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Steroidal Ureas as Enantioselective Receptors for an *N*-Acetyl α-Amino Carboxylate

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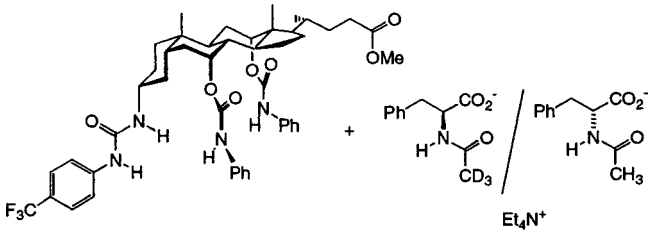
ABSTRACT



Cholic acid has been elaborated into three regioisomeric bis-carbamoylureas, which have been investigated as enantioselective receptors for *N*-acetyl phenylalanine. L/D selectivities, peaking at 5:1, have been determined by a sensitive and rapid MS-based extraction method that should be generalizable to related systems.

Enantioselective recognition is a long-standing issue in supramolecular chemistry.[1] Despite much research, it continues to provide interesting challenges. Success for some substrate classes remains elusive, and there are still few systems of any type which exhibit selectivities of ≥90% ee.[2] Moreover, good enantiodiscrimination often requires careful matching of substrate and receptor. Effective discrimination for a range of substrates may therefore require an equally wide range of receptors.

Modular receptor-like architectures provide an attractive approach to this area. Given suitable screening methods,[3] they could be elaborated into libraries from which the best variant for any particular substrate could be selected. Podand structures are especially easy to vary, and "cholapod"[4] architectures such as **1** have particular advantages. The steroidal framework, derived from cholic acid **2**, provides a chiral scaffold that positions the codirected legs so as to create a binding site for a small-to-medium size molecule. The side chain may be used to control solubility, or to link the scaffold to a polymer backbone for solid-phase synthesis. Cholapod libraries prepared by "split-and-mix" solid-phase

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(1) Webb, T. H.; Wilcox, C. S. *Chem. Soc Rev.* **1993**, *22*, 383. Zhang, X. X.; Bradshaw, J. S.; Izatt, R. M. *Chem. Rev.* **1997**, *97*, 3313. Ogoshi, H.; Mizutani, T. *Acc. Chem. Res.* **1998**, *31*, 81.

(2) Selected examples: Peacock, S. C.; Domeier, L. A.; Gaeta, F. C. A.; Hegelson, R. C.; Timko, J. M.; Cram, D. J. *J. Am. Chem. Soc.* **1978**, *100*, 8190. Jeong, K. S.; Muehldorf, A. V.; Rebek, J. *J. Am. Chem. Soc.* **1990**, *112*, 6144. Hong, J.-I.; Namgoong, S. K.; Bernardi, A.; Still, W. C. *J. Am. Chem. Soc.* **1991**, *113*, 5111. Yoon, S. S.; Still, W. C. *J. Am. Chem. Soc.* **1993**, *115*, 823. Pirkle, W. H.; Murray, P. G.; Rausch, D. J.; McKenna, S. T. *J. Org. Chem.* **1996**, *61*, 4769. Martín, M.; Raposo, C.; Almaraz, M.; Crego, M.; Caballero, C.; Grande, M.; Morán, J. R. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2386. Botana, E.; Ongeri, S.; Arienzo, R.; Demarcus, M.; Frey, J. G.; Piarulli, U.; Potenza, D.; Gennari, C.; Kilburn, J. D. *Chem. Commun.* **2001**, 1358.

(3) For a method applicable to one-bead-one-compound libraries, see: Weingarten, M. D.; Sekanina, K.; Still, W. C. *J. Am. Chem. Soc.* **1998**, *120*, 9112.

(4) Ayling, A. J.; Pérez-Payán, M. N.; Davis, A. P. *J. Am. Chem. Soc.* **2001**, *123*, 12716.

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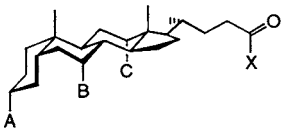
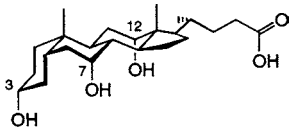
synthesis have already been used for sequence-selective peptide recognition[5] and for studies in enzyme modeling.[6]

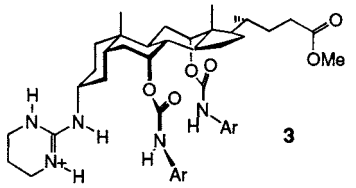


1



2

As part of our general program on receptors derived from cholic acid,[4,7] we have recently shown that guanidinium cations of form **3** can extract *N*-acetyl α-amino carboxylates from an aqueous phase into chloroform with up to 80% ee.[8] Moreover, a lipophilic analogue has been found to transport *N*-acetyl phenylalanine through bulk liquid membranes with high turnover numbers and moderate-to-good enantioselectivities.[9] These results suggest that the cholapod architecture is intrinsically suitable for enantioselective recognition. However, further exploration of this system was hampered by problems in synthesizing the guanidinium centers and handling the cationic products. We therefore decided to investigate urea **4**, an electroneutral analogue of **3**, as a potential model for receptor libraries. We now report the synthesis and recognition properties of **4**, and also of regioisomers **5** and **6**. The enantioselectivities of **4**−**6** have been measured by using a straightforward, rapid, and accurate MS-based procedure that may prove broadly useful in screening for enantiodiscrimination by small quantities of potential receptors.



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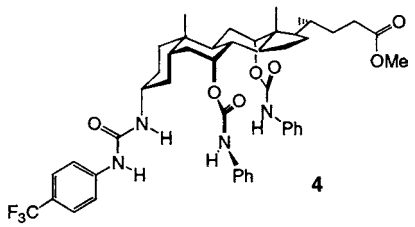

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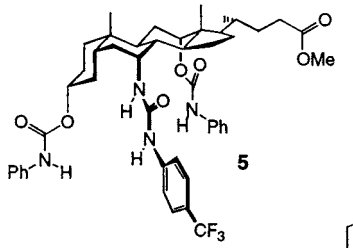

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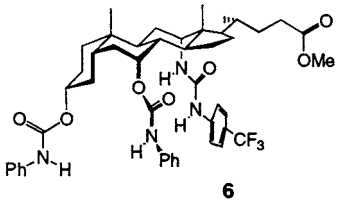
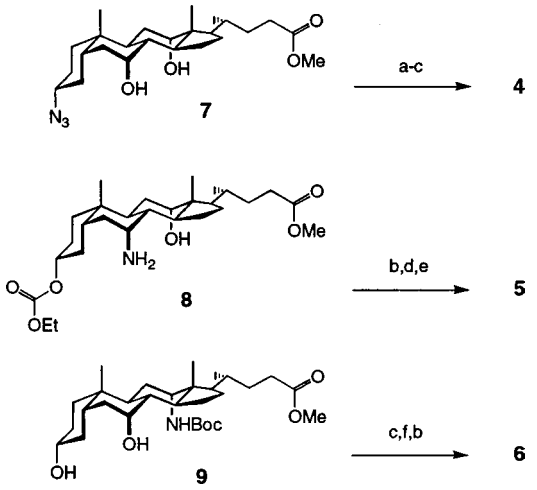


6

The syntheses of receptors **4**−**6** are summarized in Scheme 1. The 3α-urea **4** was prepared from azido-diol **7**, previously

Scheme 1. Preparation of **4**−**6**[a]



[a] Reagents: (a) Zn, AcOH; (b) *p*-$CF_3C_6H_4NCO$, Et_3N, DCM; (c) PhNCO, DCM, cat. TMSCl; (d) NaOMe, MeOH; (e) PhNCO, $CHCl_3$, cat. TMSCl; (f) TFA, DCM.

used for the synthesis of **3**.[8b] 7α-Urea **5** was synthesized via amine **8**, previously reported by Kasal and co-workers.[10] Finally 12α-urea **6** was prepared from diol **9**, an intermediate in an earlier synthesis of a differentially protected 3,12-diamine.[11] A *p*-trifluoromethyl substituent was incorporated in the urea moieties to acidify the ArN*H*, hopefully promoting the formation of doubly H-bonded urea-carboxylate motifs.

The enantioselective recognition of carboxylates can be studied through the extraction of racemic substrates from aqueous into organic phases. Enantioselectivities are obtained simply by measuring the ratios of substrate enantiomers present in the organic phase. This procedure is convenient, and directly relevant to potential applications in separation systems. The method is more obviously applicable to cationic

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(7) Reviews: (a) Davis, A. P. *Chem. Soc. Rev.* **1993**, *22*, 243. (b) Davis, A. P.; Bonar-Law, R. P.; Sanders, J. K. M. In *Comprehensive Supramolecular Chemistry*; Murakami, Y., Ed.; Pergamon: Oxford, UK, 1996; Vol. 4 (Supramolecular Reactivity and Transport: Bioorganic Systems), pp 257. (c) Davis, A. P. In *Supramolecular Science: Where It Is and Where It Is Going*; Ungaro, R., Dalcanale, E., Eds.; Kluwer: Dordrecht, The Netherlands, 1999; pp 125−146. Recent contributions: (d) Ayling, A. J.; Broderick, S.; Clare, J. P.; Davis, A. P.; Pérez-Payán, M. N.; Lahtinen, M.; Nissinen, N. J.; Rissanen, K. *Chem. Eur. J.* **2002**, *8*, 2197. (e) Lambert, T. N.; Boon, J. M.; Smith, B. D.; Pérez-Payán, M. N.; Davis, A. P. *J. Am. Chem. Soc.* **2002**, *124*, 5276−5277.

(8) (a) Davis, A. P.; Lawless, L. J. *Chem. Commun.* **1999**, 9. (b) Lawless, L. J.; Blackburn, A. G.; Ayling, A. J.; Pérez-Payán, M. N.; Davis, A. P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1329.

(9) Baragaña, B.; Blackburn, A. G.; Breccia, P.; Davis, A. P.; de Mendoza, J.; Padrón-Carrillo, J. M.; Prados, P.; Riedner, J.; de Vries, J. G. *Chem. Eur. J.* **2002**, *8*, 2931.

(10) Kasal, A.; Kohout, L.; Lebl, M. *Collect. Czech. Chem. Commun.* **1995**, *60*, 2147.

(11) Barry, J. F.; Davis, A. P.; Pérez-Payán, M. N.; Elsegood, M. R. J.; Jackson, R. F. W.; Gennari, C.; Piarulli, U.; Gude, M. *Tetrahedron Lett.* **1999**, *40*, 2849.

receptors such as **3**, but can be employed for electroneutral receptors provided a cation of suitable lipophilicity is also present. In our previous work on **3**, the measurements were performed by ^{1}H NMR, effectively employing the cation as a chiral shift reagent. However, for the present work, we wanted to develop a method that would be more general, and could be applied routinely to receptors synthesized on very small scales.

Mass spectroscopy on isotopically labeled "pseudoracemates" provides a sensitive and general method for measuring enantioenrichments.[12] This technique seemed readily adaptable to the study of enantioselective extractions. A receptor in one phase may be equilibrated with an excess of pseudoracemate in the second phase. The mass-differentiated pseudoenantiomers are extracted in a ratio that reflects the enantioselectivity, and a single MS analysis of the receiving phase can yield this figure. A potential complication is that receptor−substrate association might perturb the analysis. However, use of LCMS, under conditions which separate receptor from substrate, removes this possibility.

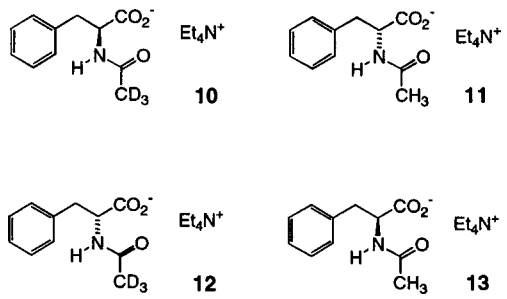
For our studies on **4**−**6**, the phenylalanine derivatives **10** (L-deuterated) and **11** (D-undeuterated) were employed as substrates. Aqueous solutions for the extraction experiments were prepared by dissolving a 1:1 mixture of the corresponding acids (to 30 mM), Et_4NOH (to 3 mM), and NaOH (to 27 mM) in a pH 7.4 phosphate buffer (0.1 M). This concentration of tetraethylammonium countercation was found to give acceptable levels of extraction without promoting background (nonselective) phase transfer. Aliquots of these aqueous solutions (2 mL) were added to receptors **4**−**6** in chloroform (1 mL, various concentrations; see Table 1).

Table 1. Extraction of **10** + **11** by Receptors **4**−**6** into Chloroform from Aqueous "Pseudoracemate"

receptor	[receptor] (mM)	ratio extracted (L-**10**/D-**11**)	amount extracted[a] (%)
4	1	5.0	
4	3	5.0[b]	
4	6	4.9[c]	17[b]
5	1	3.7	
5	3	3.6[b]	
5	6	3.8[b]	17
5	12	3.7	
6	1	3.1[b]	
6	3	3.1[b]	
6	6	3.1[b]	16

[a] Mol %, with respect to receptor. [b] Average of two measurements. [c] Average of three measurements.

The mixtures were stirred vigorously for 1 h, then allowed to separate. The organic phases were collected, passed through hydrophobic filter paper, then evaporated, and the residues were dissolved in acetonitrile for analysis by LCMS (for details, see the Supporting Information). In some cases quantitative analysis by NMR, after addition of an internal standard, was used to obtain estimates of the total amounts extracted.

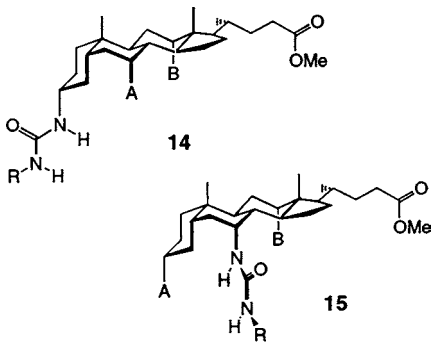


The results of these experiments are summarized in Table 1. All three receptors extracted ~17 mol % of **10**/**11** under these conditions, and each showed a significant preference for the L-derivative **10**. The selectivities ranged from ~5:1 for **4** to ~3:1 for **6**. The validity and reliability of the method were confirmed as follows. First, control mixtures of **10** and **11** were made up in known ratios and analyzed by LCMS. The analyses gave the predicted results to good accuracy.[13] Second, repeat measurements of enantioselectivity gave consistent results to within ±1%. Third, the measured enantioselectivities were independent of receptor concentration (see Table 1). This confirmed the absence of significant background extraction, which should degrade the apparent selectivity at low receptor concentrations. Finally, the complementary pseudoracemate **12** + **13** was prepared and tested against receptor **5**. The measured L/D ratio of 3.9 corresponds closely to those given in Table 1, confirming that isotope effects are negligible in this system.

The enantioselectivities obtained for these receptors are quite encouraging. Certainly, receptor **4** compares quite well with its cationic analogues **3**. Arguably, the greater ease of synthesis and handling of ureas vs guanidinium cations provides sufficient compensation for the modest drop in selectivity. As it stands receptor **4** has the potential for enantioselective transport through nonpolar membranes,[9] while elaboration into libraries of general form **14** should be feasible and could yield high levels of enantiodiscrimination. The selectivities obtained for **5** and **6** may be thought slightly disappointing. There were grounds for hoping that these receptors might out-perform **4**. The urea groups are axial in **5** and **6**, restricting rotation about the C(7/12)−N bonds and directing the NH groups inward beneath the α-face of the steroid.[4] These compounds might therefore appear better preorganized for binding chiral carboxylates, and likely to show higher selectivities. In fact, both showed lower enantiodiscrimination than **4**. However, their selectivities are still appreciable, and there is every reason to suppose that the corresponding libraries **15** and **16** will contain highly selective receptors.

(12) Recent examples: (a) Reetz, M. T.; Becker, M. H.; Klein, H. W.; Stockigt, D. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1758. (b) Welch, C. J.; Pollard, S. D.; Mathre, D. J.; Reider, P. J. *Org. Lett.* **2001**, *3*, 95. (c) Diaz, D. D.; Yao, S. L.; Finn, M. G. *Tetrahedron Lett.* **2001**, *42*, 2617.

(13) For a similar study giving parallel results, see ref 12b.



In conclusion, we have shown that steroidal ureas **4**−**6** can extract an *N*-acetyl amino acid salt from aqueous into organic phase with significant enantioselectivities. These receptors should serve as models for more complex podands, which may ultimately yield greatly improved performance. The selectivities were measured by using a MS-based method that is readily applicable to small quantities of electroneutral, organic-soluble receptors.

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Supporting Information Available: Experimental details for the synthesis of **4**−**6** and for the enantioselectivity measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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