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Synthesis and evaluation of α-helix mimetics based on a trans-fused polycyclic ether: sequence-selective binding to aspartate pairs in α-helical peptides

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Abstract—Inspired by the topological similarity between ladder-like cyclic ether skeletons and α-helical peptides, a trans-fused 6/6/6/6 tetracyclic ether containing two hydroxyl groups separated by a distance of 4.8 Å was designed as a scaffold for a nonpeptidic α-helix mimetic. Two alkyl guanidinium groups were attached to the hydroxyl groups to develop a synthetic receptor for the specific recognition of $i+4$ spaced aspartate pairs on the surface of an α-helical peptide. A circular dichroism (CD) titration showed that this mode of molecular recognition stabilizes α-helical structures of peptides containing $i+4$ spaced aspartate pairs.

The rational design of small molecules that recognize protein surfaces and subsequently disrupt protein–protein interactions is an ongoing challenge.[1] One approach to meeting this challenge is the design of molecular scaffolds that mimic the surface functionality projected along one face of an α-helix. As well as investigations of cross-linked interfacial peptides,[2] β-peptides,[3] and oligoamide foldamers,[4] synthetic approaches to developing nonpeptidic molecules of α-helix mimetics have been reported.[5] In this context, we recently designed a structurally defined polycyclic ether scaffold as an α-helix mimetic,[6] inspired by the topological similarity between ladder-like cyclic ether marine toxins and α-helical peptides.[7] In the 6/6/6/6 trans-fused polycyclic ether system shown in Figure 1a, the distance between skeletal oxygen atoms on the same side (4.8 Å) of the cyclic ethers is almost identical to the interval between the side-chains of α-helical peptides in the canonical i, $i+4$ relationship (ca. 5 Å). In addition, it is thought that incorporation of the oxygen atoms on the skeleton results in moderate aqueous solubility. Despite extensive efforts toward the total synthesis of complex marine toxins,[8] attempts to design functional molecules based on a trans-fused cyclic ether framework, harnessing the potential of privileged molecular properties, have been limited to date.[9] In the present report, we describe the synthesis of a 6/6/6/6 tetracyclic ether scaffold **1** possessing two equatorial hydroxyl groups (C4 and C10) separated by a distance of 4.8 Å (Fig. 1b). An α-helix mimetic **2**, which has two guanidinium groups linked to **1** through two hydroxyl groups, has also been developed. By exploiting the ability of alkylguanidinium groups to bind strongly to carboxylates in polar solvents (Fig. 1a),[10] the sequence selective binding of the synthetic receptor **2** to aspartate pairs in α-helical peptides were evaluated by circular dichroism (CD) spectroscopy.

To construct the trans-fused 6/6/6/6 tetracyclic ether skeleton, we planned to assemble two tetrahydropyrans

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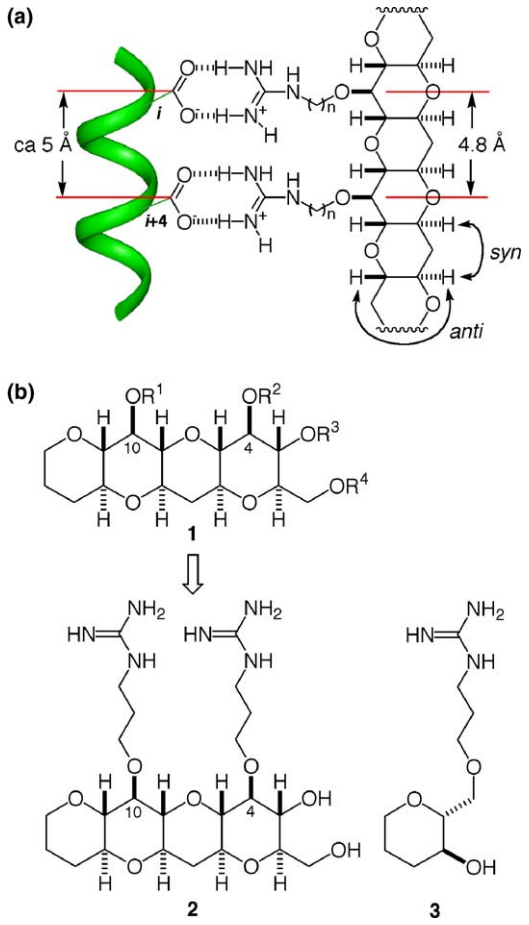
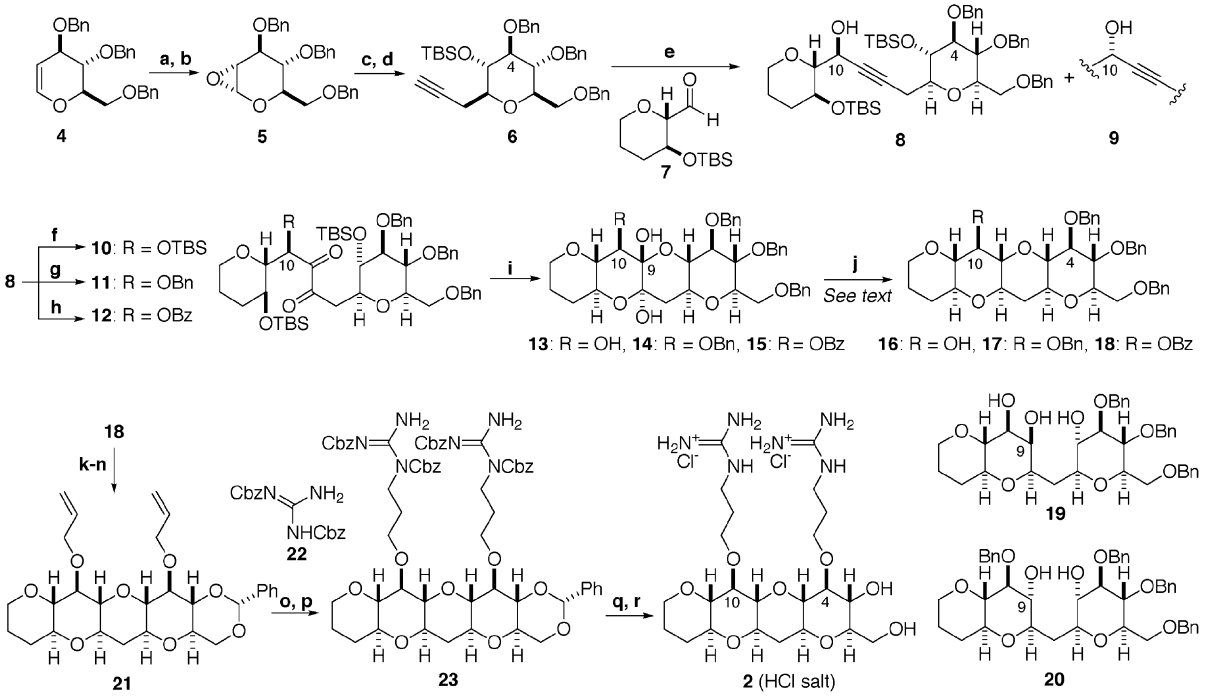


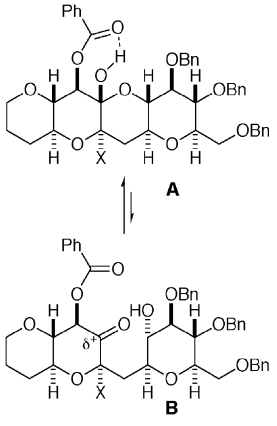
Figure 1. (a) Illustration of the binding of a cyclic ether-based synthetic receptor to an α-helical peptide through simultaneous hydrogen bondings between guanidinium and carboxylate groups. (b) Design of a cyclic ether scaffold **1**, a synthetic receptor **2** containing two guanidinium groups, and a half-receptor **3**.

based on the convergent strategy developed by Fujiwara et al.[11] Nakata and co-workers,[12] and Mori et al.[13] As shown in Scheme 1, this assembly involves (i) acetylide-mediated connection of two tetrahydropyrans (**6** + **7** → **8**), (ii) oxidation of the alkyne group to an α-diketone (**8** → **10**), (iii) double cyclization to tetracyclic dihemiacetal (**10** → **13**), and (iv) stereocontrolled reduction of the dihemiacetal, leading to the tetracyclic ether framework (**13** → **16**). To incorporate the two equatorial hydroxyl groups into the C4 and C10 positions of the tetracyclic ether skeleton, the coupling component, acetylene **6** containing the C4 hydroxyl group was synthesized from tri-*O*-benzyl-D-glucal **4**. Epoxidation of **4** using Spilling's method, followed by addition of propargyl Grignard reagent $HCCCH_2MgBr$, yielded **6** in 53% yield (3 steps).[14] The C10 hydroxyl group was installed by stereoselective addition of the lithium acetylide of **6** to aldehyde **7**,[15] resulting in a mixture of separable diastereomers (**8**:**9** = 2.7:1.0) in quantitative yield. Protection of the resulting hydroxyl group of **8** and subsequent oxidation of the internal acetylene with RuO_2–$NaIO_4$ gave diketones **10**–**12**. Exposure of the diketones to CSA in $MeOH/CH_2Cl_2$ (4:1) effected the removal of the TBS groups and in situ bishemiacetal formation to afford tetracyclic products **13**–**15** in good yields.[16] It was anticipated that the Et_3SiH/TMSOTf-mediated reduction of bishemiacetals **13**–**15** to form **16**–**18** would be affected by participation of the neighboring C10 hydroxyl group. In fact, the reduction of **13** (R = OH) failed to provide the desired tetracyclic ether **16**; instead, triol **19** was obtained in 89% yield.[17] The C10 hydroxyl group was thus protected as a benzyl ether, inhibiting the ring-opening to form **19**. Reduction of **14** under identical conditions afforded the desired tetracyclic ether **17** in moderate yield (61%) along with substantial amounts of diol **20** (26%).[17] After considerable experimentation, the optimized result was attained by reduction of **15**, which contains a benzoyl ester group at C10. Upon treatment of **15** with Et_3SiH–TMSOTf at 0 °C, the desired tetracyclic ether **18** was obtained in 91% yield in a stereocontrolled manner without the formation of products having C9 hydroxyl groups corresponding to **19** and **20**.[18,19] It is likely that hydrogen bond formation between the carbonyl oxygen and the C10 hemiacetal group, along with the electron-withdrawing nature of the benzoyl group, stabilize the hemiacetal (**A**) and facilitate reduction leading to **18** (Scheme 2).[20] Protecting group manipulation of **18** and subsequent allylation of the resulting secondary alcohols gave **21**. The allyl groups were subjected to hydroboration, followed by reaction with *N*,*N*′-bis(benzyloxycarbonyl)guanidine **22** under Mitsunobu conditions to give **23** in 77% yield.[21] Hydrogenolysis of the benzyloxycarbonyl and the benzylidene acetal groups liberated the hydrochloride salt of **2** in 88% yield (2 steps).

After obtaining the tetracycle **2**, interactions with α-helical peptides were evaluated by CD spectroscopy. We prepared a family of 16-mer peptides with two aspartate groups at different positions ($i+3$, $i+4$, $i+5$, and $i+11$) along the chain, which included C- and N-terminal capping (Fig. 2).[22] All peptides were used as their bistetramethylammonium salts in 10% H_2O/90% MeOH at 25 °C and were designed to possess significant α-helical character under the conditions used.[23] Upon addition of increasing amounts of receptor **2** (up to 4 equiv: 1.23 mM) into the solution of the peptide $i+4$, the CD spectrum showed a marked increase (5%) in α-helicity (minimum at 222 nm), as shown in Figure 3. In comparison, the increase in helicity for the $i+3$ peptide was far less obvious, and no substantial alterations of the peptide structures of $i+5$ and $i+11$ were observed upon addition of 4 equiv of receptor **2** to the peptides (Fig. 3b). To verify the effect of single-point binding on peptide conformational stability, a similar CD titration of the $i+4$ peptide was carried out using the half-receptor **3**. The change in the peptide structure was not clear. Thus, these results suggest that receptor **2** binds preferentially to the helically oriented $i+4$ peptide, in which the rigid cyclic ether scaffold orients two guanidinium groups to interact simultaneously



Scheme 1. Reagents and conditions: (a) NBS, THF/H_2O (10/1), 0 °C; (b) $KN(SiMe_3)_2$, toluene, −78 °C; (c) $HCCCH_2MgBr$, 53% (3 steps); (d) TBSCl, imidazole, DMF, 60 °C, 89%; (e) *n*-BuLi, THF, −80 °C, then **7**, −80 to −20 °C, **8** (73%), **9** (27%); (f) TBSCl, imidazole, DMF, 40 °C, 86%, then RuO_2, $NaIO_4$, CCl_4/MeCN/H_2O (1/1/1.5), **10** (61%); (g) benzyl bromide, NaH, THF/DMF (3/1), 99%, then RuO_2, $NaIO_4$, CCl_4/MeCN/H_2O (1/1/1.5), **11** (70%); (h) benzoyl chloride, DMAP, pyridine, 96%, then RuO_2, $NaIO_4$, CCl_4/MeCN/H_2O (1/1/1.5), **12** (73%); (i) CSA, MeOH/CH_2Cl_2 (4/1), **13** (73%), **14** (90%) or **15** (96%); (j) Et_3SiH, TMSOTf, MeCN/CH_2Cl_2 (3/1), −40 to 0 °C; (k) H_2, $Pd(OH)_2$, AcOEt/MeOH (5/1); (l) $PhCH(OMe)_2$, CSA, DMF, 95% (2 steps); (m) K_2CO_3, MeOH; (n) allyl bromide, NaH, THF/DMF (4/1), 85% (2 steps); (o) $(Sia)_2BH$, THF, 0 °C, then H_2O_2, NaOH 71%; (p) **22**, DEAD, PPh_3, THF, 77%; (q) H_2, $Pd(OH)_2$/C, MeOH/AcOEt (1/3); (r) MeOH, concd HCl (10/1), 88% (2 steps).



Scheme 2.

with two carboxylates spaced by 4–5 Å in an approximately parallel arrangement, as illustrated in Figure 1a. The resulting binding curves of the $i+4$ peptide with **2** were fitted by a 1:1 binding model,[25] and the association constants were calculated as $K_a = 1.00 \times 10^3\ M^{-1}$.[26]

In conclusion, a small-molecule receptor for specific recognition of $i+4$ spaced aspartate pairs on the surface of α-helical peptides has been developed. We also showed that this mode of molecular recognition promotes α-helicity of the peptide in aqueous media. Further efforts will be directed to exploit the structurally defined cyclic ether scaffold for the development of cell-permeable small molecules that bind to a larger area of the protein surface and disrupt protein–protein interactions.

***i* + 3** : Ac-Ala-Ala-Gln-**Asp**-Ala-Ala-**Asp**-Ala-Ala-Ala-Ala-Ala-Gln-Ala-Ala-Tyr-NH_2
***i* + 4** : Ac-Ala-Ala-Gln-**Asp**-Ala-Ala-Ala-**Asp**-Ala-Ala-Ala-Ala-Gln-Ala-Ala-Tyr-NH_2
***i* + 5** : Ac-Ala-Ala-Gln-**Asp**-Ala-Ala-Ala-Ala-**Asp**-Ala-Ala-Ala-Gln-Ala-Ala-Tyr-NH_2
***i* + 11** : Ac-Ala-Ala-Gln-**Asp**-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Gln-Ala-Ala-**Asp**-Tyr-NH_2

Figure 2. Substrate peptide sequences; target aspartates are shown in bold.

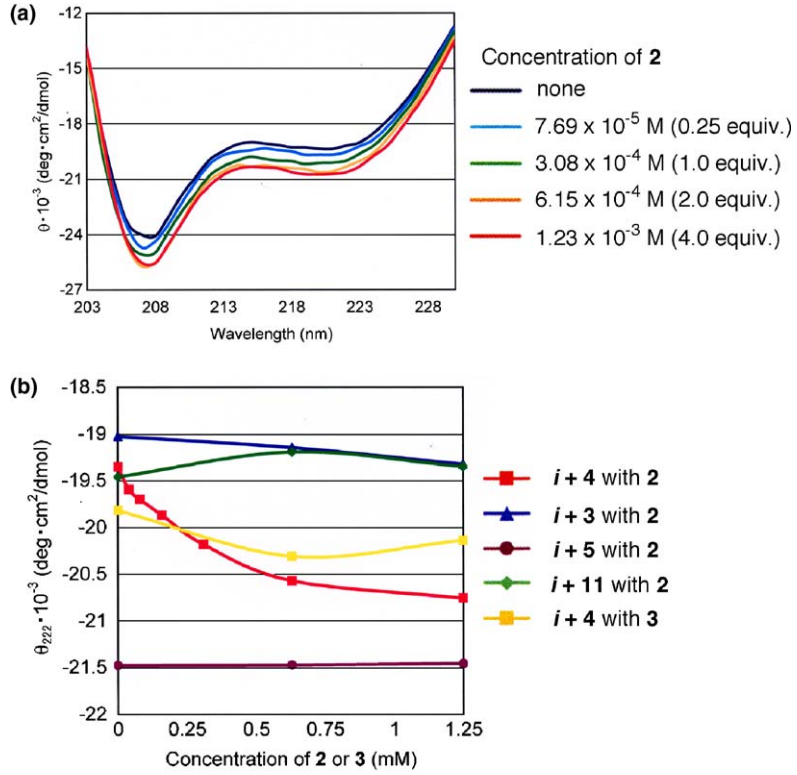


Figure 3. (a) CD spectra of peptide $i+4$ (0.314 mM) in the absence or presence of the receptor **2** (0–1.23 mM (4 equiv)) in 10% water/methanol at 25 °C. (b) Ellipticity at 222 nm for each peptide in the presence of increasing amounts of receptor **2**: (■) $i+4$ (0.314 mM), (▲) $i+3$ (0.341 mM), (●) $i+5$ (0.299 mM), and (◆) $i+11$ (0.273 mM) in 10% water/methanol at 25 °C. Control titration of $i+4$ (0.314 mM) with the half-receptor **3** is also shown (■). Uncertainties in all CD measurements were estimated to be ±10%.

Acknowledgments

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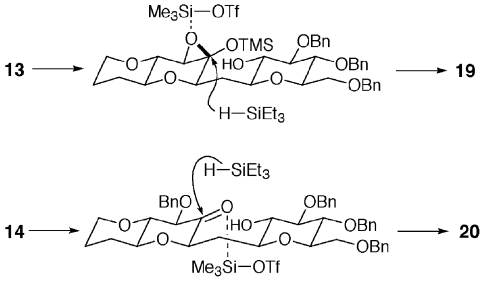
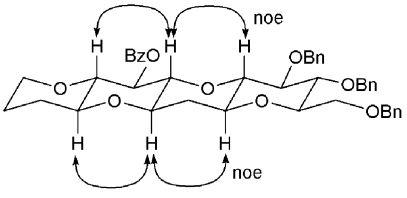
16. Attempts to convert bishemiacetal **14** into bismethylketals by treatment with CSA and $CH(OMe)_3$ in $MeOH/CH_2Cl_2$ were unsuccessful.
17. The stereochemistries of **19** and **20** were unambiguously determined by NMR analysis after acetylation. The coupling constants between H9 and H10 for the triacetate of **19** and the diacetate of **20** were $J = 6.0$ and 9.0 Hz, respectively. A plausible explanation for the stereochemical outcome at C9 is as follows.



18. The stereochemistry of **18** was unambiguously determined by NOE experiments.



19. Mori et al. reported that similar reduction of the tetracyclic bishemiacetal without the C10 hydroxyl group resulted in the formation of a complex mixture or an unexpected skeletal rearrangement yielding a spiroketal, see Ref. 13a.
20. Reduction of the corresponding acetate in place of benzoate **15** resulted in the formation of the desired tetracyclic ether in a lower yield (36%). This suggests that the electron-withdrawing nature of the benzoate group plays an important role in this conversion.
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23. Ellipticity is reported as the mean residue ellipticity. The degree (fraction, *f*) of helicity was calculated from $f = -(\Theta_{222} + 2340)/30300$.[24] The percentages of helicity of the peptides were determined as $i+3$ (63%), $i+4$ (56%), $i+5$ (71%), and $i+11$ (64%) by monitoring the ellipticity at 222 nm using the average of 120 points collected over 60 s.
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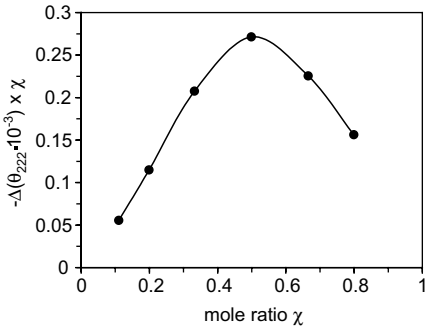

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