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N-Linked Oligosaccharide Processing Enzymes as Molecular Targets for Drug Discovery

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Abstract: *N*-Linked oligosaccharide processing enzymes are key enzymes in the biosynthesis of *N*-linked oligosaccharides. These enzymes are a molecular target for inhibition by anti-viral agents that interfere with the formation of essential glycoproteins required in viral assembly, secretion and infectivity. We think that the molecular recognition of three kinds of glucosidases (family 13 and family 31 α-glucosidases and endoplasmic reticulum glucosidases) are different. Therefore, glycon and aglycon specificity profiling of glucosidases was an important approach for the research of glucosidase inhibitors. We carried out the profiling of glucosidases using small molecules as a probe. Moreover, we designed and synthesized three types of glucosidase inhibitors. These compounds were evaluated with regard to their ability to inhibit glucosidases *in vitro*, and were also tested in a cell culture system. We found some compounds having glucosidase inhibitory activity and anti-viral activity.

Key words: α-glucosidase, ER glucosidase, inhibitor, anti-viral activity

α-Glucosidases (EC 3.2.1.20) are also exo-acting carbohydrases, catalyzing the release of α-D-glucopyranose from the non-reducing ends of various substrates,[1,2] and on the basis of amino acid sequence similarities, α-glucosidases are classified into two families, family 13 and family 31.[3,4] Endoplasmic reticulum (ER) glucosidases, glucosidase I (EC 3.2.1.106) and glucosidase II (EC 3.2.1.84), are key enzymes in the biosynthesis of asparagine-linked oligosaccharides that catalyze the first processing event after the transfer of $Glc_3Man_9GlcNAc_2$ to proteins. These enzymes are a target for inhibition by anti-viral agents that interfere with the formation of essential glycoproteins required in viral assembly, secretion and infectivity.[5] Many papers reported that inhibitors of α-glucosidases are potential therapeutics for the treatment of such diseases as viral diseases, cancer and diabetes.[5,6] However, many screenings of α-glucosidase inhibitors did not use enzymes from target tissues or organs. We think that the molecular recognitions of three kinds of glucosidases (family 13, family 31 α-glucosidases and ER glucosidases) are different. Therefore, the glycon and aglycon specificity profiling of glucosidases has been an important approach for the research of glucosidase inhibitors.

In this research, we first describe the glycon and aglycon specificity profiling of glucosidases using small molecules as probes. Next, compounds designed and synthesized as glucosidase inhibitor candidates were evaluated with regard to their ability to inhibit three kinds of glucosidases. Finally, the glucosidase inhibitor candidates were tested for their anti-viral activities in a cell culture system.

Glycon specificity profiling of glucosidases using chemically modified substrates.

Chemically modified substrates are effective methods in the study of substrate specificity profiling. We have applied this approach to family 13 and family 31 α-glucosidases,[7–10] ER glucosidases,[11,12] α-galactosidases[8,13] and α-mannosidases[8,14] using partially substituted monosaccharides. We used all of the monodeoxy analogs of *p*-nitrophenyl α-D-glucopyranoside (PNP α-Glc) 1–4 (Fig. 1) as chemically modified substrates for glycon specificity profiling. We investigated the hydrolytic activities of family 13 and family 31 α-glucosidases and ER glucosidase II of PNP α-Glc and its deoxy derivatives 1–4, and checked the inhibitory activities of ER glucosidase I of PNP α-Glc and probes 1–4, so that PNP α-Glc was not a substrate for ER glucosidase I. These results are shown in Table 1.[11,12] Clearly, of the four deoxy derivatives of PNP α-Glc 1–4, family 31 α-glucosidases and ER glucosidase II hydrolyzed the 2-deoxy glucopyranoside (1); its activity with 1 appeared to be substantially higher than that with PNP α-Glc. Kinetic studies of the hydrolysis of PNP α-Glc, 1 and 2 were also carried out (Table 2).[9,11] The V_{max}/K_m or k_{cat}/K_m values of family 31 α-glucosidases

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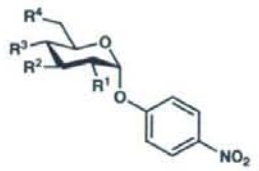
and ER glucosidase II for 1 was about twice as great as PNP α-Glc, which indicated that probe 1 was a good substrate for the enzymes. These reaction velocities to probe 1 increased to 3–28 fold that of PNP α-Glc. PNP Glc and probes 1–4 inhibited ER glucosidase I by 56.2, 71.7, 18.5, 22.2 and 32.3% at 5 mM, respectively. These results also indicated that ER glucosidase II might have properties similar to those found in family 31 α-glucosidases.

Aglycon specificity profiling and inhibition of glucosidases using heptitol derivatives.

For aglycon specificity profiling, we designed and synthesized eight probes, 5–12, including 1-amino-2, 6-anhydro-1-deoxy-D-*glycero*-D-*ido*-heptitol, which might mimic to a great extent the topography of α-D-glucopyranoside and modified aglycon of α-glucopyranoside (Fig. 2).[11] These probes do not have the specific functional groups for glycosidase inhibition, electrostatic interactions (*e.g.* 1-deoxynojirimycine), transition state mimetic structure (*e.g.* D-gluconolactone), or covalent bond formation with the enzyme catalytic site (*e.g.* conduritol B epoxide). The structures of α-glucosidase inhibitors are summarized in Fig. 3. We investigated the inhibitory activities of family 13 and family 31 α-glucosidases, ER glucosidases, and other glycosidases (β-glucosidase, α- and β-mannosidase, α- and β-galactosidase) against probes



1: R^1=H, R^2=R^3=R^4=OH
2: R^1=R^3=R^4=OH, R^2=H
3: R^1=R^2=R^4=OH, R^3=H
4: R^1=R^2=R^3=OH, R^4=H

Fig. 1. Chemical structure of glycon profiling probes 1–4.

5–12, and their aglycon specificity profiling was discussed. The values of the % inhibition and IC_{50} are summarized in Table 3.[12] Probe 8 indicated specific inhibitions of *Saccharomyces* (*S.*) *cerevisiae* (IC_{50}=55.5 μM) and *Bacillus* (*B.*) *stearothermophilus* (IC_{50}=415 μM) α-glucosidases. Probe 11 inhibited α-glucosidase from *S. cerevisiae* (IC_{50}=449 μM). Honey bee isozyme I (HBG I) was inhibited by probe 5 (IC_{50}=851 μM). Family 13 α-glucosidases and ER glucosidases were inhibited by the specific probes. On the other hand, family 31 α-glucosidases were broadly inhibited by probes 5–12. All probes did not inhibit β-glucosidase, α- or β-mannosidases, or α- or β-galactosidases at a 5-fold concentration. These facts indicated that aglycon specificities of α-glucosidases differed greatly among family 13 α-glucosidases, family 31 α-glucosidases and ER glucosidases. Moreover, each aglycon specificity of family 13 α-glucosidases is different in spite of the highly conserved amino acid sequences in the catalytic site.[13] In the kinetic studies on the inhibitions of 8 and 11 and the hydrolysis of PNP α-Glc by *S. cerevisiae* and *B. stearothermophilus* α-glucosidases, the values of *Ki* and K_m (mM) were calculated from Dixon plots and Michaelis-Menten plots, respectively, and these values and inhibition types are summarized in Table 4.[12] Probes 8 and 11 were competitive type inhibitors of the *S. cerevisiae* enzyme (*Ki*=0.13 mM and 0.50 mM). Probe 8 was a mixed type inhibitor of *B. stearothermophilus* enzyme (*Ki*=0.58 mM). The affinities of 8 against both enzymes were higher than PNP α-Glc as a substrate. These results indicated that probe 8 formed a specific hydrogen bond between the primary hydroxyl group of aglycon moiety and *S. cerevisiae* enzyme, and that probe 11, with a terminal phenyl group, formed a hydrophobic interaction with the *S. cerevisiae* enzyme.

Inhibition of α-glucosidase by reactive oxygen species.

The reactive oxygen species (ROS) generated com-

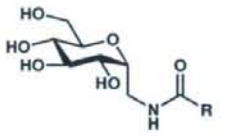
Table 1. Hydrolytic activities and inhibitory activities of probes 1–4 against glucosidases.[11,12]

Enzyme source	Relative rate of hydrolysis (%) / % Inhibition				
	PNP α-Glc	PNP 2D α-Glc (1)	PNP 3D α-Glc (2)	PNP 4D α-Glc (3)	PNP 6D α-Glc (4)
ER Processing glucosidase					
Rat microsome					
Glucosidase I	– / 56.2	– / 71.7	– / 18.5	– / 22.2	– / 32.3
Glucosidase II	100 / HD	189 / HD	– / –	– / –	– / –
	Relative rate of hydrolysis (%)				
α-Glucosidase family 13					
S. cerevisiae	100	–	–	–	–
B. stearothermophilus	100	–	–	–	–
Honey bee I	100	–	–	–	–
Honey bee II	100	–	–	–	–
Honey bee III	100	–	–	–	–
α-Glucosidase family 31					
Rice	100	175	–	–	–
Sugar beet	100	244	–	–	–
Flint corn	100	231	3.7	–	–
A. niger	100	259	11.9	–	–

Relative rate of hydrolysis was expressed by comparison with the amount of *p*-nitrophenol that was released from PNP α-Glc, which was taken as 100%. Assay of glucosidase I inhibitory activities used [^{3}H] glucose-labeled VSV glycoprotein as a substrate. –, Hydrolytic or inhibitory activity was not detected, HD, Hydrolyzing activity was observed.

Table 2. Kinetic study of hydrolysis of family 31 α-glucosidases and ER glucosidase II.[9,11]

Enzyme / Substrate	K_m (mM)	V_{max} (μmol/min/U)	V_{max}/K_m
ER glucosidase II			
PNP α-Glc	0.92	1.12	1.23
PNP 2D α-Glc (1)	0.76	3.44	4.53
Enzyme / Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat} / K_m
Rice α-glucosidase			
PNP α-Glc	2.62	43.8	16.7
PNP 2D α-Glc (1)	6.66	237	35.6
Sugar beet α-glucosidase			
PNP α-Glc	1.04	0.071	0.068
PNP 2D α-Glc (1)	5.70	0.64	0.11
Flint corn α-glucosidase			
PNP α-Glc	0.88	2.00	2.27
PNP 2D α-Glc (1)	7.38	17.0	2.30
PNP 3D α-Glc (2)	9.98	0.44	0.044
A. niger α-glucosidase			
PNP α-Glc	0.59	3.44	5.83
PNP 2D α-Glc (1)	6.09	96.9	15.9
PNP 3D α-Glc (2)	10.2	4.23	0.41



5: R=CH_2OH 9: R=$(CH_2)_5OH$
6: R=$(CH_2)_2OH$ 10: R=Me
7: R=$(CH_2)_3OH$ 11: R=Ph
8: R=$(CH_2)_4OH$ 12: R=$C(CH_3)_3$

Fig. 2. Chemical structure of aglycon profiling probes 5–12.

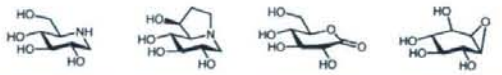


Fig. 3. Chemical structure of typical α-glucosidase inhibitor.

pounds, 13–24, shown in Fig. 4, were assessed as inhibitors of glycoside hydrolase family 13 α-glucosidases and family 31 α-glucosidases,[16] and the results are listed in Table 5 (Preparation for publication). Compounds 18 and 24, with a terminal α-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). We reasoned that the enzymatic liberation of the aglycon from compounds 18 and 24 might be followed by the ejection of a sulfinate anion with the concomitant formation of *p*-benzoquinone and *p*-benzoquinone imine, which would then generate ROS in the enzyme active site, leading to enzyme deactivation.[16,17] Therefore, the effects of compounds 18 and 24 on ROS-mediated DNA breakage were investigated. DNA strand scission in the super coiled pBR322DNA was induced by ROS in the presence of *p*-benzoquinone or *p*-benzoquinone imine, metal ion, and NADH.[17] Compound 24 induced DNA strand breakage condition in the above conditions (data not shown). We suggest that ROS-generated enzyme inhibition might be a new approach for the development of an enzyme inhibitor.

Inhibition of α-glucosidase by catechin derivatives.

The catechin derivatives 25–33 shown in Fig. 5 were assessed as inhibitors of family 13 and family 31 α-glucosidases, and the results are listed in Table 6.[18] A comparison of the results against family 13 and family 31 α-glucosidases shows that family 13 α-glucosidases were remarkably inhibited by catechin derivatives compared with family 31 α-glucosidases. The potent inhibition of family 13 α-glucosidases, *S. cerevisiae* and *B. stearothermophilus*, shown by catechin derivative 25 (IC_{50} = 1.2 μM and IC_{50} = 0.7 μM) and 30 (IC_{50} = 0.9 μM and IC_{50} = 1.1 μM), are in contrast to the weak activity shown by cate-

Table 3. Inhibitory activities of probes 5–12 against glycosidases.[12]

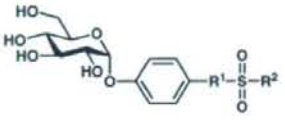
Enzyme source	% Inhibition (IC_{50})							
	5	6	7	8	9	10	11	12
Family 13 α-glucosidase								
S. cerevisiae	<1.0	21.1	<1.0	100 (55.5 μM)	<1.0	<1.0	67.4 (449 μM)	6.1
B. stearothermophilus	<1.0	<1.0	<1.0	100 (415 μM)	<1.0	<1.0	<1.0	<1.0
Honey bee I	52.3 (851 μM)	<1.0	<1.0	37.5	<1.0	10.4	4.6	<1.0
Honey bee II	4.4	2.7	3.6	21.4	4.4	<1.0	12.3	<1.0
Honey bee III	<1.0	3.2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Family 31 α-glucosidase								
Rice	10.7	8.5	7.6	18.3	26.0	21.8	16.0	3.8
Sugar beet	6.9	1.7	3.6	3.1	11.9	8.8	9.8	3.2
Flint corn	29.1	14.1	18.5	37.0	44.6	31.0	49.2	5.6
A. niger	6.6	2.6	<1.0	6.8	<1.0	23.3	14.0	1.2
ER processing glucosidase								
Glucosidase I	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	18.2	<1.0
Glucosidase II	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	5.9	<1.0
β-Glucosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
α-Mannosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
β-Mannosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
α-Galactosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
β-Galactosidase	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

Probe concentrations (family 13 and 31 α-glucosidases: 1 μmol/mL, ER processing α-glucosidases: 2 μmol/mL, β-glucosidase, mannosidases and galactosidases: 5 μmol/mL). Substrate (family 13 and 31 α-glucosidases, ER glucosidase II: PNP α-Glc, ER glucosidases I: [^{3}H] glucose-labeled vesicular stomatitis virus glycoprotein, β-glucosidase: PNP β-Glc, α-mannosidase: PNP α-Man, β-mannosidase: PNP β-Man, α-galactosidase: PNP α-Gal, β-galactosidase: PNP β-Gal).

Table 4. Kinetic studies of the inhibition of family 13 α-glucosidases.[12]

Probe	*S. cerevisiae*		*B. stearothermophilus*	
	Ki (mM)	Inhibition type	*Ki* (mM)	Inhibition type
8	0.13	Competitive	0.58	Mixed
11	0.50	Competitive	–	–
PNP α-Glc	0.35*	–	1.16*	–

*K_m value.

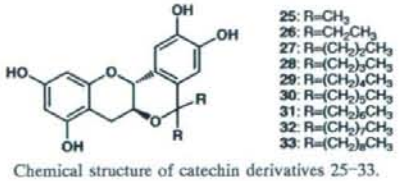


13: R^1=O, R^2=NO_2
14: R^1=O, R^2=Cl
15: R^1=O, R^2=CF_3
16: R^1=O, R^2=CH_3
17: R^1=O, R^2=$C(CH_3)_3$
18: R^1=O, R^2=α-naphthyl
19: R^1=NH, R^2=NO_2
20: R^1=NH, R^2=Cl
21: R^1=NH, R^2=CF_3
22: R^1=NH, R^2=CH_3
23: R^1=NH, R^2=$C(CH_3)_3$
24: R^1=NH, R^2=α-naphthyl

Fig. 4. Chemical structure of ROS-generated compounds 13–24.

Table 5. Inhibitory activity of ROS-generated compounds 13–24 against α-glucosidases.

Compound	IC_{50} (μM)		
	Glycoside hydrolase family 13		Glycoside hydrolase family 31
	S. cerevisiae	*B. stearothermophilus*	Rice
13	499	>500	>500
14	437	>500	>500
15	407	>500	>500
16	499	>500	>500
17	391	>500	>500
18	51.7	60.1	>500
19	239	218	>500
20	200	254	>500
21	146	244	>500
22	231	325	>500
23	136	237	>500
24	74.1	89.1	>500



25: R=CH_3
26: R=CH_2CH_3
27: R=$(CH_2)_2CH_3$
28: R=$(CH_2)_3CH_3$
29: R=$(CH_2)_4CH_3$
30: R=$(CH_2)_5CH_3$
31: R=$(CH_2)_6CH_3$
32: R=$(CH_2)_7CH_3$
33: R=$(CH_2)_8CH_3$

Fig. 5. Chemical structure of catechin derivatives 25–33.

chin derivative 26, which has one methylene group long alkyl side chain compared with 25 (IC_{50}=47.5 μM and IC_{50}=26.8 μM) and catechin derivative 33 which has three methylene groups long alkyl side chain compared with 30 (IC_{50}=64.0 μM and IC_{50}=28.1 μM). From these results, it is thought that the inhibition mechanism of catechin derivative 25 and the inhibition mechanism of catechin derivative 30 are different. The IC_{50} values of typical α-glucosidase inhibitor 1-deoxynojirimycine (see Fig. 3) and catechin derivative 30 against *S. cerevisiae* α-glucosidase

Table 6. Inhibitory activity of catechin derivatives 25–33 against α-glucosidases.[18]

Compound	IC_{50} (μM)			
	Glycoside hydrolase family 13		Glycoside hydrolase family 31	
	S. cerevisiae	*B. stearothermophilus*	Rice	*A. niger*
Catechin	>500	>500	>500	>500
25	1.2	0.7	>500	>500
26	47.5	26.8	>500	>500
27	37.5	28.4	>500	>500
28	2.1	14.2	>500	>500
29	5.3	6.8	248	>500
30	0.9	1.1	>500	>500
31	4.9	21.1	>500	>500
32	33.2	13.8	>500	>500
33	64.0	28.1	>500	>500

were 3.3[19] and 0.9 μM, respectively. This result indicated that catechin derivative 30 is about 3.6 times more potent than 1-deoxynojirimycine when their IC_{50} values are compared.

Anti-viral activity of α-glucosidase inhibitors.

Compounds 1–33 were assayed with regard to their ability to inhibit glycoprotein processing at the cellular level. Vesicular stomatitis virus glycoprotein (VSV G) was prepared from VSV-infected and probe-treated baby hamster kidney (BHK) cells.[11] 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 compounds 1–24 except for catechin derivatives (25–33) inhibited processing glycosidases. The catechin derivatives had the possibility of inhibition of processing glycosidases (data not shown). Then, we assayed the anti-virus activities by effects of the catechin derivatives of processing glycosidases on virus glycoprotein synthesis and syncytium formation after newcastle disease virus (NDV) infection, and effects on synthesis and cell surface expression of NDV glycoprotein, hemagglutinin-neuraminidase (HANA) glycoprotein in whole cell lysates were quantified. Moreover, viral infectivity was determined by a plaque assay in BHK cells.[20] In the above assays, catechin derivative 30 showed potent inhibition of the viral infectivity (Table 7, Preparation for publication).

Conclusion and perspectives.

The discovery of glucosidase inhibitors may help us to understand the roles of the oligosaccharides of glycoproteins and glycolipids in cellular functions, and pharmaceutical applications. From this study, it is better to use enzymes of target tissues or organs for the screening of agents for viral diseases, cancer and diabetes. Moreover, in applying glucosidases as inhibitors of glycoprotein processing, inhibitory action of many inhibitors at the cellular levels is not so remarkable, as expected based on their action at the enzyme level. This was speculated to be caused by the difficulty for inhibitors to be able to access the site of action. We think that high throughput

Table 7. Anti-viral activity of catechin derivatives at the cellular level.

Compound	Conc. (μM)	% HAU	SF	% PFU	CPU
Catechin	500	100	+	95	+
	250	100	+	100	+
	125	100	+	NT	+
	63	100	+	NT	+
25	500	0	−	0	+
	250	6	−	14	+
	125	100	+	100	+
	63	100	+	100	+
26	500	0	−	0	−
	250	0	−	0	+
	125	100	+	24	+
	63	100	+	85	+
27	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	100	+	90	+
28	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	100	+	90	+
29	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	100	+	50	−
	31	100	+	100	+
	16	100	+	100	+
30	500	0	−	0	−
	250	0	−	0	−
	125	0	−	0	−
	63	9	+/−	25	−
	31	100	+	95	+
	16	100	+	100	+
33	500	0	−	0	−
	250	0	−	0	−
	125	25	−	50	−
	63	100	+	100	+

screening assays using specific probes and enzymes of target tissues or organs and highly effective design and synthesis of inhibitors *in silico* are necessary for the development of new and potent glucosidase inhibitors.

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N-結合型糖鎖プロセッシング酵素を分子標的とした創薬

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現在，新 H5N1 型インフルエンザや SARS など続々と出現する新興ウイルス感染症や鳥インフルエンザのヒトへの伝播等，新興ウイルス感染症は人類の脅威となっている．しかし，ウイルス感染症に対する有効な薬剤の開発は，細菌感染症の抗生物質に比べ遅れている．そこで，ウイルス共通の感染機序に基づいた薬剤の開発が重要と考え，外被を有する多くのウイルスの感染・増殖には複合型の *N*-結合型糖鎖が関与している知見を基にして，小胞体 *N*-結合型糖鎖プロセシング酵素を標的酵素とした分子標的薬の開発を目指して研究を行っている．分子標的薬の開発には，標的酵素である糖鎖プロセシング酵素の基質特異性の解明が必要であると考えた．そこで，合成プローブを用いて *N*-結合型糖鎖プロセシングの第 1 段階を担うプロセシンググルコシダーゼ I (EC 3.2.1.106)と第 2 段階を担うプロセシンググルコシダーゼ II (EC 3.2.1.84)のグリコンおよびアグリコン特異性を調べ，α-グルコシダーゼ (EC 3.2.1.20, GH13 and GH31) のそれと比較した．その結果，グルコシダーゼ I のグリコン特性は GH13 α-グルコシダーゼと，グルコシダーゼ II のグリコン特性は GH31 α-グルコシダーゼと同様であった．またグルコシダーゼ I とグルコシダーゼ II のアグリコン認識は同様であり，GH13 および GH31 α-グルコシダーゼとは異なっていた．そこで，プロセシンググルコシダーゼ I および II を標的として，酵素阻害剤候補化合物の設計と合成を行った．これら候補化合物の *in vitro* 酵素阻害活性と細胞レベルでのウイルス外被糖タンパク質の合成・成熟・転送阻害およびプラーク法による感染性ウイルス数の測定を行った．その結果，*in vitro* においてヘプチトール誘導体，スルフォニル誘導体の一部に IC_{50} 約 50 μM の阻害活性を，カテキン誘導体の一部に IC_{50} 0.9 μM の強力な阻害活性を見いだした．さらに，細胞レベルではカテキン誘導体の一部にプロセシンググルコシダーゼ阻害を作用点とするとみられる比較的強い抗ウイルス活性を見いだした．今後，ウイルス外被糖タンパク質の糖鎖構造解析等により詳細な作用機序の解明を行う予定である．

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〔質 問〕 食総研 徳安

1) 安全性の高いカテキン骨格をリード化合物として，カテキン骨格を含む阻害剤の設計と合成を行っていますが，その阻害剤の「安全性が高い」という理由はなにか．

2) ウイルスに対してカテキン誘導体の効果があったが，α-グルコシダーゼに対して作用した結果なのか．

〔答〕

1) 誘導体合成前のカテキンの安全性が高いからといって，カテキン骨格を有する誘導体の安全性が高いということはできません．しかし，安全性の高い骨格を創薬リード化合物として用いることは，毒性を回避するという目的において理にかなっていると考えています．また，本誘導体はカテキン骨格をほぼ維持しているので，毒性発現の可能性を低く抑えられるのではないかと考えております．

2) α-グルコシダーゼに対する阻害効果なのかどうか，直接の証拠はありませんが，*in vitro* での強い阻害活性およびウイルスを感染させた培養細胞の形態から α-グルコシダーゼ阻害を作用機序とする抗ウイルス作用であると考えております．今後，ウイルス粒子を回収し，そのウイルス外被糖タンパク質の糖鎖解析を行うことにより，作用点を解明したいと考えております．

〔質 問〕 食総研 北岡

1) グルコシダーゼ阻害剤のリード化合物として，数ある安全性の高い物質の中から，カテキンを選択した理由はなにか．

2) グルコシダーゼ以外の糖質加水分解酵素の阻害剤になっている可能性はあるのでしょうか．

〔答〕

1) 安全性の高い物質は他にもたくさんありますが，カテキンには弱いながらも血糖上昇抑制作用が報告されており，腸管グルコシダーゼ阻害が示唆されておりますので，リード化合物として選択いたしました．

2) グルコシダーゼ阻害以外の阻害活性があることは否定できません．現時点では，一部の α-マンノシダーゼに対する阻害活性がないことだけ確認しております．

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Regular Paper

Design and Synthesis of α-Glucosidase Inhibitor Having DNA Cleaving Activity

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Abstract: Apoptosis, or programmed cell death, is a mechanism by which cells undergo death to control cell proliferation or in response to DNA damage. The present study was designed to explore small molecule apoptosis inducers for antitumor agents. The synthesis of 4-sulfonylphenyl α-D-glucopyranoside derivatives 1–6 and 4-(sulfonylamino)phenyl α-D-glucopyranoside derivatives 7–12, endoplasmic reticulum (ER)-targeted small molecules that were designed to induce apoptosis from ER stress by ER glucosidase inhibition and DNA damage is described. 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). Moreover, compound 12 strongly induced the DNA strand breakage condition. When compounds 1–12 were assayed for their ability to inhibit processing by glucosidases at the cellular level, no effects on glycoprotein processing were observed.

Key words: α-glucosidase, inhibitor, DNA cleavage, apoptosis, ER stress

The cell is perturbed by environmental stress conditions. In order to avoid cell death from the stress, cells must sense and respond to stress, including viral infection, genetic mutation, chemical insult, and nutrient depletion.[1] In the ER, stress is a condition that accumulates misfolded or unfolded proteins by disturbing these ER circumstances. Specific response programs are activated to circumvent each type of stress. The ER stress induces a coordinated adaptive program called the unfolded protein response (UPR).[2] The UPR is activated upon disruption of the ER environment by such events as the inhibition of *N*-linked oligosaccharide processing, which results in the accumulation of unfolded or misfolded proteins in the ER.[3] *N*-Linked oligosaccharide processing is carried out by ER glucosidases I and II. Both enzymes are key enzymes in the biosynthesis of *N*-linked oligosaccharides that catalyze the first processing event after the transfer of $Glc_3Man_9GlcNAc_2$ to proteins.[4] The inhibition of ER glucosidases induces the accumulation of unfolded proteins in the ER, and increases ER stress. The UPR caused by ER stress is insulted due to DNA damage, and the cell is led to apoptosis. Apoptosis targets are currently being explored for antitumor agent discovery, such as the tumor-necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptors, the BCL2 family of anti-apoptotic proteins, and inhibitor of apoptosis (IAP) proteins.[1,5]

We think that the inhibition of ER glucosidases can be used to trigger ER stress, and that the ER stress may trigger the UPR. Further, following interruption of the UPR by DNA damage, the cell is led to apoptosis. We think that compounds that have α-glucosidase inhibitory activity and DNA breakage activity may be developed into an ER-targeted small molecule apoptosis inducer for use as an antitumor agent. We have already elucidated the molecular recognition properties[6–13] and the inhibition[13,14] of α-glycosidases necessary for the molecular design of glycosidase inhibitors using synthetic probes. Based on our knowledge, we designed compounds 1–12 to have α-glucosidase inhibitory activity and DNA breakage activity (Fig. 1). The enzymatic liberation of the aglycon from compounds 1–12 might be followed by the ejection of a R^2SO_2H with the concomitant formation of *p*-benzoquinone or *p*-benzoquinone imine,[15] which would then generate reactive oxygen species (ROS), leading to DNA breakage,[16] shown in Fig. 2. The group of Taylar *et al.* has developed a series of 4-(sulfonylamino)phenyl α-D-glucopyranosides.[15] These compounds have been reported to act as competitive yeast α-glucosidase inhibitors. We suspect that these compounds may also be enhanced in their inhibitory activity by changing the sulfonamide of 4-(sulfonylamino)phenyl α-D-glucopyranoside to sulfonate, since the liberation of *p*-benzoquinone is easier than that of *p*-benzoquinone imine.

In this report, we first describe the design and synthesis series of 4-sulfonylphenyl α-D-glucopyranoside derivatives 1–6 and 4-(sulfonylamino)phenyl α-D-glucopyranoside derivatives 7–12. These compounds 1–12 were evaluated with regard to their ability to inhibit three kinds of α-glucosidases, and the effects of α-glucosidase triggered

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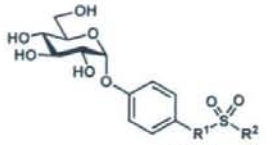

1: R^1=O, R^2=4-NO_2-C_6H_4
2: R^1=O, R^2=4-Cl-C_6H_4
3: R^1=O, R^2=4-CF_3-C_6H_4
4: R^1=O, R^2=4-CH_3-C_6H_4
5: R^1=O, R^2=4-$C(CH_3$-$C_6H_4)_3$
6: R^1=O, R^2=2-naphthyl
7: R^1=NH, R^2=4-NO_2-C_6H_4
8: R^1=NH, R^2=4-Cl-C_6H_4
9: R^1=NH, R^2=4-CF_3-C_6H_4
10: R^1=NH, R^2=4-CH_3-C_6H_4
11: R^1=NH, R^2=4-$C(CH_3)_3$-C_6H_4
12: R^1=NH, R^2=2-naphthyl

Fig. 1. Chemical structure of target compounds 1–12.

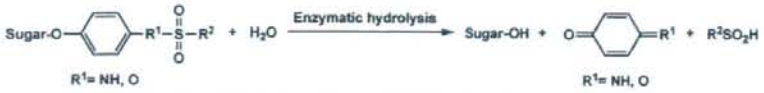

Fig. 2. Schematic diagram of enzymatic liberation of quinone derivatives.

ROS-mediated DNA breakage. Finally, these compounds were also tested in a cell culture system.

MATERIALS AND METHODS

General methods. Optical rotations were measured with a JASCO DIP-370 digital polarimeter at 25°C. The NMR spectra were recorded with a Varian Mercury 400 spectrometer (400 MHz for ^{1}H). Chemical shifts were expressed in ppm downfield relative to Me_4Si. Low resolution mass spectra were obtained with a Waters MicroMass ZQ instrument under positive and negative ion ESI conditions. Column chromatography was performed on silica gel 60 (0.063–0.200 mm, Merck). The progress of all reactions was monitored by thin-layer chromatography on silica gel 60 F_{254} (0.25 mm, Merck).

Method A. To the solution of α-arbutin (1, 0.5 g, 1.8 mmol) in 100 mL of dry acetone was added triethylamine (NEt_3, 10 mL) and the sulfonyl chloride derivative (2.8 mmol). After the mixture was stirred for 15 min, the resulting salt was removed by filtration through a cotton filter, and the solvent was concentrated.

Method B. To the solution of α-arbutin (1, 1.0 g, 3.7 mmol), in 50 mL of dry acetone was added dry potassium carbonate (K_2CO_3, 1.52 g, 11 mmol) and the sulfonyl chloride derivative (5.5 mmol). After the mixture was stirred overnight, the K_2CO_3 was removed by filtration through Celite, and the solvent was concentrated.

Method C. To the solution of *p*-nitrophenyl α-D-glucopyranoside (14, 1.0 g, 3.3 mmol), in pyridine (50 mL) at room temperature was added acetic anhydride (10 mL). The mixture was stirred overnight and poured into water. The product was extracted with AcOEt (3 × 50 mL) and washed with water, 1 M HClaq, satd. $NaHCO_3$aq and brine, and then dried over Na_2SO_4. The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford a quantitative yield of 15. A mixture of compound 15 (1.6 g, 3.3 mmol) in ethanol (EtOH, 100 mL) was hydrogenated under H_2 with 20% palladium hydroxide on carbon (150 mg). After the mixture was stirred for 2 h, the palladium charcoal was removed by filtration through Celite and the solvent was concentrated. The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 1.5 g (93.9%) of 16.

Method D. To the solution of compound 16 (0.5 g, 1.2 mmol), in pyridine (20 mL) at room temperature was added sulfonyl chloride derivative (1.36 mmol). The mixture was stirred for 10 min and poured into water. The product was extracted with AcOEt (3 × 50 mL) and washed with water, 1 M HClaq, satd. $NaHCO_3$aq and brine, and then dried over Na_2SO_4, and the solvent was concentrated.

Method E. A mixture of methanol (MeOH) : NEt_3 : H_2O (5:1:1, 60 mL) was added to a stirred solution of the sulfonyl derivative (0.85 mmol). After the mixture was stirred for 6 h at room temperature, the solvent was evaporated.

Synthesis of compounds 1–12.

4-(4-Nitrobenzenesulfonyl)phenyl α-D-glucopyranoside (1). According to method A, compound 1 was prepared from 13 (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 dichloromethane (CH_2Cl_2)-MeOH) to afford 0.46 g (54.8%) of 2: $[\alpha]_D$ +14.9° (*c* 0.93, MeOH); ^{1}H NMR (CD_3OD) δ 3.38 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=10.0 Hz, H-4), 3.55 (dd, 1H, J_{1-2}= 3.8 Hz, J_{2-3}=9.8 Hz, H-2), 3.58 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=5.2 Hz, J_{5-6b}=2.4 Hz, H-5), 3.67 (dd, 1H, J_{5-6a}=5.0 Hz, J_{6a-6b}=11.8 Hz, H-6a), 3.73 (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), 5.42 (d, 1H, *J*=3.6 Hz, H-1), 6.95 (d, 2H, *J*=8.8 Hz, -OC_6H_4O-), 7.12 (d, 2H, *J*=9.2 Hz, -OC_6H_4O-), 8.07 (d, 2H, *J*=9.2 Hz, -$SO_2C_6H_4NO_2$-), 8.44 (d, 2H, *J*=9.2 Hz, -$SO_2C_6H_4NO_2$), MS: 480 (M+Na)$^+$.

4-(4-Chlorobenzenesulfonyl)phenyl α-D-glucopyranoside (2). According to method A, compound 2 was prepared from 13 (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.59 g (72.5%) of 3: $[\alpha]_D$ +13.8° (*c* 1.51, MeOH); ^{1}H NMR (CD_3OD) δ 3.40 (dd, 1H, J_{3-4}=9.0 Hz, J_{4-5}=9.8 Hz, H-4), 3.55 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}=9.6 Hz, H-2), 3.59 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=5.4 Hz, J_{5-6b} =2.2 Hz, H-5), 3.67 (dd, 1H, J_{5-6a}=5.4 Hz, J_{6a-6b}=12 Hz, H-6a), 3.73 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.81 (dd, 1H, J_{2-3}=9.6 Hz, J_{3-4}=9.2 Hz, H-3), 5.43 (d, 1H, *J*=3.6 Hz, H-1), 6.92 (d, 2H, *J*=9.6 Hz, -OC_6H_4O-), 7.12 (d, 2H, *J*=9.2 Hz, -OC_6H_4O-), 7.62 (d, 2H, *J*=8.8 Hz, -$SO_2C_6H_4Cl$), 7.78 (d, 2H, *J*=8.8 Hz, -$SO_2C_6H_4Cl$), MS: 469 (M+Na)$^+$.

4-(4-Trifluorobenzenesulfonyl)phenyl α-D-glucopyranoside (3). According to method A, compound 3 was prepared from 13 (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.59 g (88.4%) of 3: $[\alpha]_D$ +11.7° (*c* 1.25, MeOH); ^{1}H NMR (CD_3OD) δ 3.40 (dd, 1H, J_{3-4}=9.0 Hz, J_{4-5}=9.8 Hz, H-4), 3.55 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}= 10.0 Hz, H-2), 3.58 (m, 1H, H-5), 3.66 (dd, 1H, J_{5-6a}=5.0

Hz, J_{6a-6b}=11.8 Hz, H-6a), 3.73 (dd, 1H, J_{5-6b}=2.4 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.81 (dd, 1H, J_{2-3}=J_{3-4}=9.4 Hz, H-3), 5.43 (d, 1H, J=3.6 Hz, H-1), 6.93 (d, 2H, J=9.2 Hz, -OC_6H_4O-), 7.12 (d, 2H, J=9.2 Hz, -OC_6H_4O-), 7.94 (d, 2H, J=8.0 Hz, -$O_2C_6H_4O$-), 8.02 (d, 2H, J=8.4 Hz, -$SO_2C_6H_4CF_3$), MS: 503 (M+Na)$^+$.

4-(4-Methylbenzenesulfonyl)phenyl α-D-glucopyranoside (4). According to method B, compound 4 was prepared from 13 (1.1 g, 5.5 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 1.03 g (63.4%) of 4: $[\alpha]_D$ +18.4° (*c* 0.97, MeOH); ^{1}H NMR (CD_3OD) δ 2.44 (s, 3H, -CH_3), 3.39 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=10.0 Hz, H-4), 3.54 (dd, 1H, J_{1-2}=3.8 Hz, J_{2-3}=9.8 Hz, H-2), 3.58 (ddd, 1H, J_{4-5}= 10.0 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), 3.73 (dd, 1H, J_{5-6b}= 2.6 Hz, J_{6a-6b}=11.8 Hz, H-6b), 3.80 (dd, 1H, J_{2-3}=9.8, J_{3-4} =9.0 Hz, H-3), 5.41 (d, 1H, J=3.6 Hz, H-1), 6.88 (d, 2H, J=9.6 Hz, -OC_6H_4O-), 7.09 (d, 2H, J=9.2 Hz, -OC_6H_4O-), 7.40 (d, 2H, J=8.0 Hz, -$SO_2C_6H_4CH_3$), 7.66 (d, 2H, J=8.4 Hz, -$SO_2C_6H_4CH_3$), MS: 449 (M+Na)$^+$.

4-(4-*tert*-Butylbenzenesulfonyl)phenyl α-D-glucopyranoside (5). According to method B, compound 3 was prepared from 13 (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.39 g (43.9%) of 5: $[\alpha]_D$ +13.6° (*c* 1.40, MeOH); ^{1}H NMR (CD_3OD) δ 1.36 (s, 9H, -C (CH_3)$_3$), 3.39 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=10.0 Hz, H-4), 3.54 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}=9.6 Hz, H-2), 3.59 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=5.0 Hz, J_{5-6b}=2.5 Hz, H-5), 3.66 (dd, 1H, J_{5-6a}=5.0 Hz, J_{6a-6b}=11.8 Hz, H-6a), 3.73 (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), 5.41 (d, 1H, J=3.6 Hz, H-1), 6.89 (d, 2H, J=9.2 Hz, -OC_6H_4O-), 7.10 (d, 2H, J=9.2 Hz, -OC_6H_4O-), 7.64 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4C$ (CH_3)$_3$), 7.72 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4C$ (CH_3)$_3$), MS: 491 (M+ Na)$^+$.

4-(2-Naphthalenesulfonyl)phenyl α-D-glucopyranoside (6). According to method B, compound 6 was prepared from 13 (0.5 g, 1.8 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.63 g (74.6%) of 6: $[\alpha]_D$ +12.3° (*c* 1.32, MeOH); ^{1}H NMR (CD_3OD) δ 3.38 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=10.0 Hz, H-4), 3.52 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}=9.6 Hz, H-2), 3.55 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=4.8 Hz, J_{5-6b} =2.8 Hz, H-5), 3.64 (dd, 1H, J_{5-6a}=4.8 Hz, J_{6a-6b}=12.0 Hz, H-6a), 3.68 (dd, 1H, J_{5-6b}=2.8 Hz, J_{6a-6b}=12.0 Hz, H-6b), 3.78 (dd, 1H, J_{2-3}=J_{3-4}=9.0 Hz, H-3), 5.38 (d, 1H, J=3.6 Hz, H-1), 6.89, 7.05 (d, 2H × 2, J=9.6 Hz, -OC_6H_4O-), 7.64–7.83, 8.00–8.11, 8.35 (m, 7H, -$SO_2C_{10}H_7$), MS: 485 (M+Na)$^+$.

4-(4-Nitrophenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (17). According to methods C and D, compound 17 was prepared from 16 (0.5 g, 1.1 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.67 g (94.6%) of 17.

4-(4-Chlorophenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (18). According to methods C and D, compound 18 was prepared from 16 (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.54 g (93.1%) of 17.

4-(4-Trifluoromethylphenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (19). According to methods C and D, compound 19 was prepared from 16 (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.65 g (99.9%) of 19.

4-(4-Methlphenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (20). According to methods C and D, compound 20 was prepared from 16 (0.5 g, 1.1 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.64 g (93.1%) of 20.

4-(4-tert-Butylphenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (21). According to methods C and D, compound 20 was prepared from 16 (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.55 g (88.5%) of 21.

4-(2-Naphthalenephenylsulfonylamino)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (22). According to methods C and D, compound 22 was prepared from 16 (0.4 g, 1.0 mmol). The product was purified by column chromatography on silica gel (1:1 hexane-AcOEt) to afford 0.53 g (92.5%) of 22.

4-(4-Nitrophenylsulfonylamino)phenyl α-D-glucopyranoside (7). According to method E, compound 7 was prepared from 17 (0.7 g, 1.1 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.41 g (83.3%) of 7: $[\alpha]_D$ +12.1° (*c* 1.23, MeOH); ^{1}H NMR (CD_3OD) δ 3.38 (dd, 1H, J_{3-4}=8.8 Hz, J_{4-5}=10.0 Hz, H-4), 3.53 (dd, 1H, J_{1-2}=3.6 Hz, J_{2-3}= 9.6 Hz, H-2), 3.59 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=5.0 Hz, J_{5-6b}=2.4 Hz, H-5), 3.65 (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}=9.2 Hz, H-3), 5.38 (d, 1H, J= 3.6 Hz, H-1), 7.0 (d, 2H, J=8.8 Hz, -OC_6H_4NH-), 7.05 (d, 2H, J=9.2 Hz, -OC_6H_4NH-), 7.91 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4NO_2$), 8.31 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4NO_2$), MS: 455 (M−H)$^-$.

4-(4-Chlorophenylsulfonylamino)phenyl α-D-glucopyranoside (8). According to method E, compound 8 was prepared from 18 (0.54 g, 0.9 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.38 g (95.2%) of 8: $[\alpha]_D$ +13.4° (*c* 1.42, MeOH); ^{1}H NMR (CD_3OD) δ 3.39 (dd, 1H, J_{3-4} =9.2Hz, J_{4-5}=10.0 Hz, H-4), 3.53 (dd, 1H, J_{1-2}=3.8 Hz, J_{2-3}=9.8 Hz, H-2), 3.60 (ddd, 1H, J_{4-5}=10.0 Hz, J_{5-6a}=4.8 Hz, J_{5-6b}=2.6 Hz, H-5), 3.66 (dd, 1H, J_{5-6a}=4.8 Hz, J_{6a-6b} =11.9 Hz, H-6a), 3.72 (dd, 1H, J_{5-6b}=2.6 Hz, J_{6a-6b}=11.9 Hz, H-6b), 3.80 (dd, 1H, J_{2-3}=J_{3-4}=9.2 Hz, H-3), 5.38 (d, 1H, J=4.0 Hz, H-1), 6.98 (d, 2H, J=9.2 Hz, -OC_6H_4NH-), 7.05 (d, 2H, J=9.6 Hz, -OC_6H_4NH-), 7.48 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4Cl$), 7.65 (d, 2H, J=8.8 Hz, -$SO_2C_6H_4Cl$), MS: 444 (M−H)$^-$.

4-(4-Trifluoromethylphenylsulfonylamino)phenyl α-D-glucopyranoside (9). According to method E, compound 9 was prepared from 19 (0.7 g, 1.0 mmol). The product was purified by column chromatography on silica gel (5:1 CH_2Cl_2-MeOH) to afford 0.44 g (90.9%) of 7: $[\alpha]_D$ +13.4°

(*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: $[\alpha]_D$ +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: $[\alpha]_D$ +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: $[\alpha]_D$ +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-

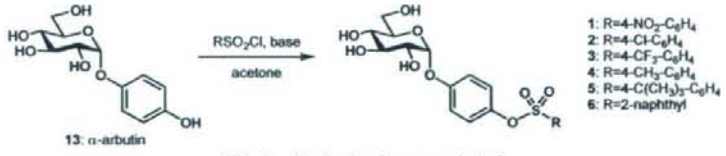

Fig. 3. Synthesis of compounds 1–6.

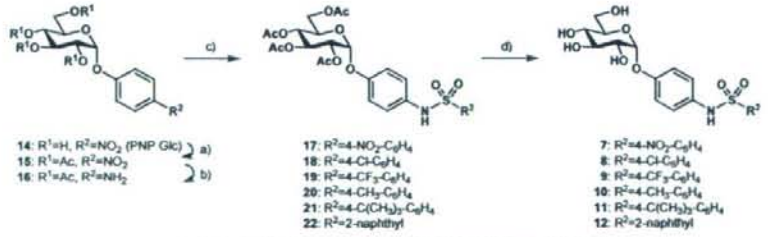

Fig. 4. Synthesis of compounds 7–12.

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.

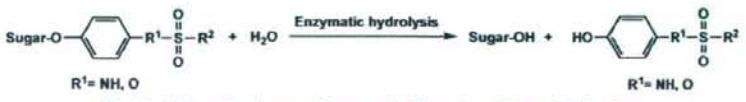

Fig. 5. Schematic diagram of enzymatic liberation of phenol derivatives.

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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The Japanese Peptide Society (2006)

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

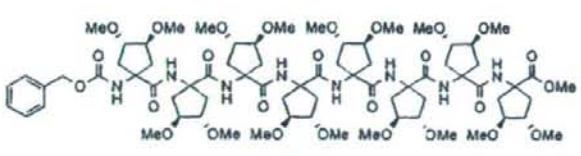
Introduction

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. difference of energies was small. There were both right- and left-handed 3_{10}-helic the solid state [4]. These results indicated computational simulation u conformational search calculations could predict the helical screw sense oligopeptides containing chiral α,α-disubstituted amino acids.



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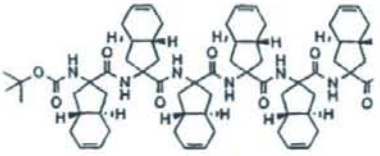

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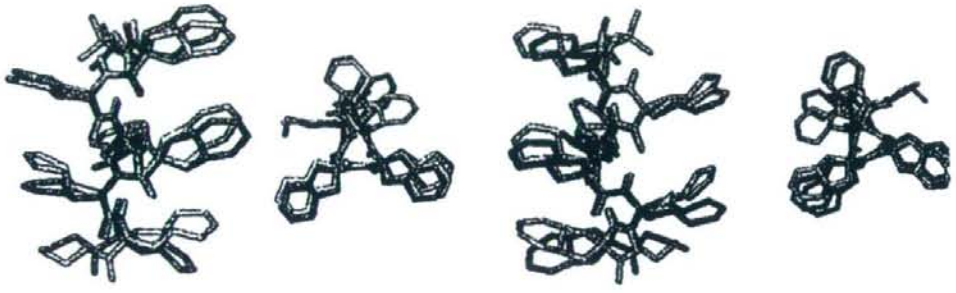
Fig. 1. Structures of peptides



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

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

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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' —— **CTTTTT**ACATGCTA —— 3'
3' —— **GAAAAA**TGTACGAT —— 5'

DNA $D^D(0)$

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

DNA D^Dmis

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

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

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^Dmis. 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.

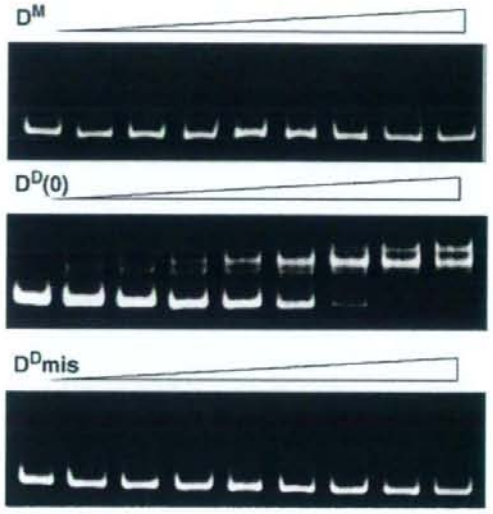

Figure 3. Polyacrylamide gel mobility shift assay showing the titration with increasing amounts of PNA **1** to dsDNA D^M, $D^D(0)$, D^Dmis. PNA concentrations were 0, 0.1, 0.19, 0.34, 0.63, 1.2, 2.2, 4.1, and 7.5 μM, respectively, from left to right. Conditions: 10 mM sodium phosphate buffer, 1 mM EDTA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

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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

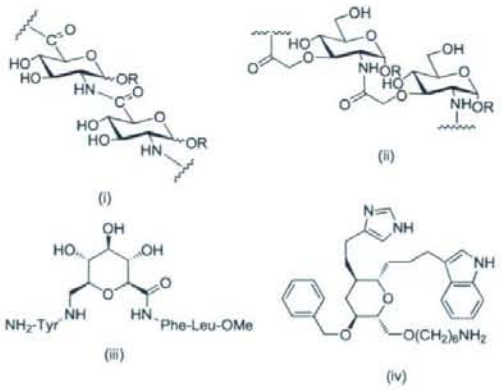

Figure 1. Amido-linked carbohydrates.

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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such as (iv) by Smith and Nicolaou et al.,[15] while the molecule was not incorporated into the peptide backbone. Furthermore, other sugar-containing amino acids, prepared with both furanose and pyranose residues, were reported.[16–19] Although those compounds have interesting structures, no suggestions of biological activity were shown in the papers.

In work directed toward designing new carbohydrate mimetics, we demonstrated a new kind of carbohydrate analogue, which has the possibility for developing solid-phase synthesis, and also for studying different kinds of new drugs. As shown in Figure 2, in the general structure of our designed amide-linked oligosaccharide analogues (i), the monosaccahride component (ii) has a C-1 carboxylate group, and one of the hydroxy groups is replaced with a protected amino group. Thus, the monosaccharide unit (iii) is regarded as an amino acid derivative, and, therefore, the chain elongation reaction becomes an easy peptide bond formation. The carbohydrate mimetics synthesized are amide-linked tetrasaccharide analogues, in which the monosaccharide residues are linked to each other via the C-1 carbonyl of one sugar to the amino group of another sugar; for example, **1**, a β(1→2)-amide-linked analogue[20] from D-glucosamine, **2**, a β(1→3)-amide-linked analogue[21] from D-glucose, **3**, a β(1→4)-amide-linked analogue[21] from D-galactose, **4**, a β(1→6)-amide-linked analogue[22] from D-glucose, as a new class of carbohydrate mimetics as shown in Figure 3. These analogues are, as expected, resistant to glycosidases (α- and β-glucosidases). We investigated the biological activities of sulfated analogues **18** and **33**, such as anti-HIV activity and inhibition of sialyl Lewis x selectin-mediated cell adhesion. The result revealed that these *O*-sulfated oligomers effectively inhibited HIV infection, sialyl Lewis x-mediated cell adhesion, and heparanase activity in a linkage-specific manner.[21] Therefore, these molecules have potential applications as drugs that block protein–protein interactions and inhibit enzyme catalysis.

Furthermore, our compounds can be assumed as non-natural amino acid analogues with additional functionality on the molecules because of their hydroxyl groups and ring oxygen. Among those analogues, which possess the structural and functional features of both carbohydrates and peptides, our analogues have both a carboxylate group at the anomeric position and an amino group replacing one of the OH groups of the sugar. The structural preferences of our compounds **1–4** have been investigated in nuclear magnetic resonance (NMR) and circular dichroism (CD) experiments.[21] As

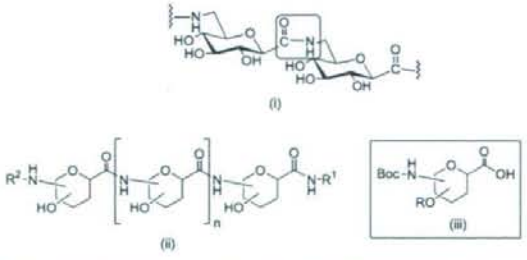


Figure 2. The general structure of our designated amido-linked oligosaccharide analogues.

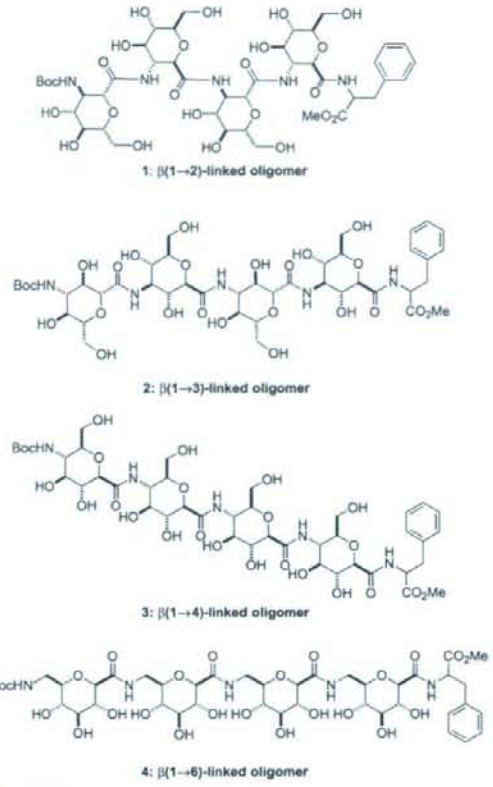


Figure 3. A new class of carbohydrate mimetics.

a result, they were found to form rigid secondary structures, in particular the β(1→2)-linked tetramer, which were very likely to form a 14-helical structure. From this information, we predicted that if our glycosamino acid monomer was polymerized, the resulting homooligomer would have the property of a unique folding peptide. The folding of a polypeptide chain into the stable three-dimensional structure of a biologically active protein is still not understood in detail; however, several research groups have recently reported successful atomistic simulations of secondary-structure formation, including the formation of helices of different types, β turns and β sheets of α- and β-peptides.[23–28] Insight into the nature of the folding state has been obtained from various studies simulating the reversible folding of peptides.[29–32] On the basis of this background, we further predicted that the various unique biological activities of our sulfated analogues were due to their three-dimensional structure. Therefore, we examined the additional conformational analysis of our oligomers with computer modeling (molecular dynamics calculations), and explored the application possibility of new physiologically active substances.

We herein report the detailed synthetis method of the tetrameric β(1→2)- and β(1→6)-carbopeptoids. Furthermore, the results of the conformational study of the homooligomer with molecular modeling calculations are reported.

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%

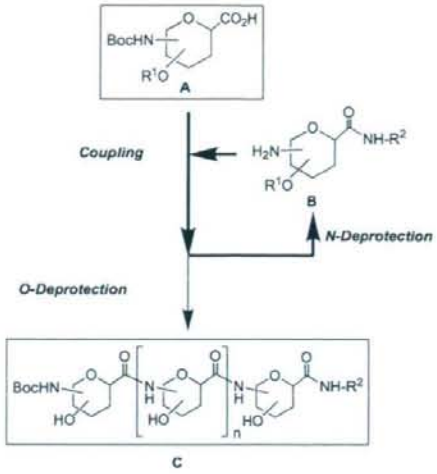
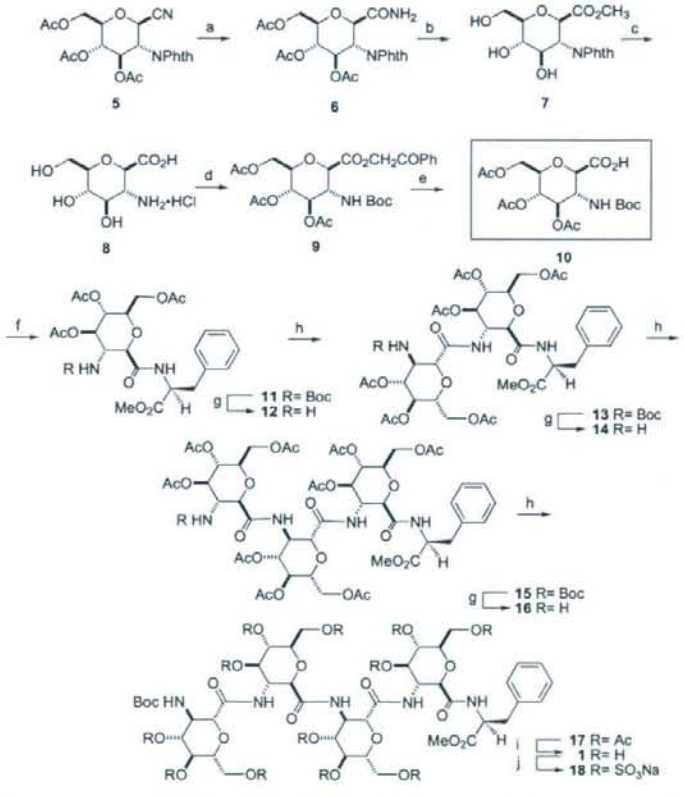


Figure 4. General synthetic strategy.



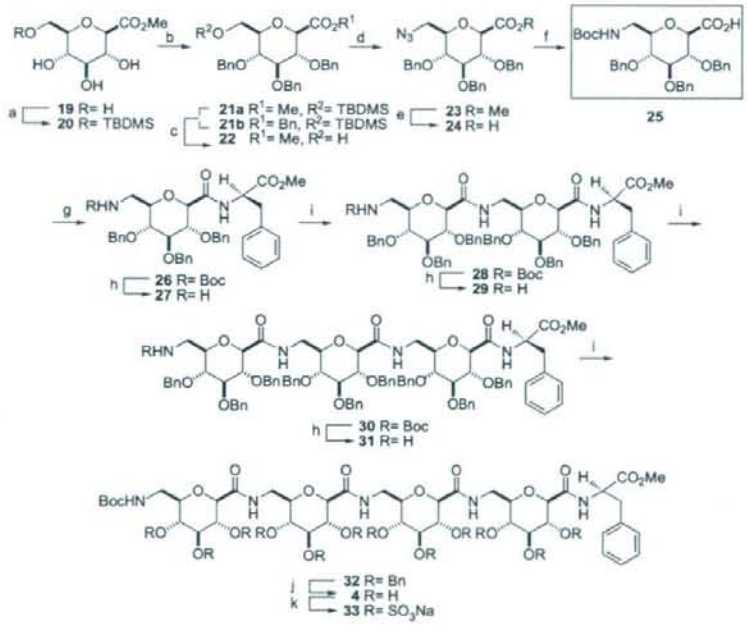
Scheme 1. Synthesis of a carbopeptoid tetramer **1**. Reagents and conditions: (a) 30% HBr–AcOH, 3 h, 0 °C to rt, 85%; (b) Dowex 50W-X8 [H^+], MeOH, 16 h, 80 °C, 97%; (c) (i) 6 equiv of LiOH, MeOH/H_2O (3:1), 16 h, 60 °C, (ii) 3 N HCl, 3 h, reflux, 95% overall; (d) (i) 2 equiv of BOC-ON, Et_3N, dioxane/H_2O (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.

HBr–AcOH, then converted to a $CONH_2$ group (**6**) in 85% yield. Hydrolysis of the amide group of **6** with Dowex 50W-X8 [H^+] in refluxing MeOH gave methyl ester derivative (**7**) in 97% yield. The removal of acetyl groups and the phthaloyl group was accomplished by successive treatment with aqueous LiOH and 3 N HCl to give an amine derivative (**8**) in 95% overall yield. Protection of the amino group was carried out with BOC-ON,[34] then esterification of the C-1 carboxylate with 2-bromoacetophenone and conventional acetylation of hydroxyl groups afforded **9** in 73% overall yield. Finally, the monomeric component (**10**) was obtained by hydrogenation of **9** in 90% yield.

To form polysaccharide mimetics, the C-terminal group of **10** was first linked to L-phenylalanine methyl ester. C-Terminal modification can be replaced by a polymer support and applied for the solid-phase synthesis of oligo- and polysaccharide mimetics. Coupling of **10** with L-phenylalanine methyl ester using diethylphosphoryl cyanide (DEPC)[35] and Et_3N gave **11** in 86% yield. Removal of the Boc group of **11** with 2 N HCl/EtOAc gave **12** in 95% yield. The elongation reaction of **14** with **10** was accomplished with BOP[36] reagent and diisopropylethylamine (DIEA) in DMF to give the coupling product in 59% yield. Repetition of the same synthetic manipulation: (i) removal of the Boc group and (ii) coupling with the monomeric component (**10**), easily produced the trimer (**15**) and tetramer (**17**). The tetramer (**17**) was *O*-deacetylated with NaOMe in MeOH to give **1** in 68% yield.

Scheme 2 summarizes the synthesis of the β(1→6)-linked homooligomer **4** starting with a known methyl D-*glycero*-D-gulo-hepturonate (**19**), which was reported by Lehmann et al.[10] For the introduction of the 6-amino group, the primary hydroxyl group 6-OH of **19** was selectively silylated with TBDMSCl to afford **20**, and the remaining 2,3,4-trihydroxyl groups of **20** were benzylated with Ag_2O and BnBr in DMF. The benzylation of **20** gave a 1:1 mixture of a methyl ester (**21a**) and the corresponding benzyl ester (**21b**) in 75% yield. Presumably, the basic condition caused an ester-exchange reaction between the CO_2Me and BnOH generated by the hydrolysis of BnBr. The silyl group of **21a** and **21b** was removed under acidic conditions, and the crude product was then treated with MeOH under basic conditions to give **22** in 78% overall yield. Tosylation of the primary OH followed by conversion to azido gave **23** in 81% overall yield. The methyl ester of **23** was hydrolyzed with aqueous LiOH, followed by successive reduction of azido with H_2/Lindlar catalyst,[37] and protection of the amino group with Boc_2O to obtain the monomeric building block **25**. The monomeric component **25** was first coupled with phenylalanine using the same method as Scheme 1 in 92% yield. The formation of polysaccharide mimetics **4** was also carried out repeating the same method as Scheme 1.

O-Sulfation of the dimer, trimer, and tetramer of each of the β-amide-linked oligosaccharide analogues **1** and **4** produced the corresponding *O*-sulfated derivatives **18** and **33**.[21] The β(1→2)- (**1**) or β(1→6)-tetramer (**4**) was treated with an



Scheme 2. Synthesis of a carbopeptoid tetramer **4**. Reagents and conditions: (a) 1.2 equiv of TBDMSCl, 2.5 equiv of imidazole, DMF, 2 h, 0 °C, 83%; (b) 9 equiv of BnBr, 6 equiv of Ag_2O, DMF, 20 h, rt, 75%; (c) (i) AcOH/THF/H_2O (3:1:1), 15 h, rt, (ii) 25% NaOMe, MeOH, 1 h, rt, 78% overall; (d) (i) TsCl, pyridine, 12 h, 0 °C to rt, (ii) 2 equiv of NaN_3, DMF, 12 h, 60 °C, 81% overall; (e) 2 equiv of LiOH·H_2O, MeOH/THF/H_2O (3:3:1), 3 h, rt, 82%; (f) (i) Lindlar catalyst, H_2, MeOH, 3 h, rt, (ii) 1.5 equiv of Boc_2O, 2 equiv of LiOH·H_2O, MeOH/H_2O (3:1), 12 h, rt, 54% overall; (g) 1.2 equiv of L-phenylalanine methyl ester, 1.5 equiv of DEPC, 3 equiv of Et_3N, 16 h, 0 °C to rt, 92%; (h) 2 N HCl in EtOAc, 3 h, 0 °C to rt; (i) 1.2 equiv of **25**, 1.5 equiv of DEPC, 3 equiv of Et_3N, 16 h, 0 °C to rt; (j) 10% Pd/C, H_2, MeOH, 16 h, rt, 86%; (k) 10 equiv of sulfur trioxide trimethylamine complex, 5 days, 50 °C.