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A steroid-based receptor for unprotected amino acids: the enantioselective recognition of L-tryptophan

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ABSTRACT

A cholapod receptor possessing urea binding sites at C3, C7, and C12 positions and with an intrinsic chiral structure was synthesized, and the binding abilities toward amino acids in both L- and D- forms (Trp, Phe, Leu, and Ala) were studied using ^{1}H NMR spectroscopy, UV–vis spectroscopy and computer simulation. Changes in ^{1}H NMR spectra of the receptor revealed that complexation with amino acids occurred via hydrogen bonding and CH–π interactions. Binding to tryptophan was especially strong, and was found to be enantioselective (K_a=480 M^{-1} for L-Trp, 260 M^{-1} for D-Trp). NOESY and computer simulations were used to investigate the structures of the diastereomeric complexes between the receptor and the tryptophan enantiomers. In the case of L-Trp the carboxylate group bound at the two ureas adjacent to C7 and C12, while D-Trp was positioned closer to the urea adjacent to C3.

1. Introduction

The molecular recognition of biological targets has formed a major part of host-guest chemistry. Amino acids and their derivatives are especially important substrates,[1] due to their central role in natural living systems. However, the molecular recognition of unprotected α-amino acids presents distinctive challenges, due to the requirement for polar solvents to dissolve the substrates and the suppression of polar interactions in such solvents. Amino acids are also relevant as archetypal chiral substrates. Much effort has been devoted to the design and synthesis of receptors for chiral recognition.[2] However, success has not been easy to achieve, and not many have shown good selectivities. There are only a few receptor systems giving enantioselectivities of more than 90% ee[3].

To obtain good enantioselectivity, a receptor must be designed carefully for 3D complementarity to its intended substrate. The steroidal framework derived from cholic acid **1** affords many advantages; (i) a large rigid unit able to span a substrate, (ii) chirality, and (iii) preorganized codirected functional groups, which can be elaborated into 'legs' to contact the substrate. This combination of properties has lead to various applications of steroids in the construction of receptors,[4] including enantioselective receptors for amino acid derivatives.[5] A successful strategy has been the conversion of cholic acid **1** into cholapods of general form **2**, in which groups A–C include NH groups for hydrogen bonding with the substrate. The NH unit is more versatile than the native OH in **1**, appearing in amides, sulfonamides, carbamates, ureas thioureas, and guanidinium groups.

Although cholapods have been used for the recognition of peptides[4a] and *N*-acetylamino acids,[5] they have not been applied to unprotected amino acids.[6] In the present work, our target was a receptor capable of enantioselective recognition of the less polar α-amino acids (L-Trp, D-Trp, L-Phe, D-Phe, L-Leu, D-Leu, L-Ala, and D-Ala) in a polar organic solvent. Our design **8** was based on the cholapod architecture with urea units attached to C-3, C-7, and C-12. Urea groups were known to interact strongly with anionic species through hydrogen bonding interactions,[7] and would be especially suitable as binding sites for the carboxylate groups of zwitterionic amino acids. By employing three ureas we would maximise the chance of binding in the polar, competitive solvent, which would be needed to dissolve the substrates. Cholapods with three urea groups had been used to bind inorganic anions,[7k] but not organic substrates. The aromatic groups terminating the 'legs' could assist binding through CH–π interactions with substrate side-chains, while the nitroazobenzene unit was included as a chromophore, which might provide an optical response to binding.

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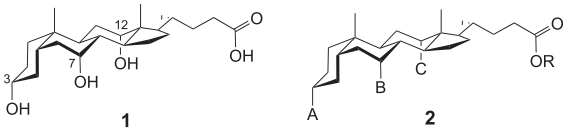
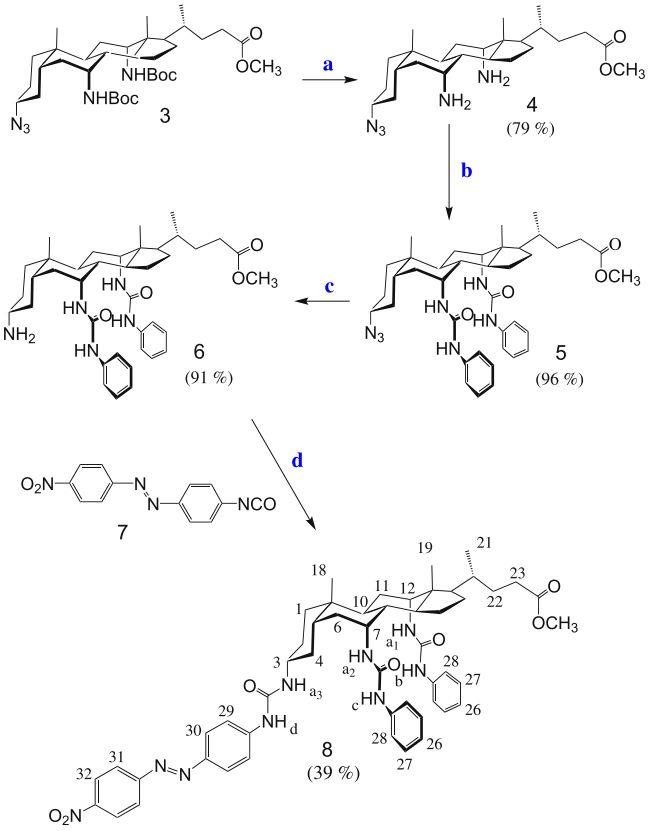


1 2

2. Results and discussion

2.1. Synthesis

The synthetic pathway to receptor **8** is summarized in Scheme 1. The synthesis of azide **3**, a key intermediate for the synthesis of steroidal podands with multiple NH groups, has been previously reported.[8] Diamine **4** was prepared by reaction of **3**[8] with TFA. Treatment of **4** with 2 equiv of phenyl isocyanate afforded 7,12-bis-urea **5**[9] in 96% yield. The third amino group was then unmasked through reduction of the azide group, giving **6**.[9]



Scheme 1. Synthesis and numbering system for receptor **8**. Reagents and conditions: (a) TFA, DCM; (b) PhNCO, Et_3N, DMAP, THF, 50 °C; (c) activated zinc, HOAc; (d) DMAP, THF.

Treatment of **6** with nitroazobenzene isocyanate **7**[10] in the presence of DMAP as a catalyst gave the desired product **8** in 39% yield.

2.2. Binding studies using ^{1}H NMR spectroscopy

The recognition of unprotected α-amino acids by **8** was investigated first by ^{1}H NMR spectroscopy. The amino acids were added in fourfold excess to the receptor in DMSO-d_6, and the resulting changes in the spectrum of the receptor were recorded to provide preliminary information on complex formation. The results are shown in Figure 1 and Table 1. Addition of the amino acids produced substantial downfield movements of the urea NH signals (H_a, H_b, H_c, and H_d), consistent with complex formation via hydrogen bonding interactions between the urea moieties and the carboxylate groups of the zwitterionic substrates. Small shifts of some aromatic protons (H_{27}, H_{28}, H_{29}, and H_{30}) were also observed, suggestive of interactions between amino acid side-chains and aromatic moieties in **8**.

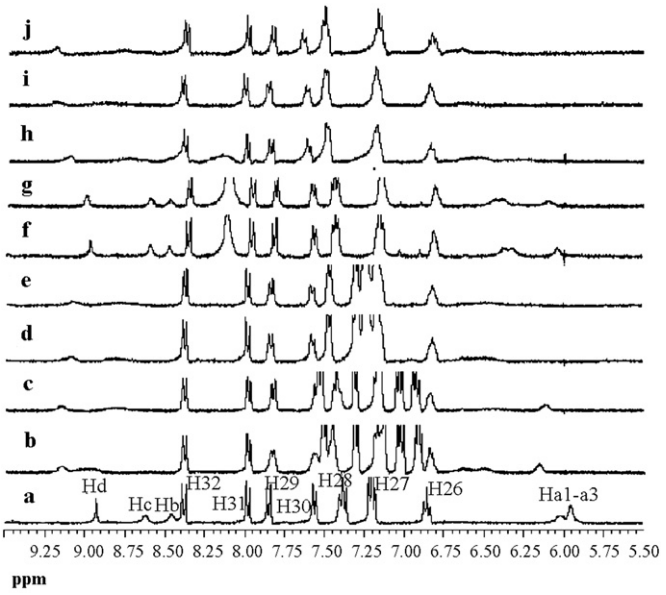


Figure 1. Partial ^{1}H NMR spectra in DMSO-d_6 of **8** (a) and the complexes of **8** with 4 equiv of L-Trp (b) D-Trp (c) L-Phe (d) D-Phe (e) L-Leu (f) D-Leu (g) L-Ala (h) D-Ala (i) Gly (j).

Table 1
Chemical shifts (ppm) of ligand **8** (4 mM) in DMSO-d_6 and the changes observed upon addition of various amino acids (4 equiv)[a]

	H_{26}	H_{27}	H_{28}	H_{29}	H_{30}	NH_a	NH_b	NH_c	NH_d
8	6.86	7.20	7.38	7.84	756	6.00	8.46	8.23	8.93
8·L-Trp	−0.02	−0.03	+0.08	−0.02	–	+0.48	+0.50	+0.34	+0.21
8·D-Trp	−0.01	−0.03	+0.04	−0.03	–	+0.31	+0.36	+0.20	+0.22
8·L-Phe	−0.03	−0.04	+0.08	–	–	–	+0.35	+0.19	+0.17
8·D-Phe	−0.03	−0.04	+0.08	−0.02	+0.02	–	–	–	+0.22
8·L-Leu	−0.03	−0.04	+0.08	−0.02	+0.03	+0.30	+0.04	–	+0.08
8·D-Leu	–	–	+0.09	–	+0.05	+0.31	+0.06	+0.02	+0.09
8·L-Ala	−0.03	−0.04	+0.09	−0.02	+0.05	–	–	–	+0.25
8·D-Ala	−0.03	−0.04	+0.09	−0.02	+0.05	–	–	–	+0.25
8·Gly	−0.03	−0.05	+0.12	−0.02	+0.07	–	–	–	+0.25

–: Values could not be determined due to signal broadening.
[a] Positive values represent downfield shifts and negative values represent upfield shifts.

The movements of the urea NH signals varied considerably depending on the amino acid added, giving preliminary indications of differential complexation properties. Especially, large movements were observed in the complex between **8** and L-Trp (**8⊂L-Trp**). In this case, the signals for H_a, H_b, and H_c were shifted downfield by +0.48, +0.50, and +0.34 ppm, respectively. The corresponding values for the diastereomeric complex **8⊂D-Trp** were significantly smaller. The shifts of urea protons (H_d) and aromatic protons (H_{26}–H_{30}) were less sensitive to the structure of the amino acid.

Complex formation was further investigated through ^{1}H NMR titrations. The results from the addition of aliquots of L-Trp to **8** in DMSO-d_6 are shown in Figure 2. Corresponding data from the experiments employing D-Trp, L-Phe, and D-Phe are shown in the Supplementary data.

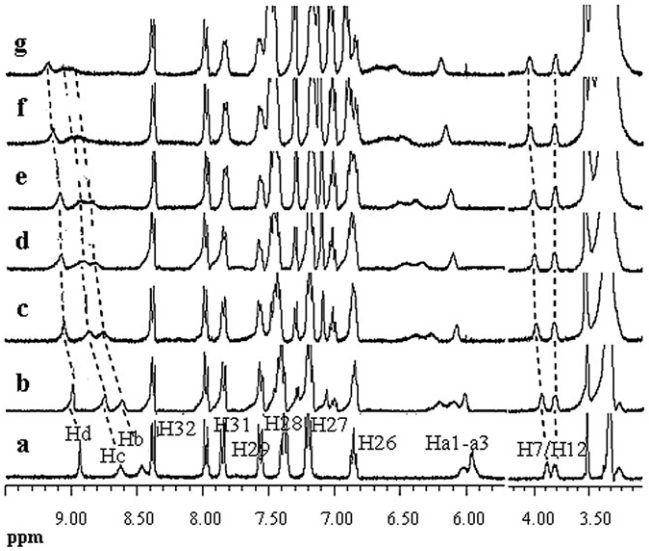


Figure 2. Partial ^{1}H NMR titration spectra of (a) **8** (4.0×10^{-3} M) upon adding L-Trp in equivalent of (b) 0.4 (c) 0.8 (d) 1.6 (e) 2.0 (f) 3.0 and (g) 4.0 in DMSO-d_6.

Initially, the NH_a urea protons in **8** appeared as two signals, one due to the NH proton adjacent to the C3 position and the other due to the two NH protons adjacent to C7 and C12. Addition of L-Trp caused all the NH_a resonances to move downfield, although the signals due to C7/12-NH moved considerably further than those due to C3–NH. A similar pattern was observed for the ArNH urea protons, where NH_b and NH_c (from the C7 and C12 ureas) were shifted further than the C3 urea proton NH_d.

It thus seems that complex formation between **8** and L-Trp occurs mainly through hydrogen bonding to the C7 and C12 ureas. Downfield shifts were also observed for all the NH protons of **8** on addition of the other amino acids. However, in these cases the signals became broadened so that detailed interpretations were problematic.

The broadening of the NH signals precluded accurate analysis to obtain binding constants. However small movements of the C7 and C12 protons at δ=3.60–3.80 ppm were also observed, and these could be followed reliably. In the case of D/L-Phe, D/L-Leu, and D/L-Ala, the changes in chemical shift were almost linear with substrate concentrations, over the range of concentrations accessible so that binding was too weak to be quantified by this methodology. In contrast, the movements of the C7-H signal caused by addition of L-Trp or D-Trp showed significant curvature when plotted against the concentrations of added guest (see Fig. 3). The shifts could be analyzed by non-linear curve-fitting according to a 1:1 binding model, to give association constants K_a of 480 M^{-1} for L-Trp and 260 M^{-1} for D-Trp. The formation of simple 1:1 complexes was confirmed by Job's plot analysis (see Supplementary data).

UV–vis titration experiments were also carried out in DMSO, in the hope of detecting binding through an optical signal. Unfortunately, the UV–vis spectrum of **8** showed only very small changes on addition of amino acids (see Supplementary data), and no significant color change was observed. Although disappointing, this was consistent with the NMR and modeling studies, which did not imply significant interactions between substrate and chromophore. We thus could not refine and investigate the binding properties of host **8** with amino acids by UV–vis spectrophotometry. If the azo group is not involved in binding, it is probable that the simpler analogues with three identical urea groups[7k] would show similar affinities and enantioselectivities.

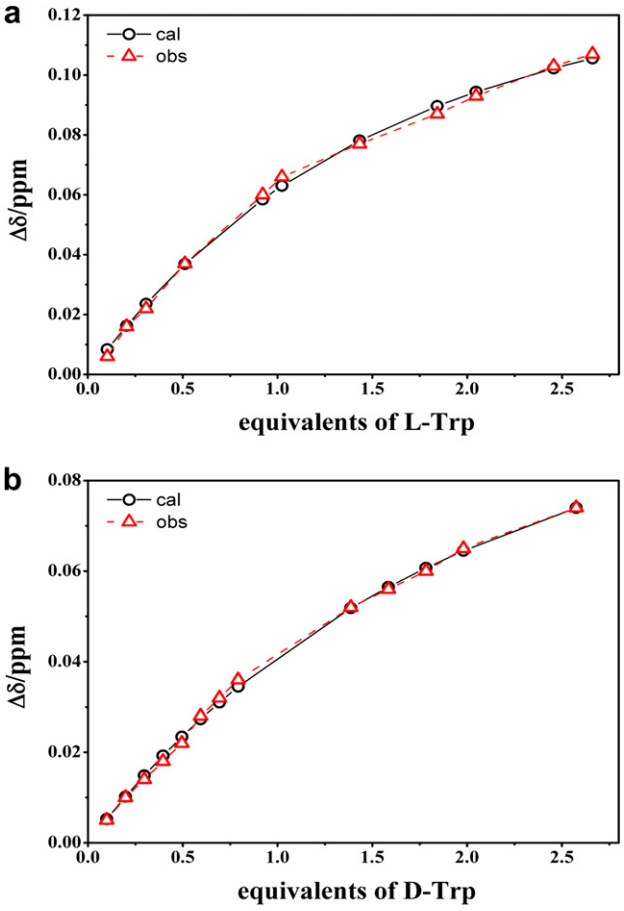


Figure 3. Theoretical (○) and experimental (△) binding curves for NMR titrations of **8** (4.0×10^{-3} M) with tryptophan enantiomers in DMSO-d_6. In each case the signal followed was due to 7βH. (a) L-Trp, K_a=480 M^{-1}. (b) D-Trp, K_a=260 M^{-1}.

2.3. NOESY results and computer simulation of the complex of Trp and host 8

The NMR results described above showed clearly that **8** binds both D- and L-Trp in DMSO, with selectivity over other amino acids and significant enantioselectivity in favor of L-Trp. We were interested in the origins of this selectivity and therefore investigated the structures of the complexes through intermolecular NOESY and computational chemistry. NOESY spectra of **8** with D- and L-Trp suggested that the structures of the diastereomeric complexes were significantly different from each other. In the case of **8** and L-Trp, several correlation peaks between protons on the phenyl rings of **8** (denoted H_x) and protons on Trp (denoted T_x) were observed (H_{28} to T_1, T_2, T_3, T_5; H_{27} to T_4; see Supplementary data and Fig. 4a). These connections suggest that the L-Trp indole unit lies in the cleft between the C7 and C12 phenylurea groups. In the case of **8**+D-Trp, the NOESY spectrum again showed correlations between Trp aryl protons (T_5 and T_2) and receptor phenylurea protons (H_{27}, H_{28}) (see Supplementary data and Fig. 4b). However cross-peaks also appeared between the D-Trp aryl protons T_2/T_5 and the azobenzene proton H_{29}. It thus seems that, while the D-Trp indole unit spends part of its time in the cleft between the C7/C12 phenylurea groups, it can also take up a position near the C3 substituent.

The correlations between the receptor phenyl protons and the tryptophan indole protons (both enantiomers) may help to explain the selectivity of **8** for Trp. The close approach of these aromatic units may be driven by CH–π interactions, which would contribute to the overall binding energies. To obtain further insight into the

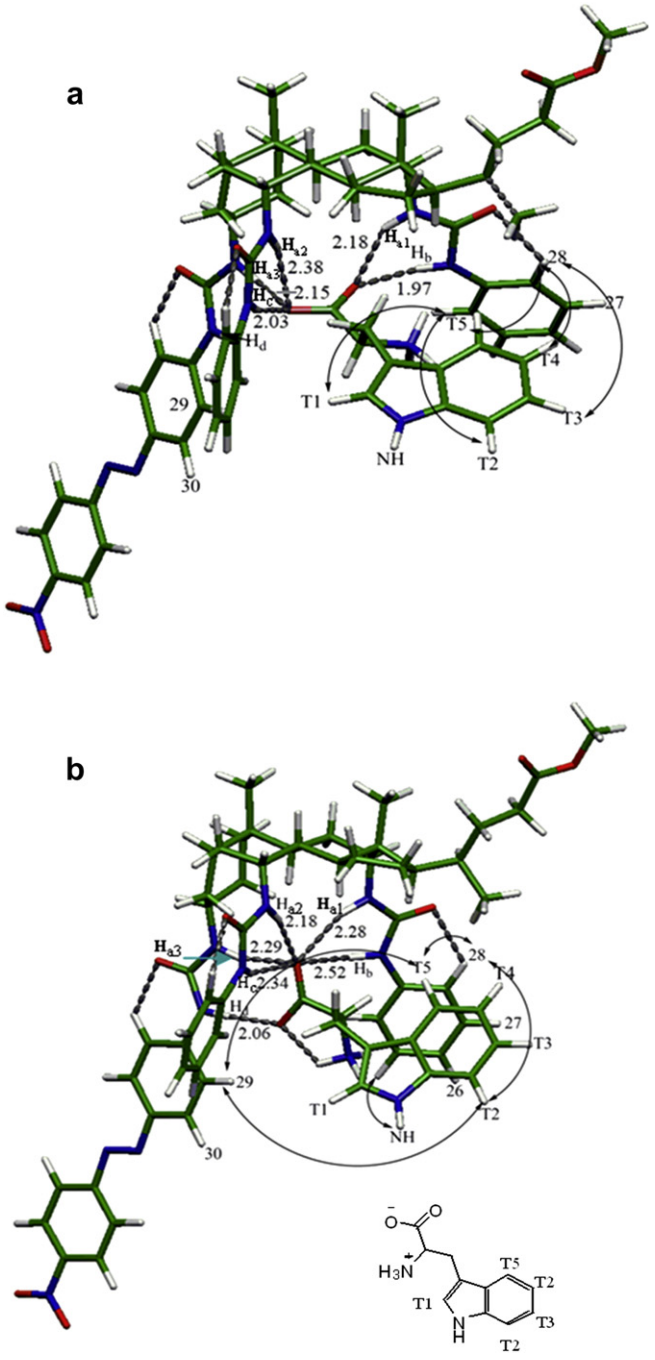


Figure 4. Structures of the (a) **8**⊂L-**Trp** and (b) **8**⊂D-**Trp** complexes derived from computer-based molecular modeling using Density Functional Theory: DFT (B3LYP/6-31G(d)) in Gaussian03 Program. Arrows represent the correlation peaks observed from NOESY spectra. Intramolecular and intermolecular hydrogens are shown as broken lines. Atoms H, C, N, and O represent in white, green, blue, and red, respectively. Inset; formula for Trp substrates with numbering used in discussion.

structures of [**8**⊂L-**Trp**] and [**8**⊂D-**Trp**], modeling studies were carried out using Density Functional Theory (B3LYP/6–31G(d) in the Gaussian03 program). Starting conformations were randomised and independent of the NOESY analyses. The lowest energy minima obtained for the diastereomeric complexes are shown in Figure 4. For [**8**⊂L-**Trp**] the carboxylate group is located close to all four C7/12 urea NH, apparently forming four strong hydrogen bonds (donor–acceptor distances of *d*[NH_{a1}, NH_{a2}, *d*NH_b and $NH_c\cdots O$]=2.18, 2.38 Å, 1.97 and 2.03 Å, respectively). A fifth hydrogen bond is formed to the C3–NH (H_{a3}) group (2.15 Å). In contrast, the carboxylate group in D-Trp was found to make two hydrogen bonds to the C3 urea group (donor–acceptor distances *d* [NH_{a3} and $NH_d\cdots O$]=2.29 and 2.06 Å, respectively), as well as four hydrogen bonds to the C7/12 ureas (donor–acceptor distances of *d* [NH_{a1}, NH_{a2}, NH_b, and $NH_c\cdots O$]=2.28, 2.18, 2.52, and 2.34 Å). However, these last four protons involve a single carboxylate oxygen. This oxygen makes five H-bonds in total (including one to the proximal C3 urea) while the second carboxylate oxygen binds only to the distal C3 urea NH. This asymmetric arrangement results in longer H-bond distances, presumably due to crowding and competition around the multiply-bonded oxygen. Such overcoordination is probably unfavorable and may account for the weaker binding of D-Trp compared to L-Trp. The movements of the C7/12 urea NH signals (Table 1) are consistent with this analysis. The signals for [**8**⊂L-**Trp**] are generally more downfield of those for [**8**⊂D-**Trp**], implying more effective H-bonding in the former case. For both structures, the modeling places the Trp indole unit between the C7 and C12 phenylurea groups as implied by the NOESY analysis. The correlations between T_2/T_5 and H_{29} in [**8**⊂D-**Trp**] are assumed to arise from an alternative conformation.

3. Conclusion

Previous research has shown that cholapods can serve as receptors for anions in non-polar solvents, and can distinguish between enantiomers of chiral carboxylates. In the present work we have shown that amino acid zwitterions can also be bound enantioselectively, even in a highly polar organic solvent (DMSO). Receptor **8** shows interesting selectivity for one particular amino acid, tryptophan, which may be ascribed to aromatic–aromatic interactions. The enantioselectivity is modest, but significant considering that the main interactions between host and guest (H-bonds) are strongly attenuated by the solvent. Improvements in scope and selectivity could result from the engineering of a more enclosed binding site, and this will be the subject of future research.

4. Experimental

4.1. Materials and methods

All reagents and solvents were obtained from commercial suppliers and used without further purification unless otherwise stated. Methanol was distilled over calcium chloride, magnesium, and iodine. DMF was obtained from Aldrich. THF and DCM were obtained from an Anhydrous Engineering Solvent Purification System (AESPS). Analytical TLC was carried out on DC-Alufolien Kieselgel 60F_{254} 0.2 mm plates (Merck) and compounds were visualized by UV fluorescence, ninhydrin solution or by charring over a Bunsen burner flame. Flash chromatography of reaction products was carried out using Silica 60A, particle size 35–70 μ (Fischer Scientific). IR spectra were recorded on a Perkin–Elmer Spectrum One spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Jeol Delta/GX270 or Delta/GX400 spectrometers, using deuterated solvents and were referenced internally to the residual solvent peak or TMS (δ_H=0.00 ppm, δ_C=0.00 ppm) signal. UV absorption spectra were obtained on a Varian Cary 50 Probe UV-Visible spectrophotometer. Unless otherwise specified, the solvents were spectroscopic grade without further purification. Samples were contained in 10 mm path length quartz cuvettes (3.5 mL volume).

4.2. Synthesis

*4.2.1. Methyl 3α-azido-7α, 12α-bis-[(phenylaminocarbonyl) amino]-5β-cholan-24-oate (**5**)*[9]. Azido-bis-carbamate **3**[8] (2.27 g, 3.52 mmol) was dissolved in dry dichloromethane (55 mL) and placed in an ice bath. Trifluoroacetic acid (34 mL) was added

dropwise, and the mixture was stirred at 0 °C for 1 h then at room temperature for 4 h. The solvent was evaporated under reduced pressure and the residue was redissolved in dichloromethane. The solution was washed with satd aq $NaHCO_3$, dried over magnesium sulfate, and evaporated under reduced pressure to give a yellow crude product. Precipitation from DCM/hexane gave diamine **4**[9] (1.24 g, 79%) as a white solid. **4**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=0.71 (3H, s, 18-H_3), 0.91 (3H, s, 19-H_3), 0.96 (3H, d, J=6.32 Hz, 21-H_3), 2.02–2.15 (2H, m), 2.18–2.27 (1H, m), 2.32–2.52 (2H, m), 3.07–3.21 (3H, m, 3β-H+7β-H+12β-H), 3.66 (3H, s, $COOCH_3$).

To a solution of diamine **4** (1.27 g, 2.84 mmol) in dry THF (30 mL) were added DMAP (0.33 g, 2.70 mmol), triethylamine (0.74 mL, 5.30 mmol), and phenyl isocyanate (620 μL, 5.68 mmol). The reaction mixture was stirred at 50 °C for 24 h and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (eluent DCM/methanolic ammonia 99:1) to provide azido-bis-urea **5** (1.86 g, 96%) as a white solid. **5**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=0.75 (3H, s, 18-H_3), 0.85 (3H, d, J=6.36 Hz, 21-H_3), 0.91 (3H, s, 19-H_3), 1.96–2.06 (1H, m), 2.25–2.32 (1H, m), 3.15 (1H, br s, 3β-H), 3.93 (1H, br s, 7β-H), 4.05 (1H, br s, 12β-H), 5.33 (1H, br s, NH), 5.63 (1H, br s, NH), 6.85 (1H, br s, NH), 6.98–7.06 (2H, m, Ar–CH), 7.25–7.39 (8H, m, Ar–CH). lit.[9] ^{1}H NMR δ=3.15 (1H, br s), 3.92 (1H, br s), 4.07 (1H, br s).

*4.2.2. Methyl 3α-amino-7α, 12α-bis-[(phenylaminocarbonyl) amino]-5β-cholan-24-oate (**6**)[9].* Activated zinc powder (3.77 g, 58.74 mmol) was added to a solution of azide **5** (2.00 g, 2.93 mmol) in glacial acetic acid (120 mL) and the mixture was stirred vigorously for 24 h. Acetic acid was completely removed by the addition of toluene followed by evaporation, repeated as necessary. The residue was dissolved in satd aq NaCl (60 mL), which was then basified with triethylamine and extracted with EtOAc. The organic phase was dried over magnesium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (eluent DCM/methanolic ammonia 97:3 to 9:1) to give amine **6** (1.76 g, 91%) as a white solid. **6**: ^{1}H NMR ($(CD_3)_2CO$) δ=0.88 (3H, s, 18-H_3), 0.90 (3H, d, J=9.76 Hz, 21-H_3), 1.04 (3H, s, 19-H_3), 3.16 (1H, br s, 3β-H), 3.54 (3H, s, $COOCH_3$), 4.04 (1H, br s, 7β-H), 4.15–4.21 (1H, br s, 12β-H), 5.86 (1H, br s, CH–N*H*–CO), 5.94 (1H, br s, CH–N*H*–CO), 6.86–6.94 (2H, m, Ar–CH), 7.18–7.26 (4H, m, Ar–CH), 7.51 (4H, d, J=5.88 Hz, Ar–CH), 8.10 (2H, d, J=8.30 Hz, Ar–N*H*–CO). lit.[9] ^{1}H NMR δ=3.24–3.34 (1H, br s), 3.96–4.02 (1H, br s), 4.17–4.23 (1H, br, s).

*4.2.3. 4-Isocyanato-4′-nitroazobenzene (**7**)[10].* Disperse orange **3**[8] (4-(4-nitrophenylazo)-aniline) (0.20 g, 0.83 mmol) was dried under high vacuum then dissolved in dry THF (15 mL). Triphosgene (2 equiv) was dried by dissolution in toluene followed by evaporation under reduced pressure, then redissolved in dry THF (15 mL) and added into the solution of disperse orange 3. The red-brown reaction mixture was stirred at room temperature for 2 h, monitoring the progress of the reaction using FT-IR (disappearance of the band at 1734 cm^{-1} due to –NHCOCl). A band due to the product **7** appeared at 2257 cm^{-1} (lit.[10] 2259 cm^{-1}). The solution was employed in the following step without purification.

*4.2.4. Methyl 3α-[(4-nitroazobenzene-4′-aminocarbonyl)ami no]-7α, 12α-bis-[(phenylaminocarbonyl)amino]-5β-cho lan-24-oate (**8**).* To the above solution of compound **7** in dry THF was added a solution of amino compound **6** (0.5 g, 0.83 mmol) and DMAP (0.13 g, 1.08 mmol) in THF. The mixture was heated for 2 h after which the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography using dichloromethane/methanolic ammonia 94:6 as eluent to provide tris-urea **8** (0.30 g, 39%) as a red solid. **8**: IR (KBr, cm^{-1}) 3385, 2941, 2867, 1676, 1544, 1439, 1341, 1228, 1139; ^{1}H NMR ($(CD_3)_2CO$) δ=0.81 (6H, s, 18-H_3+21-H_3), 0.99 (3H, s, 19-H_3), 3.26 (1H, m, 3β-H), 3.48 (3H, s, $COOCH_3$), 3.99 (1H, br s, 7β-H), 4.09 (1H, br s, 12β-H), 5.56 (1H, d, J=5.85 Hz, CH–NH_a–CO), 5.79 (1H, m, CH–NH_a–CO), 6.82–6.86 (2H, m, Ar_{phenyl}–CH_{26}), 7.21–7.15 (4H, m, Ar_{phenyl}–CH_{27}), 7.40–7.44 (4H, s, Ar_{phenyl}–CH_{28}), 7.56–7.58 (2H, d, J=9.0 Hz, Ar_{org3}–C_{29}), 7.79–7.83 (3H, d, J=4.8 Hz, Ar_{org3}–CH_{30}+Ar_{phenyl}–NH_c–CO), 7.96–7.98 (2H, d, J=9.0 Hz, Ar_{org3}–CH_{31}), 8.03 (1H, br s, Ar_{phenyl}–NH_b–CO), 8.16 (1H, br s, Ar_{org3}–NH_d–CO), 8.33–8.35 (2H, d, J=9.0 Hz, Ar_{org3}–CH_{32}); ^{13}C {^{1}H} NMR (100 MHz, $(CD_3)_2CO$, 25 °C, TMS) δ=12.27, 15.71, 21.82, 22.31, 25.74, 26.17, 26.86, 29.45, 29.80, 31.92, 33.78, 34.15, 34.73, 35.56, 36.45, 41.14, 43.65, 43.78, 45.22, 47.28, 49.67, 50.44, 51.84, 116.73, 116.86, 120.29, 120.36, 122.14, 123.82, 127.75, 139.98, 144.26, 146.11, 147.42, 153.18, 153.41, 153.59, 155.13, 172.62. Anal. Calcd for $C_{52}H_{63}N_9O_7$+1.5H_2O: C, 65.54; H, 6.93; N, 13.24. Found C, 65.48; H, 6.87; N, 13.03; MS (ESI): m/z for $C_{52}H_{63}N_9O_7$=948 $[M+Na]^+$, 926 $[M+H]^+$.

4.3. ^{1}H NMR titrations of host 8 and amino acids

Typically, a 4.0 mM solution of the ligand **8** (1.4×10^{-3} mmol) in DMSO-d_6 (0.35 mL) was prepared in a 5-mm NMR tube. An initial ^{1}H NMR spectrum of the solution of the ligand was recorded. A stock solution of guest compound (7.48×10^{-3} mmol, 18 mM) in DMSO-d_6 (0.4 mL) was prepared in a vial. The solution of guest (0.3 mL) was added portionwise via microsyringe (10 and 50 μL portions) to the NMR tube. After each addition the sample was shaken to ensure mixing and the ^{1}H NMR spectrum was recorded.

4.4. UV–vis titrations of host 8 and amino acids

All UV–vis experiments were carried out in DMSO with tetrabutylammoniumhexafluorophosphate as supporting electrolyte. The solution of **8** (2.00×10^{-4} mmol, 0.02 mM) was prepared and gradually added with the solution of guests (1.067×10^{-2} mmol, 1.067 mM) until the system reached the equilibrium point observed by a small change in UV–vis spectrum. Each addition was recorded from 300 to 550 nm at room temperature.

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Supplementary data

The synthetic procedure of **8** and the analysis of the synthesized compounds including of ^{1}H NMR titration, 2D NMR spectra and Job's plots of complexes are provided as supplementary data. Supplementary data associated with this article can be available in the online version at doi:10.1016/j.tet.2010.06.069.

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