overcome problem of incorrect bindings that inevitably arises when targeting long DNA sequences.

CONCLUSION

We found a short PNA cooperatively binds to double-stranded DNA with excellent sequence specificity. This strategy would be useful to target a longer site and eventually enable the application of triplex invasion to anti-gene therapy.

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Efficient synthesis of carbopeptoid oligomers: insight into mimicry of β-peptide

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Abstract—The ready access to a new class of carbohydrate mimetics was demonstrated by the synthesis of tetrameric carbopeptoids, in which glycosidic bonds were replaced with amide linkages. We herein describe the detailed synthetis method of β(1→2)- and β(1→6)-linked carbopeptoids starting from each D-glucosamine and D-glucose derivative. The building blocks were polymerized using BOP reagent and DIEA to form a homooligomer. These produced carbopeptoids are resistant to glycosidases and have interesting biological activity. With conformational analysis by molecular modeling calculation, β(1→2)-linked decamer showed a typical 16-helix form as a mimic of β-peptide. Therefore, our polysaccharide analogues have potential as peptide foldamers.

1. Introduction

It has been revealed that carbohydrates are responsible for a variety of diseases and intercellular recognitions, including cell adhesion, viral infection, and cancer metastasis.[1] Due to the rapidly growing importance of carbohydrates in biology, several carbohydrate analogues, including pseudosugars[2] (a cycloalkane instead of sugar ring), C-[3] and S-glycosides,[4] and other mimetics[5–8] have been prepared and their stability against glycosidases has been assessed; however, those analogues have many problems such as synthesis of the unit, deprotection of protective groups, purification and so on. Therefore, new carbohydrate mimetics are still under consideration. We anticipate that if monosaccharides are linked via amide bonds instead of glycosidic bonds, the resulting oligosaccharide analogues may have the same biological activity as the original oligosaccharide because of their similar structure to the oligosaccharide.

Until recently, various amide-linked carbohydrates have been synthesized. One was first demonstrated by Yoshimura et al.[9] in the synthesis of (2→6)-amide-linked disaccharide derivatives: coupling of D-glucosamine and D-mannosamine to 2-amino-2-deoxy-D-mannuronic and D-glucuronic acids such as (i) in Figure 1. Meanwhile, Lehmann et al.[10] also reported a polysaccharide analogue in 1975 in which glycosidic bonds were replaced with amide linkage. This analogue

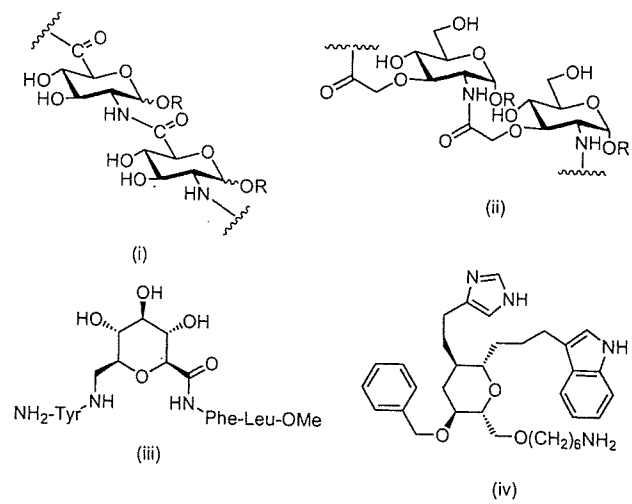

Figure 1. Amido-linked carbohydrates.

was later reported and named 'carbopeptoid' by the Nicolaou group[11] in 1995; however, no experiment was reported. Furthermore, the Hoffman-La Roche group reported the synthesis of a tetramer of (i) by a solid-phase-type elongation reaction[12] and of another amide-linked oligosaccharide composed of nor-muramic acid (ii).[13] The application of carbohydrates as amino acid analogues has also recently been reported: von Roedern and Kessler[14] prepared a glucose homologue with both amino and carboxyl groups and incorporated it into a peptide to mimic a proline β-turn residue, as shown in (iii). In a related work, D-glucose has already been used as a scaffold molecule to mimic a cyclic peptide

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2. Results and discussion

As shown in Figure 4, our general synthetic strategy both in the solution phase and in the solid phase is as follows: the monomeric building block (**A**), having a free C-1 carboxylic acid and Boc-protected amino group, couples with **B**, which has a free amino group, in the presence of a peptide coupling reagent to give a coupled product. The coupled product is treated with acid for N-deprotection (removal of *N*-Boc group) to generate another free amino group-carrying compound (**B**) with additional monomeric residue, which is again coupled to the monomeric building block **A**. After the chain elongation sequence is completed, the coupled product is O-deprotected to give the oligomeric analogue (**C**). The tetrameric compound was sulfated by a known method to afford the sulfated analogue (**D**).

Scheme 1 outlines the synthesis of the β(1→2)-linked homo-oligomer **1** starting from D-glucosamine hydrochloride. We first synthesized the known 1-cyano-2-phthalimide derivative (**5**), which was obtained according to the published procedure.[33,34] The C-1 CN group was hydrated with 30%

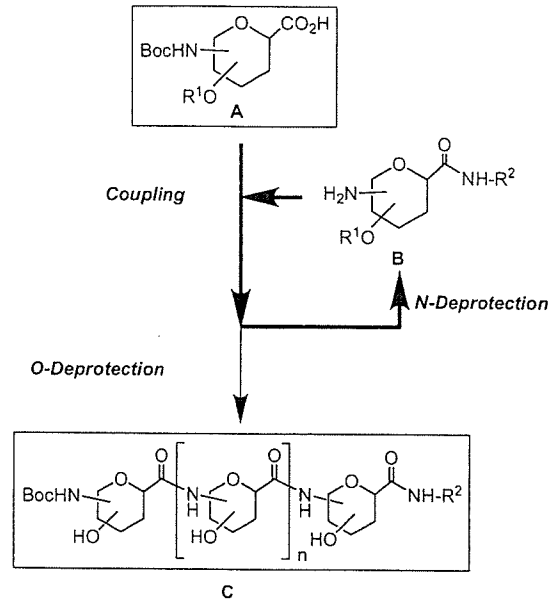
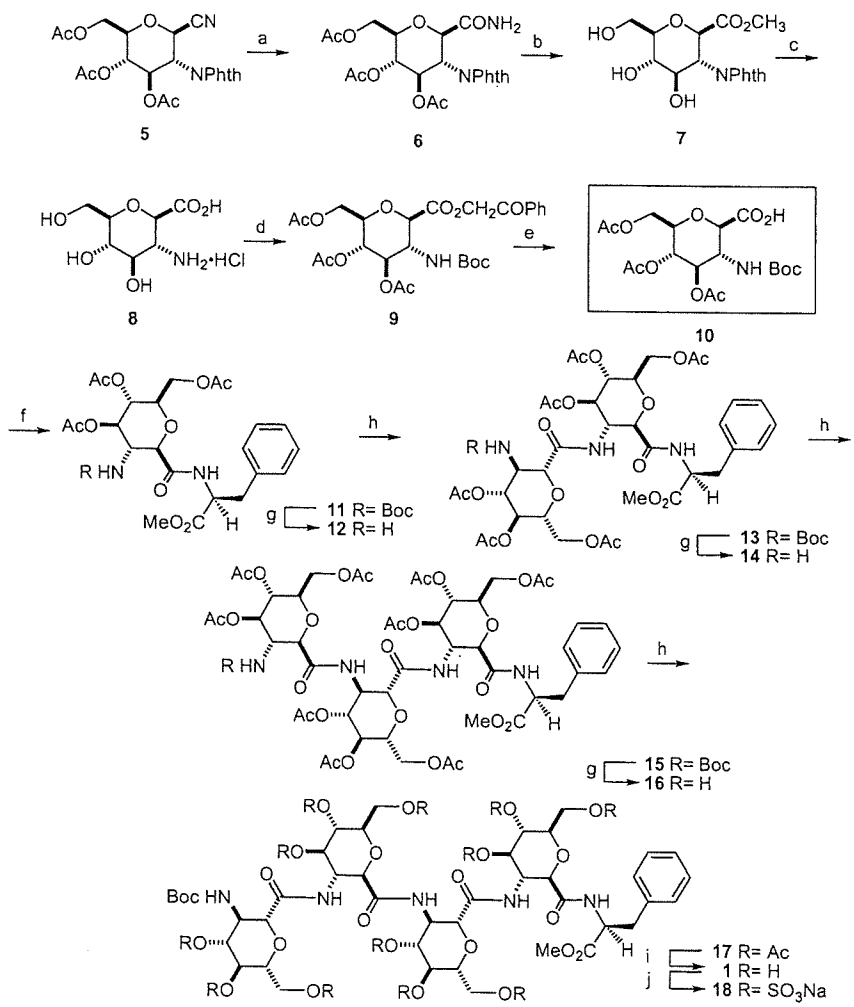


Figure 4. General synthetic strategy.



Scheme 1. Synthesis of a carbopeptoid tetramer **1**. Reagents and conditions: (a) 30% HBr–AcOH, 3 h, 0 °C to rt, 85%; (b) Dowex 50W-X8 [H^+], MeOH, 16 h, 80 °C, 97%; (c) (i) 6 equiv of LiOH, MeOH/H_2O (3:1), 16 h, 60 °C, (ii) 3 N HCl, 3 h, reflux, 95% overall; (d) (i) 2 equiv of BOC-ON, Et_3N, dioxane/H_2O (1:1), 12 h, rt, (ii) 2-bromoacetophenone, Et_3N, DMF, 4 h, rt, (iii) Ac_2O, pyridine, 12 h, rt, 73% overall; (e) H_2, Pd/C, AcOEt/EtOH (2:1), 16 h, rt, 90%; (f) L-phenylalanine methyl ester, DEPC, Et_3N, 16 h, 0 °C to rt, 86%; (g) 2 N HCl in EtOAc, 3 h, 0 °C to rt, 95%; (h) 1.2 equiv of **10**, BOP, DIEA, DMF, 16 h, rt, 59%; (i) MeONa in MeOH (pH 11), 2 h, rt, 68%; (j) 10 equiv of sulfur trioxide trimethylamine complex, 5 days 50 °C.

excess amount of $SO_3 \cdot NMe_3$ in anhydrous DMF at 50 °C and the reaction progress was monitored by TLC (disappearance of the starting material and if possible for product formation). However, in this case, we expected high efficiency of a complete O-sulfation, and it was quite impossible to observe the fully *O*-sulfated compound in TLC. Instead, we stopped the reaction by cooling down and separated the product from the reagents with a Sephadex G-15 column eluted with water. Fractions stained with Azure reagent[38] were combined and concentrated to dryness. Then, we lyophilized the fractions and took an NMR. If the reaction was not completed, we observed several peaks corresponding to the methyl ester of **18** or **33**. After stirred at 50 °C for 5 days, we were able to see the single peak for the methyl ester of sulfated compounds. The sulfur content of the sulfated compounds was calculated by elemental analysis.

The β(1→2)- (**1**) and β(1→6)-tetramers (**4**) showed interesting and potent biological activities as mentioned. We anticipated that those activities might relate to their conformation. In our previous study, CD spectroscopy data of a β(1→2)-linked hexamer suggested the possibility of a 14-helical structure.[21] In addition, a β(1→2)-linked oligomer can be particularly assumed as a unique β-peptide with functionality on molecules such as hydroxyl groups and ring oxygen. Therefore, we focused on a β(1→2)-linked oligomer for conformational study among our carbopeptoid oligomers **1–4**. Conformational search calculations were performed with the package of MacroModel ver. 8.1 (Schrödinger Inc.) on an SGI workstation. The Monte Carlo Multiple Minimum (MCMM) method and AMBER* force field were used to find the global and local minimum energy conformation. As the initial structure, an extended structure was used. More than 10,000 conformers were optimized. Figure 5 shows the result of conformational analysis of a β(1→2)-linked decamer with a molecular modeling calculation. Interestingly, it revealed that a typical right-handed 16-helix was the most stable conformation. The 16-helix structure was stabilized with a hydrogen bond between oxygen of the C-1 carbonyl group and nitrogen of the C-2 amino group of another unit as shown in Figure 5. Although we examined various calculations, β(1→2)-linked oligomers from dimer to decamer have never had left-handed conformation except tetramers. This finding corresponds to the result of β-amino acid oligomer reported by Gellman et al.[23,24] In the case of the tetramer, one reason why it possessed sinistral structure might be steric hinderance caused by amino acids.

This unique conformation of a β(1→2)-linked oligomer may give various functions such as protein–protein interaction. In an earlier work in this field, Seebach demonstrated that β-peptide hairpins could bind somatostatin receptors with high affinity and specificity.[39,40] Seebach and co-workers,[41] DeGrado and co-workers,[42,43] and Gellman and co-workers[44–46] demonstrated that amphiphathic β-peptides could perform a variety of functions including the inhibition of cholesterol and fat uptake,[41] potent antibacterial activity,[42–45] and RNA binding.[46] Recently, some groups reported the synthesis and investigation of the helical structure of α, β, and γ-peptide including molecular dynamics simulation.[47–51] Some evaluated biological activity, for example, Kritzer et al. studied protein–protein interaction inhibitors, which were made from 14-helical β-peptide.[47] Meanwhile Porter et al. reported structure–activity trends among

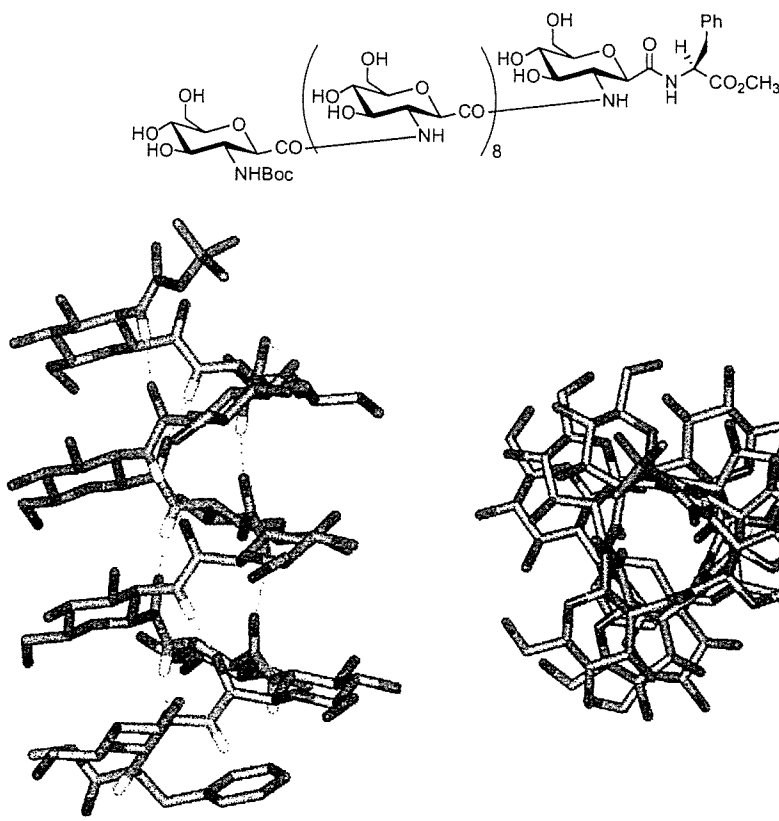


Figure 5. Conformational analysis of β(1→2)-amide-linked analogue.

eluted with 1 L of 1 N aqueous NH_4OH. Ninhydrin-active fractions were collected and concentrated, then the resin was suspended in 50 mL of 1 N hydrochloric acid and evaporated. Lyophilization with a small amount of water gave **8** (8.10 g, 33.2 mmol; 95%) as a colorless amorphous powder: ^{1}H NMR (300 MHz, D_2O): δ 4.10 (d, 1H, J=10.5 Hz, H-1), 3.63 (1H, H-6a), 3.51–3.42 (m, 2H, H-4, 6b), 3.31–3.16 (m, 2H, H-2, 5), 3.10 (t, 1H, J=10.4 Hz, H-3); ^{13}C NMR (72.5 Hz, D_2O): δ 171.8 (CO_2H), 80.5, 74.02, 73.93, 70.2, 61.4, 53.7 (C-1,2,3,4,5,6).

4.1.4. (2*R*,3*R*,4*R*,5*S*,6*R*)-2-Oxo-2-phenylethyl 4,5-diacetoxy-6-(acetoxymethyl)-3-(*tert*-butoxycarbonylamino)-tetrahydro-2*H*-pyran-2-carboxylate (9). To a stirred solution of **8** (4.2 g, 12.0 mmol) and Et_3N (7.21 mL, 51.7 mmol) in water (30 mL) was added a solution of BOC-ON (6.43 g, 25.9 mmol) in 1,4-dioxane (30 mL). After 3 h at room temperature, the solution was concentrated, and the residue was dissolved in 50 mL of water. The water solution was then adjusted to neutral using Dowex 50W-X8 [H^+] and washed with 50 mL of CH_2Cl_2, followed by evaporation of the aqueous layer. To a DMF solution (50 mL) of the residue was added 2-bromoacetophenone (phenacylbromide) (5.25 g, 25.8 mmol) and Et_3N (3.6 mL, 25.8 mmol). The mixture was stirred at room temperature for 3 h, then pyridine (60 mL) and acetic anhydride (30 mL) were added to the solution and stirred at room temperature for 12 h. The mixture was poured into ice water and extracted three times with EtOAc (200 mL each), and organic layers were washed with 0.5 N hydrochloric acid, water, saturated aqueous $NaHCO_3$, and brine. After drying ($MgSO_4$), the solution was filtered and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc 5:1) to afford **9** (4.81 g, 8.70 mmol; 73% overall) as a white powder, mp 194–197 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (2H, Ph), 7.62 (1H, Ph), 7.49 (2H, Ph), 5.47 (t, 1H, J=9.5 Hz, H-3), 5.40 (t, 2H, J=16.3 Hz, CH_2COPh), 5.10 (t, 1H, J=9.6 Hz, H-4), 4.45 (d, 1H, J=10.4 Hz, H-1), 4.27 (dd, 1H, J=5.2, 12.5 Hz, H-6a), 4.15 (dd, 1H, J=2.2, 12.4 Hz, H-6b), 3.88 (1H, H-2), 3.80–3.75 (m, 1H, H-5), 2.08, 2.06, 2.03 (3s, 3H each, 3×CH_3CO), 1.40 (s, 9H, NHCO$_2$*t*Bu); ^{13}C NMR (72.5 Hz, $CDCl_3$): δ 170.5, 170.4, 169.4, 167.1 (CO), 133.9, 128.8, 127.7 (Ph), 76.8, 75.9, 72.7, 68.7, 66.7, 62.3 (C-1,2,3,4,5,6), 28.1 (*t*-Bu), 20.6, 20.51, 20.45 (3×CH_3CO); mass spectrum (EI) *m/e* 569.2349 (M^+, calcd 569.2347).

4.1.5. (2*R*,3*R*,4*R*,5*S*,6*R*)-4,5-Diacetoxy-6-(acetoxymethyl)-3-(*tert*-butoxycarbonylamino)-tetrahydro-2*H*-pyran-2-carboxylic acid (β(1→3)-linked monomer) (10). Compound **9** (2.70 g, 4.90 mmol) was dissolved in EtOAc/EtOH 2:1 (60 mL), and 10% Pd/C (400 mg) was added to the solution. The resulting mixture was stirred at room temperature for 16 h under H_2. Analysis of the reaction mixture by TLC ($CHCl_3$/MeOH, 2:1) indicated the disappearance of the starting material. The catalyst was filtered off and washed with MeOH. The combined solution was concentrated in vacuo to provide a crude product. Column chromatography ($CHCl_3$/MeOH, 20:1) of the residue gave compound **10** (1.91 g, 4.38 mmol; 90%) as a white powder, mp 193–195 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 5.17 (t, 1H, J=9.4 Hz, H-3), 4.99 (t, 1H, J=9.6 Hz, H-4), 4.29 (dd, 1H, J=5.0, 12.4 Hz, H-6a), 4.10 (dd, 1H, J=2.0, 12.3 Hz, H-6b), 3.97–3.86 (m, 2H, H-1, 2), 3.79–3.75 (m, 1H, H-5), 2.04, 2.00, 1.99 (3s, 3H each, 3×CH_3CO), 1.40 (s, 9H, NHCO$_2$*t*Bu); ^{13}C NMR (72.5 Hz, CD_3OD): δ 172.5 (CO_2H), 171.9, 171.4 (3×CH_3CO), 157.6 (NHCO_2*t*Bu), 80.9, 76.9, 75.9, 70.3, 63.8, 54.4 (C-1,2,3,4,5,6), 28.7 (*t*-Bu), 20.7, 20.68, 20.61 (3×CH_3CO); mass spectrum (EI) *m/e* 451.1928 ($(M+NH_4)^+$, calcd 451.1928).

4.1.6. (2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-5-(*tert*-butoxycarbonylamino)-6-(1-methoxy-1-oxo-3-phenylpropan-2-ylcarbamoyl)-tetrahydro-2*H*-pyran-3,4-diyl diacetate (11). DEPC (524 μL, 3.21 mmol) was added to a stirred solution of compound **10** (1.16 g, 2.68 mmol), L-phenylalanine methyl ester (692 mg, 3.21 mmol), and Et_3N (821 μL, 5.89 mmol) in DMF (30 mL) at 0 °C under argon. The mixture was stirred at 0 °C for 1 h, and then at room temperature for 12 h. The reaction mixture was diluted with EtOAc (200 mL), and then washed successively with water (200 mL), and saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous $MgSO_4$, and concentrated to dryness in vacuo. The residue was chromatographed on silica gel using toluene/EtOAc 5:1 to give **11** (1.23 g, 2.30 mmol; 86%) as a white powder, mp 231–234 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27–7.12 (m, 5H, Ph), 6.87 (d, 1H, J=8.1 Hz, NH), 5.27 (m, 2H, H-1, 3), 5.03 (t, 1H, J=9.7 Hz, H-4), 4.80 (m, 1H, CHCO$_2$CH$_3$), 4.21 (dd, 1H, J=5.0, 12.4 Hz, H-6a), 4.14 (dd, 1H, J=1.2, 11.9 Hz, H-6′), 4.03 (d, 1H, J=9.9 Hz, H-6b), 4.03 (d, 1H, J=9.9 Hz, NH), 3.81–3.70 (m, 5H, H-2, 5, CO$_2$CH_3), 3.18–3.02 (m, 2H, CH_2Ph of L-Phe), 2.07, 2.06, 2.02 (3s, 3H each, 3×CH_3CO), 1.39 (s, 9H, NHCO$_2$*t*Bu); ^{13}C NMR (72.5 MHz, $CDCl_3$): δ 171.1, 170.4, 169.2 (3×CH_3CO), 170.2 (CO_2CH_3), 166.8 (NHCO), 154.9 (NHOCO*t*Bu), 135.6, 129.0, 128.2, 126.9 (Ph), 79.5, 76.8, 75.2, 73.1, 68.4, 62.0 (C-1,2,3,4,5,6), 52.6 (CHCO$_2$CH$_3$), 52.0 (CO$_2$CH_3), 37.6 (CH_2Ph of L-Phe), 27.9 (*t*-Bu), 20.44, 20.38, 20.33 (3×CH_3CO); mass spectrum (EI) *m/e* 595.2513 ($(M+H)^+$, calcd 595.2503).

4.1.7. β(1→2)-Linked dimer (13). A solution of 4 N HCl in EtOAc (10 mL) was added to a stirred solution of compound **11** (887 mg, 1.49 mmol) in EtOAc (10 mL) at 0 °C. The mixture was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was washed with ether to give **12** as a white powder. This compound was reacted without further purification. Compound **12**, BOP reagent (792 mg, 1.79 mmol), and DIEA (572 μL, 3.94 mmol) were added to a solution of compound **10** (776 mg, 1.79 mmol) in DMF (20 mL) at room temperature under argon. The reaction mixture was stirred at room temperature for 16 h, then diluted with EtOAc (200 mL), and successively washed with water, saturated aqueous $NaHCO_3$, and brine (150 mL each). The organic layer was dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated and the residue was chromatographed on silica gel using toluene/EtOAc 5:1–1:1 to afford **13** (800 mg, 879 μmol; 59%) as a white powder, mp 198–203 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29–7.11 (m, 5H, Ph), 7.01 (d, 1H, J=7.9 Hz, NH), 6.70 (d, 1H, J=7.1 Hz, NH), 5.38 (t, 1H, J=9.7 Hz, 1H-3), 5.21 (t, 1H, J=9.5 Hz, 2H-3), 5.07–4.98 (m, 3H, 1H-4, 2H-1, 4), 4.72 (dd, 1H, J=6.3, 14.0 Hz, CHCO$_2$CH$_3$), 4.29–4.08 (m, 5H, 1H-6a, 6b, 2H-1, 6a, 6b), 3.97–3.87 (m, 2H, 1H-2, 5), 3.77–3.55 (m, 5H, 2H-2, 5, CO$_2$CH_3), 3.18–3.06 (m, 2H, CH_2Ph of L-Phe), 2.08, 2.06, 2.05, 2.02

($MgSO_4$) and concentrated in vacuo. The residue was chromatographed on silica gel (heptane only to 5:1 heptane/EtOAc) to give mixture of **21a** and **21b** (1:1) (12.2 g, 75%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.17–7.00 (m, 30H, Ph), 5.03–4.34 (m, 12H, CH_2Ph), 3.89–3.28 (m, 14H), 3.39 (3H, CO_2CH_3), 0.83 (s, 18H, $(CH_3)_3CSi(CH_3)_2$), 0.006, −0.013 (s, 12H, $(CH_3)_3CSi(CH_3)_2$).

4.1.14. (2*R*,3*R*,4*S*,5*R*,6*R*)-Methyl 3,4,5-tris(benzyloxy)-tetrahydro-6-(hydroxymethyl)-2*H*-pyran-2-carboxylate (22). Compounds **21a** and **21b** (10.0 g) were dissolved in AcOH/THF/H_2O (3:1:1) (500 mL) at room temperature, and the solution was stirred for 15 h at room temperature. The mixture was concentrated in vacuo and the residue was azeotropied with toluene twice. The resin was then dissolved in MeOH (200 mL), and 30% NaOMe in MeOH (2.0 mL) was added to the solution. The reaction mixture was stirred at room temperature for 15 min, and then concentrated. Purification by silica gel chromatography (20:1 toluene/EtOAc to 5:1) afforded **22** (7.60 g, 15.4 mmol, 78% overall) as a white powder, mp 76–78 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 7.31–7.21 (m, 15H, Ph), 4.88–4.52 (m, 6H, CH_2Ph), 3.97–3.66 (m, 7H), 3.62 (s, 3H, CO_2CH_3); ^{13}C NMR (72.5 MHz, CD_3OD): δ 169.8 (CO_2CH_3), 138.3, 137.8, 137.7 (*C*Ph), 128.52, 128.47, 128.44, 128.04, 127.96, 127.90, 127.7 (Ph), 86.1, 80.0, 79.9, 78.0, 77.4 (C-1,2,3,4,5), 75.6, 75.2, 75.1 (CH_2Ph), 61.8 (C-6), 52.5 (CO_2CH_3); mass spectrum (FAB) *m/e* 493.2 ((M+H)$^+$, calcd 493.2).

4.1.15. (2*R*,3*R*,4*S*,5*R*,6*R*)-Methyl 6-(azidomethyl)-3,4,5-tris(benzyloxy)-tetrahydro-2*H*-pyran-2-carboxylate (23). To a solution of **22** (7.60 g, 15.4 mmol) in CH_2Cl_2 (100 mL) was added TsCl (5.94 g, 31.7 mmol) and pyridine (3.74 mL, 46.2 mmol) at 0 °C under argon, and the reaction mixture was stirred for 12 h at room temperature. The solution was poured into stirred ice and water (200 mL), and immediately extracted with chloroform (2×200 mL). The combined extracts were successively washed with 0.5 N hydrochloric acid (100 mL), water (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was used for the next coupling reaction without further purification. Sodium azido (2.03 g, 31.7 mmol) was added to the residue (prepared from 15.4 mmol of **22**) in DMF (50 mL) at room temperature under Ar, and the reaction mixture was stirred for 12 h at 60 °C. The reaction mixture was diluted with EtOAc (300 mL) and washed successively with water (300 mL), saturated aqueous $NaHCO_3$ (300 mL), and brine (300 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on silica gel (5:1 to 3:1 hexane/EtOAc) to give **23** (6.50 g, 12.6 mmol; 81% overall) as white needles, mp 76–77 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36–7.19 (m, 15H, Ph), 4.95–4.77 (m, 4H, CH_2Ph), 4.62 (d, 1H, *J*=9.3 Hz, CH_2Ph), 3.95 (d, 1H, *J*=9.5 Hz, H-1), 3.84 (t, 1H, *J*=8.6 Hz, H-3), 3.77–3.71 (t, 1H, *J*=8.1 Hz, H-4), 3.61–3.48 (m, 2H, H-6a, 6b), 3.33 (dd, 1H, *J*=5.5, 13.6 Hz, H-2); ^{13}C NMR (72.5 MHz, $CDCl_3$): δ 169.2 (CO_2CH_3), 138.3, 137.80, 137.75 (*C*Ph), 128.54, 128.50, 128.44, 128.04, 128.00, 127.9, 127.8, 127.5 (Ph), 86.1, 79.9, 78.7, 78.1 (C-1,2,3,4,5), 75.6, 75.2, 75.1 (CH_2Ph), 52.4 (CO_2CH_3), 51.1 (C-6); mass spectrum (FAB) *m/e* 490.2 ((M−N_2+H)$^+$, calcd 490.2).

4.1.16. (2*R*,3*R*,4*S*,5*R*,6*R*)-6-(Azidomethyl)-3,4,5-tris(benzyloxy)-tetrahydro-2*H*-pyran-2-carboxylic acid (24). To the solution of **23** (4.40 g, 8.50 mmol) in MeOH/THF/H_2O (3:3:1) was added LiOH·H_2O (713 mg, 17.0 mmol), and the reaction mixture was stirred at room temperature for 12 h. The solution was evaporated and the residue was chromatographed on silica gel (10:1 EtOAc/MeOH) to afford **24** (3.51 g, 6.97 mmol; 82%) as a white powder, mp 68–70 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 7.35–7.07 (m, 15H, Ph), 4.8–4.53 (m, 12H, CH_2Ph), 3.85–3.64 (m, 3H), 3.58–3.31 (m, 4H); ^{13}C NMR (72.5 MHz, CD_3OD): δ 138.9, 137.8, 138.4 (*C*Ph), 129.0, 128.9, 128.7, 128.5, 128.4, 128.3, 128.2 (Ph), 86.6, 82.2, 81.1, 78.4, 76.0, 75.5, 75.3; mass spectrum (FAB) *m/e* 526.2 ((M+Na)$^+$, calcd 526.2).

4.1.17. (2*R*,3*R*,4*S*,5*R*,6*R*)-6-(*tert*-Butoxycarbonylaminomethyl)-3,4,5-tris(benzyloxy)-tetrahydro-2*H*-pyran-2-carboxylic acid (25). A mixture of **24** (3.51 g, 6.97 mmol) and Lindlar catalyst (2.0 g) in MeOH (100 mL) was stirred in an H_2 atmosphere at room temperature for 3 h, and then filtered through Celite. The filtrate was evaporated, then the resin was dissolved in MeOH/H_2O (3:1). To the solution were added LiOH·H_2O (585 mg, 13.9 mmol) and BOC_2O (2.35 g, 10.5 mmol), and the reaction mixture was stirred at room temperature for 12 h. The solution was evaporated and the residue was chromatographed on silica gel (10:1 EtOAc/MeOH) to give **25** (2.17 g, 3.76 mmol; 54% overall) as white needles, mp 110–112 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 7.34–7.22 (m, 15H, Ph), 4.87 (d, 1H, *J*=11.3 Hz, CH_2Ph), 4.83 (d, 1H, *J*=13.2 Hz, CH_2Ph), 4.78 (d, 1H, *J*=3.9 Hz, CH_2Ph), 4.75 (d, 1H, *J*=2.5 Hz, CH_2Ph), 4.66 (d, 1H, *J*=9.9 Hz, CH_2Ph), 4.62 (d, 1H, *J*=10.1 Hz, CH_2Ph), 3.78 (t, 1H, *J*=4.8 Hz, H-3), 3.71–3.62 (m, 2H, H-1, 4), 3.54 (dd, 1H, *J*=3.8, 13.9 Hz, H-6a), 3.47–3.29 (m, 3H, H-2, 5, 6b), 1.45 (s, 9H, $NHCO_2tBu$); ^{13}C NMR (72.5 MHz, CD_3OD): δ 172.5 (C-1), 166.5 (NH*C*O), 154.7 (NHO*C*O*t*Bu), 138.3, 137.78, 137.66, 135.6 (*C*Ph), 129.2, 128.7, 128.5, 128.4, 128.3, 128.0, 127.8, 127.6, 127.4 (Ph); mass spectrum (FAB) *m/e* 600.3 ((M+Na)$^+$, calcd 600.3), 478.2 ((M−Boc+2H)$^+$, calcd 478.2).

4.1.18. (*S*)-Methyl 2-((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-(*tert*-butoxycarbonylaminomethyl)-tetrahydro-2*H*-pyran-2-carboxamido)-3-phenylpropanoate (26). DEPC (339 μL, 1.93 mmol) was added to a cooled solution of **25** (800 mg, 1.38 mmol), L-phenylalanine methyl ester (358 mg, 1.67 mmol), and Et_3N (579 μL, 4.15 mmol) in DMF (10 mL) at 0 °C under Ar, and the mixture was stirred for 1 h at 0 °C, and then for an additional 12 h at room temperature. The reaction mixture was diluted with EtOAc (200 mL), and successively washed with water (200 mL), saturated aqueous $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on silica gel (toluene/EtOAc 5:1) to give **26** (941 mg, 1.27 mmol; 92% yield) as a white powder, mp 111–115 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.23 (m, 18H, Ph), 7.10 (2H, Ph), 6.57 (d, 1H, *J*=7.5 Hz, NH), 4.92–4.77 (m, 4H, CH_2Ph), 4.67–4.56 (m, 3H, CH_2Ph, C*H*CO_2CH_3), 3.80 (d, 1H, *J*=9.0 Hz, H-1), 3.73–3.53 (m, 7H, H-3, 4, 6a, 6b, CO_2CH_3), 3.41–3.31 (m, 2H, H-2, 5), 3.25 (1H, NH), 3.15 (dd, 1H, *J*=5.8, 14.0 Hz, CH_2Ph of L-Phe), 3.10 (dd, 1H, *J*=6.2, 14.0 Hz, CH_2Ph of L-Phe), 1.47 (s, 9H, $NHCO_2tBu$); ^{13}C NMR (72.5 MHz, $CDCl_3$): δ 171.6, 168.3, 155.7 (C-1,

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Methyl-introduced A-ring Analogues of 1α,25-Dihydroxyvitamin D_3: Synthesis and Biological Evaluation

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Abstract. *The hormonally-active metabolite of vitamin D, 1α,25-dihydroxyvitamin D_3 (1), has a wide variety of biological activities, which makes it a promising therapeutic agent for the treatment of cancer, psoriasis and osteoporosis. Insights into the structure-activity relationships of the A-ring of 1 are needed to assist the development of more potent and selective analogues, as well as to define the molecular mode of action. All possible A-ring stereoisomers of 2-methyl-1,25-dihydroxyvitamin D_3 and 2,2-dimethyl-1,25-dihydroxyvitamin D_3, which differ in stereochemistry at the C1-, C2- and C3-positions, were designed and efficiently synthesized by employing the convergent method. Biological evaluation of the analogues, in terms of the vitamin D receptor-binding affinity and HL-60 cell differentiation-inducing activity, as well as the transcriptional potency in ROS 17/2.8 cells, revealed the importance of substituents at the C2-position in certain orientations.*

Cholecalciferol, known as vitamin D_3, is metabolized *via* 25-hydroxyvitamin D_3 to produce the hormonally-active form, 1α,25-dihydroxyvitamin D_3 (**1**; Figure 1), the formation of which is strictly regulated (1). In addition to its classic role in calcium and phosphorus homeostasis, 1α,25-dihydroxyvitamin D_3 dominates the cell cycle in many malignant cells, regulating proliferation, differentiation and apoptosis. Most of the biological activities of **1** are considered to be mediated by a ligand-inducible transcriptional factor, the vitamin D receptor (VDR), which belongs to the nuclear receptor superfamily. The specific interaction of the ligands with the ligand-binding domain of VDR has been a major focus of attention, since it triggers the whole sequence of biological responses: conformational change of the VDR, particularly of the AF-2 domain, heterodimerization with retinoid X receptors (RXRs), recruitment of co-activators and binding to the DNAs. Insights into the structure-function relationships of a variety of ligands are essential to understand how the subtype-free, singular VDR can deliver the diverse biological activites of **1**, as well as allowing the development of potent therapeutic agents with selective activity profiles for the treatment of cancers or ostereoporosis.

Structural modification of **1** in the A-ring, which possesses two critical hydroxyl groups at the C1- and C3-positions, has become of interest in recent years, because the other three stereoisomers have proven to exhibit unique activity profiles, being different from the natural hormones (2, 3). Our study of all eight possible A-ring stereoisomers of 2-methyl-1,25-dihydroxyvitamin D_3 and their 20-epimers showed that introduction of a simple methyl group into the parent **1** yielded analogues with distinct activity profiles (4-6). These methyl-introduced analogues, which differ in stereochemistry at the C1-, C2- and C3-positions, exhibited cell differentiation- or apoptosis-inducing activity towards HL-60 cells, depending on their A-ring structures (7). Some of the synthesized 2α-substituted analogues of **1** showed remarkably high affinity for VDR (8-10). 2β-Methyl introduction into the A-ring, on the other hand, in combination with the 1β-hydroxy or 3α-hydroxy groups, resulted in antagonists of the nongenomic, but not genomic, actions in NB-4 cells (11).

The X-ray crystal structure of VDR complexed with **1** (12) indicated the presence of an extra space in the vicinity of the A-ring, suggesting that the substituents of synthetic A-ring analogues could occupy this additional space. Our study of the 2-methyl analogues of **1** revealed that 2α-methyl-1α,25-dihydroxyvitamin D_3 (**2a**) was a four-fold

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Key Words: Vitamins, hormones, receptors, chemical synthesis.

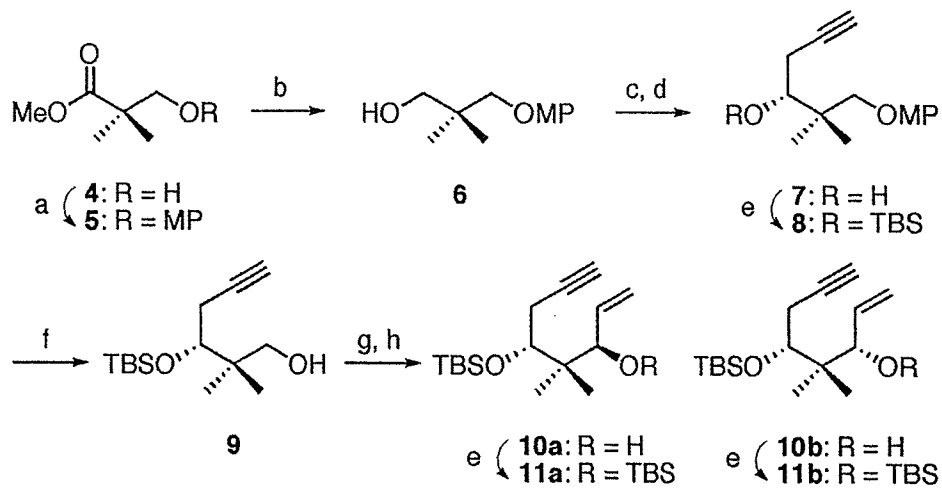

Figure 2. *Reagents and conditions: (a) 4-methoxyphenol, DEAD, Ph_3P/ THF, 98%; (b) $LiAlH_4$/ THF, 97%; (c) PDC, 4A MS/ CH_2Cl_2, 89%; (d) allenylmagnesium bromide/ ether, 68%; (e) TBSOTf, 2,6-lutidine/ CH_2Cl_2, 81% for 8, quant. for 11a,b; (f) CAN/ CH_3CN-H_2O, 77%; (g) TPAP, NMO, 4A MS/ CH_2Cl_2, 69%; (h) vinylmagnesium bromide/ toluene, 60%. (MP: 4-methoxyphenyl, TBS: t-butyldimethylsilyl).*

induce distinctive conformational changes in the VDR to modulate the stability of the transcriptional machinery in certain cells. The differential stability of the protein complexes caused by ligands would be an interesting approach for separating the activities of **1**. The results will be reported elsewhere in detail.

Acknowledgements

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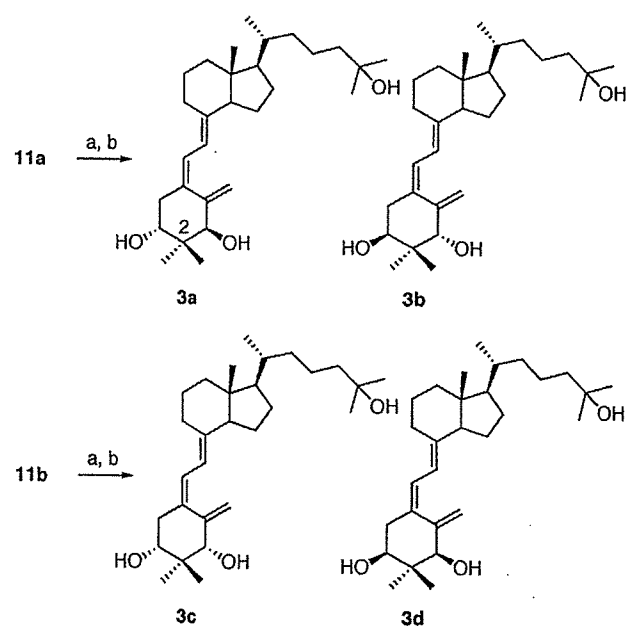

Figure 3. *Reagents and conditions: (a) CD-ring portion, $(Ph_3P)_4Pd$/ Et_3N-toluene, 63-66%; (b) TBAF/ THF, 29-63%.*

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Synthesis of Various Chiral Cyclic α,α-Disubstituted Amino Acids and Conformational Analysis of Their Peptides

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A chiral cyclic α,α-disubstituted α-amino acid; {(3R,4R)-1-amino-3,4-diazidocyclopentanecarboxylic acid; (R,R)-Ac_5c^{dN3}} was synthesized starting from dimethyl L-(+)-tartrate. The amino acid (R,R)-Ac_5c^{dN3} could be converted into several cyclic α,α-disubstituted α-amino acids having various functional groups.

Keywords: α,α-disubstituted α-amino acid, side-chain chiral center, peptide conformation, cyclic amino acid

Introduction

Helices shown in peptides and proteins, as secondary structures, almost always form the right-handed (*P*) helical screw direction. Scientists undoubtedly believe that the right-handedness is a result of the asymmetric center on the α-carbon atom of terrestrial L-α-amino acids. Among proteinogenic L-α-amino acids, isoleucine and threonine possess an additional chiral center, besides the α-carbon atom, on the side chain of amino acid. However, so far no attention has been paid as to how the chiral centers on the side-chain of amino acids affect the secondary structure of their peptides. Recently, we have reported that side-chain chiral centers of amino acids affect the secondary structures of their peptides [1]. That is, we designed and synthesized chiral cyclic α,α-disubstituted α-amino acids (dAAs); (*S*,*S*)-Ac_5c^{dOM} and (*R*,*R*)-$Ab_{5,6=}c$, in which the α-carbon atoms are not chiral centers but the asymmetric centers exist at the side-chain cycloalkane skeleton. The conformational analysis revealed that the (*S*,*S*)-Ac_5c^{dOM} homopeptides form left-handed (*M*) 3_{10}- and α-helices, and the (*R*,*R*)-$Ab_{5,6=}c$ homopeptides form both right-handed (*P*) and left-handed (*M*) 3_{10}-helices. These results indicate that the side-chain chiral environment (bulkiness or flexibility) may be important for control of the helical-screw sense of peptides. Thus herein, we report the synthesis of a chiral cyclic dAA; (3*R*,4*R*)-1-amino-3,4-diazidocyclopentanecarboxylic acid {(*R*,*R*)-Ac_5c^{dN3}}, in which the azido group can be converted into several functional groups, such as amino, triazole, and amide groups. Furthermore, we describe the peptides containing (*R*,*R*)-Ac_5c^{dN3} in Aib residues.

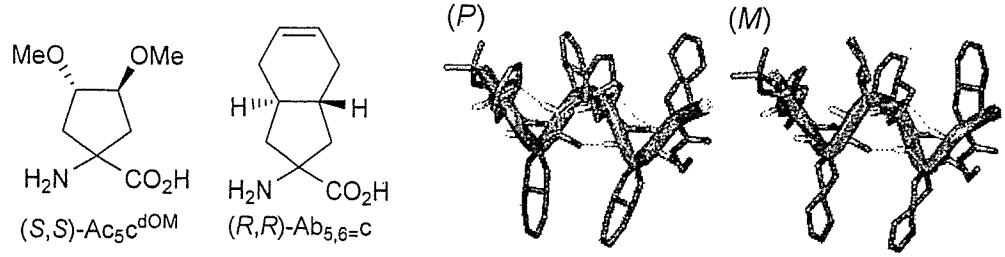

Fig. 1. Structures of (S,S)-Ac_5c^{dOM} and (R,R)-$Ab_{5,6=}c$, and X-ray structure of (R,R)-$Ab_{5,6=}c$ hexapeptide.

Results and Discussion

We synthesized an optically active (*R*,*R*)-Ac_5c^{dN3} starting from dimethyl L-(+)-tartrate. After conversion of dimethyl L-(+)-tartrate to diiodide (**1**), dimethyl malonate was bisalkylated with diiodide **1** to give a chiral cyclic diester (**2**). Deprotection of the MOM ether, conversion of diol into diazido function, and subsequent Curtius rearrangement generated the chiral cyclic (*R*,*R*)-Ac_5c^{dN3}. As expected, the amino acid (*R*,*R*)-Ac_5c^{dN3} could be efficiently converted into several dAAs having various functional groups, such as amino, triazole, amide functional groups.

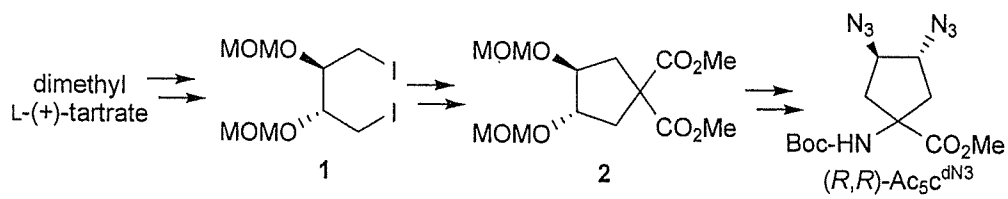

Fig. 2. Synthesis of (R,R)-Ac_5c^{dN3} from dimethyl L-(+)-tartrate.

We prepared peptides containing (*R*,*R*)-Ac_5c^{dN3} in Aib sequences. These peptides seemed to form 3_{10}-helical structures both in solution and in the solid state. The conformational analysis in detail will be published elsewhere.

Acknowledgments

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Chiral Cyclic α,α-Disubstituted α-Amino Acids Bearing Two Chiral Centers and Conformation of Their Peptides

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Two diastereomeric cyclic α,α-disubstituted α-amino acids {(1S,3S)-Ac_5c^{OM} and (1R,3S)-Ac_5c^{OM}} having two chiral centers at the side-chain cyclopentane ring and on the backbone α-carbon have been synthesized. The conformational analysis of their peptides seemed to form helical secondary structures.

Keywords: α,α-disubstituted α-amino acids, side-chain chiral center, helical-screw sense, secondary structure, peptide conformation

Introduction

Helical structures in proteins almost always form a right-handed (*P*) helical-screw sense, which is believed to result from the asymmetric center at the α-position of L-α-amino acids. Besides an asymmetric center at the α-position, L-Ile and L-Thr possess an additional chiral center at the side-chain β-position. However, no attention has been paid as to how the asymmetric center on the side chain affects the secondary structure of peptides. Recently, we have reported that the chiral centers on the side chain of cyclic α,α-disubstituted α-amino acids (dAAs) affect the helical secondary structures of their peptides [1]. That is to say, we synthesized chiral cyclic dAAs; (*S,S*)-Ac_5c^{dOM} and (*R,R*)-$Ab_{5,6=}c$, in which the α-carbon atoms are not chiral centers but the asymmetric centers exist at the side-chain cycloalkane skeleton, and studied the conformation of their peptides. The X-ray crystallographic analysis revealed that the (*S,S*)-Ac_5c^{dOM} homopeptides form left-handed (*M*) 3_{10}- and α-helices, while the (*R,R*)-$Ab_{5,6=}c$ homopeptides form both right-handed (*P*) and left-handed (*M*) 3_{10}-helices. Herein, we wish to report the synthesis of chiral cyclic dAAs; (1*S*,3*S*)-1-amino-3-methoxycyclopentanecarboxylic acid {(1*S*,3*S*)-Ac_5c^{OM}} and (1*R*,3*S*)-1-amino-3-methoxycyclopenatnecarboxylic acid {(1*R*,3*S*)-Ac_5c^{OM}}, which have two chiral centers at the side-chain cyclopentane ring and on the backbone α-carbon, and the conformational study of their homopeptides.

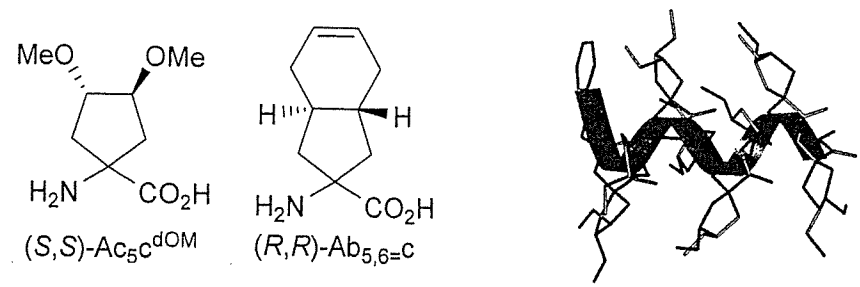

Figure 1. Structure of (S,S)-Ac_5c^{dOM} and (R,R)-$Ac_{5,6=}c$, and the X-ray structure of (S,S)-Ac_5c^{dOM} octapeptide.

Results and Discussion

Chiral cyclic dAAs (1*S*,3*S*)-Ac_5c^{OM} and (1*R*,3*S*)-Ac_5c^{OM} were synthesized starting from L-(-)-malic acid. That is to say, at first, the malic acid was converted to diiodide (**1**) by esterification, methylation of alcohol, reduction of esters, and substitution with iodide. Then, bisalkylation of dimethyl malonate with the diiodide **1** gave diester (**2**). Hydrolysis of monoester in **2**, and subsequent Curtius rearrangement afforded the chiral cyclic dAAs (1*S*,3*S*)-Ac_5c^{OM} and (1*R*,3*S*)-Ac_5c^{OM} in the ratio of 3 : 1. Recrystallization from hexane/ether afforded the pure chiral cyclic (1*S*,3*S*)-Ac_5c^{OM}.

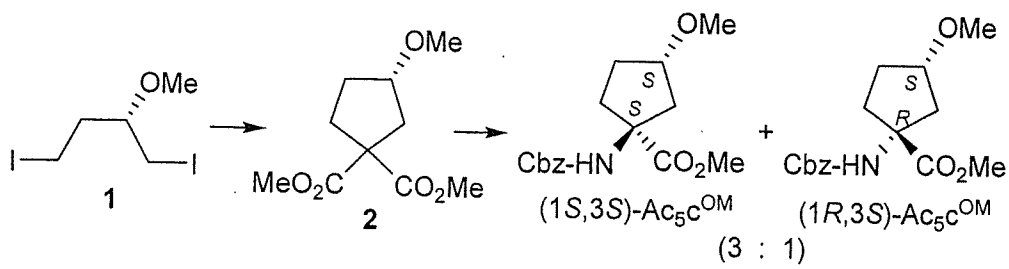

Figure 2. Synthesis of (1S,3S)-Ac_5c^{OM} and (1R,3S)-Ac_5c^{OM} from L-(-)-malic acid

We prepared (1*S*,3*S*)-Ac_5c^{OM} homopeptides by solution-methods, and studied the conformation by using the ^{1}H NMR, FT-IR, and X-ray crystallographic analysis. The peptides seemed to preferentially form helical structures. The detail of conformation analysis will be published elsewhere.

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science, and also by the Sasakawa Scientific Research Grant from the Japan Science Society.

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