Highly Substituted *ter*-Cyclopentanes as Receptors for Lipid A

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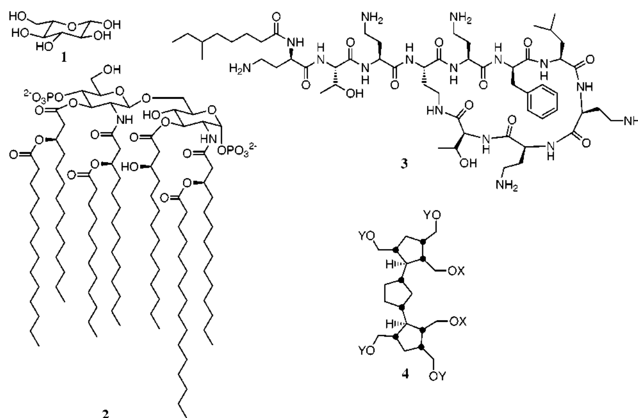
The design and synthesis of simple organic receptors capable of high-affinity binding to carbohydrates in aqueous solution presents a substantial challenge to our understanding of molecular structure and the processes which underly molecular recognition.[1] In essence, a simple sugar like glucose (**1**) is no more than an oriented collection of hydroxy groups, differing little from bulk water. Because of this, the vast majority of receptors for carbohydrates reported thus far have been studied only in organic solvents,[2] since competition between the solvent and the targeted carbohydrate for binding is not as much of a problem. Of the carbohydrate receptors examined in aqueous solution thus far, the best bind simple sugars with affinities (dissociation constants, K_D) in the millimolar range.[3]

As part of a continuing program in the development of new methods for the recognition of biologically significant structures,[4] we became interested in the possibility of employing highly substituted, stereoregular oligomers of cyclopentane as receptors for simple carbohydrates, liposaccharides, and oligosaccharides. In particular, lipid A (**2**) represented an attractive target for binding, both because of its structural features and due to its biological role as the conserved portion of lipopolysaccharide (LPS). LPS is a primary constituent of the outer cellular membrane of Gram-(−) bacteria,[5] and is perhaps better known as bacterial endotoxin, the causative agent of sepsis.[6] Antibiotics such as the polymyxin family of cyclic peptides[7] (i.e. polymyxin B, **3**) derive their effectiveness against sepsis through an ability to bind to and neutralize bacterial endotoxin, primarily mediated by interactions with lipid A.[8] However, polymyxin is problematic as a therapeutic agent both because of its complex structure (rendering it impractical to synthesize in quantity) and because of its side effects (acute renal toxicity,[9] among others). Therefore, the development of new lipid A-binding, endotoxin-neutralizing compounds is a problem of potential medical significance[10] as well as a fundamental problem in molecular recognition. Our contention that highly substituted oligocyclopentanes such as **4** might be attractive receptors for carbohydrates was based on extensive molecular mechanics simulations,[11] which suggested that such structures would be conformationally rigid, and able to present functionality in a spatially defined manner. For example, molecular models of **4** docked to lipid A suggested good surface complimentarity between the two structures. It was anticipated that variation of the acyl groups (X, Y) would allow for tuning of the structure to optimize affinity; in particular, we anticipated that employing amino acids as the "Y" groups would improve the solubility of **4** in water, while positioning amino groups optimally for hydrogen bonding to occur between **4** and the phosphate, amide, and ester moieties of **2**.



Our synthesis of **4** was designed with an eye toward both producing its three rings and 10 stereocenters in as few steps as possible and developing methodology that would be amenable to both large-scale and combinatorial library synthesis. As shown in Scheme 1, we began by converting norbornene (**5**) to the known *cis*-cyclopentane-1,3-dialdehyde[12] (**6**) via Sharpless dihydroxylation[13] followed by sodium periodate-metiated oxidative cleavage of the diol.[14] Subsequent treatment of the dialdehyde with Horner−Wadsworth−Emmons reagent **7** under standard conditions (potassium *tert*-butoxide as base in THF) provided the bis α,β-unsaturated ester **8** in 90% yield and >20:1 *EE*:*EZ* selectivity. Our initial attempts to employ **8** as the dienophile in a bidirectional Diels−Alder reaction with cyclopentadiene using standard Lewis acid catalysts were unsuccessful; however, a hybrid 0.5:0.05 $AlCl_3-Al(CH_3)_3$ catalyst system we have previously described[15] provided for the smooth conversion of **8** to double Diels−Alder cycloadduct **9** in 87% yield and 94:6 endo,endo−endo,exo selectivity.

With all of the carbocyclic skeleton and stereogenic centers of **4** installed, it remained for us to set the peripheral functionality. Reduction of the cycloadduct **9** with $LiAlH_4$ provided a diol in 53% yield, which was derivatized with benzoyl chloride to yield

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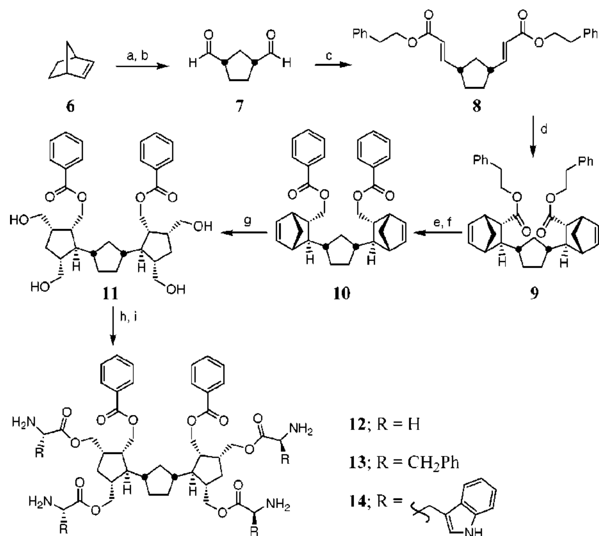
Scheme 1[a]



[a] Conditions: (a) $K_3Fe(CN)_6$, $K_2OsO_4{\cdot}2H_2O$, quinuclidine, K_2CO_3, *t*-BuOH−H_2O 1:1; (b) $NaIO_4$, THF−H_2O 3:1, 0 °C to room temperature (63%); (c) $(i\mathrm{PrO})_2P(O)CH_2C(O)CH_2CH_2Ph$, *t*-BuOK, THF, 0 °C to room temperature, 2 h, then: **7**, −78 to 4 °C (90%); (d) $(CH_3)_3Al$ (0.05 equiv) 10 min, then $AlCl_3$ (0.50 equiv), CH_2Cl_2, 0 °C, 10 min, then: cyclopentadiene (10 equiv), 4 °C (87%); (e) $LiAlH_4$ (6.0 equiv), THF, room temperature; (f) Bz-Cl (3.6 equiv), Et_3N (4.0 equiv), CH_2Cl_2, room temperature (50%, two steps); (g) O_3, CH_3OH/Ch_2Cl_2 1:1, −78 °C, then $NaBH_4$ (10 equiv), 0 °C to room temperature; (h) Boc-Gly-OH, Boc-Phe-OH, or Boc-Trp-OH (5.6 equiv), DCC (6.4 equiv), DMAP (3.7 equiv), CH_2Cl_2−DMF 7:3, room temperature, 18 h; (i) CF_3CO_2H−Et_3SiH−CH_2Cl_2 20:10:70, 12 h.

bis-benzoate **10** (94%). Ozonolysis of **10** followed by a reductive workup with $NaBH_4$ gave a tetrol, **11**, in 60% yield. This compound was acylated with N-Boc-glycine, N-Boc-phenylalanine, or N-Boc-tryptophan[16] using DCC as the coupling reagent in the presence of DMAP. Deprotection of the amino acids to provide **12**, **13**, or **14** was accomplished by using standard conditions.

Lipid A is a very weak absorber of ultraviolet light; therefore, we measured the affinities of **12**−**14** for lipid A by UV titration. Both **13** and **14** bind to lipid A with dissociation constants of comparable magnitude (587 and 592 nM, respectively, in phosphate-buffered saline, pH 7.4) to the reported polymyxin−lipid A interaction.[17] Providing further indication of the importance of the amino acid side chains of **4** in the recognition process, a saturation point for binding of lipid A to **12** was not reached at concentrations below the critical micelle concentration (cmc) for lipid A,[18] although changes in the UV absorbance clearly indicated the presence of some interaction.

The method of continuous variations[19] was employed to further examine the binding of **14** to lipid A in aqueous solution. Two inflection points are clearly observable for **14** binding to **2** (Figure 1), the first indicating the formation of a 1:1 **14**−**2** complex. The presence of the second inflection point, indicative of a 1:2 **14**−**2** complex, is somewhat puzzling; however, it is possible that this is in part due to changes in the aggregation state of **2** as its concentration is increased over the course of the experiment. To further clarify the mode of interaction between **12**−**14** and lipid A, we titrated dipalmitoyl phosphatidylcholine (DPPC) into

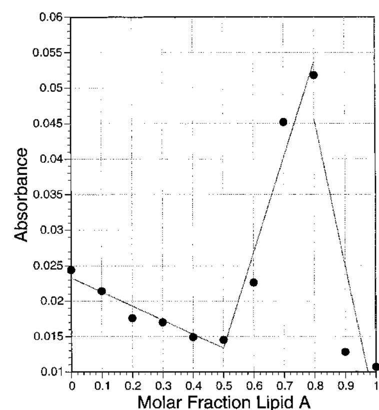


Figure 1. Job plot of **14** and lipid A.

solutions of **13** or **14**. While some absorbance changes were observed in both cases, these were very weak (on the order of 10% overall), and did not display a standard saturation profile.[20] This suggests that while there may be some interaction between **12**−**14** and the phospholipid tails of lipid A, it is likely nonspecific, and only weakly contributes to the overall affinity constant. To ensure that interactions were not simply due to the amino acid-derived functionality of **12**−**14**, we also examined the binding of lipid A to the methyl ester of tryptophan. In aqueous solution, we saw a linear increase in the tryptophan indole ring absorbance on addition of increasing concentrations of lipid A. However, this increase was not saturable at the concentrations tested (>20 μM in **2**), indicating a strictly nonspecific interaction.

Finally, further verification of a strong interaction between **14** and lipid A was provided by titrating diphosphoryl lipid A into a 1.5 mM solution of **14** in 95% D_2O, 5% DMSO, and recording the 500 MHz ^1H NMR spectrum at each point. Addition of as few as 0.1 molar equiv of diphosphoryl lipid A causes the appearance of a new peak downfield in the aromatic region of the spectrum, and subtle changes in the fine structure of resonances throughout the spectrum. As additional amounts of lipid A were added, resonance intensity decreased, culminating in substantial broadening of all resonances and the appearance of a flocculent precipitate in the NMR tube. While these data unfortunatey suggest that it will not be possible to obtain high-resolution solution structural information about the complex formed between **14** and lipid A in water, they are consistent with results obtained by others in the examination of lipid A-binding compounds.[21]

In conclusion, the highly substituted oligocyclopentanes presented herein represent a new class of compounds which demonstrate high levels of affinity for lipid A in aqueous solution. Efforts to examine the solution structures of **13** and **14**, and toward the development of a more complete structural understanding of the factors allowing **13** and **14** to function as high-affinity receptors for lipid A, are currently underway. Given the clinical importance of lipopolysaccharide as the causative agent of bacterial sepsis, the ability of **12**−**14** to neutralize bacterial endotoxin is currently under examination.

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Supporting Information Available: Selected spectra for UV titration data, spectra, NMR titration of **2** into **14**, and Skatchard analyses (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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