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Convergent and Stereospecific Synthesis of Molecules Containing α-Functionalized Guanidiniums via α-Guanidino Acids

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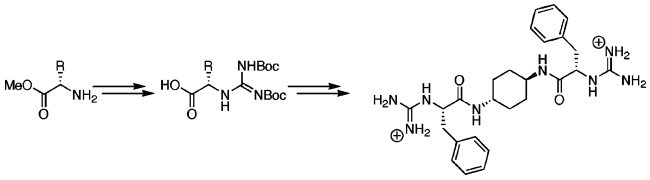

To introduce chirality and functional groups adjacent to guanidiniums to modulate specificity and affinity in recognition, *N*,*N*′-bis(Boc)-α-guanidino acids were synthesized from α-amino acid methyl esters. Protected α-guanidino acids coupled to cyclohexylamine and *trans*-1,4-diaminocyclohexane in good yield and with retention of stereochemistry. Boc deprotection was conducted under mild acidic conditions (0.5 M HCl/EtOAc) to minimize epimerization. The deprotected guanidinium is configurationally stable under more acidic conditions. This approach represents a practical, convergent, stereospecific methodology to introduce chiral α-substituted guanidinium groups into molecules.

Guanidiniums are widely used to specifically recognize diverse protein, DNA, and RNA targets.[1] Guanidiniums achieve affinity and specificity by virtue of potentially bidentate electrostatic and hydrogen-bonding interactions, yielding particularly favorable interactions with phosphates and carboxylates.[2] Guanidiniums have been generally applied in the development of synthetic receptors[3] and have been broadly applied in medicinal chemistry as components of ligands for complex biomedical targets, particularly as mimics of RGD motifs to bind integrin receptors.[4,5]

Many techniques have been developed to synthesize complex guanidiniums.[6−9] However, one of the simplest guanidinium motifs containing complex functionality is that obtained by guanylation of the amino group of an α-amino acid, generating an α-guanidino acid (Chart 1).[5,7,9] We were particularly intrigued by the use of α-substituted guandiniums derived from α-guanidino acids to achieve high affinity and high specificity in guanidinium-mediated biomolecular recognition.

In a particularly noteworthy example highlighting the potential of α-substituted guanidiniums for macromolecular recognition, the groups of Wells and Arkin at Sunesis have incorporated a single α-guanidino acid moiety into a series of high-affinity small-molecule ligands for interleukin-2 (IL-2).[5] In this work, both the functional group present and the stereochemistry at the α-position exquisitely impacted affinity for the target protein. For example, in structure−activity studies they observed a greater than 25-fold increase in IL-2 affinity for a molecule derived from D-valine over molecules derived from either L-valine or glycine.[5d] X-ray crystallography of the complexes with IL-2 revealed extensive contacts between IL-2 and both the guanidinium and side chain.[5a−c,e] This work highlights the

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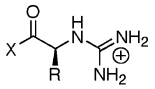
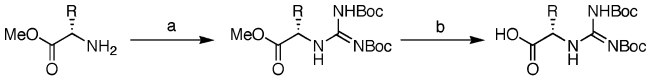
CHART 1. α-Guanidino Acids and Derivatives

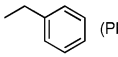
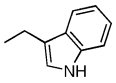


X = OH, OR, NHR

TABLE 1. Synthesis of Bis(Boc)-Protected α-Guanidino Acids[a]



1 a-e **2 a-e** **3 a-e**

entry	substrate	–R		product 2	yield (%) 2	product 3	yield (%) 3
1	**1a**	–H	(Gly)	**2a**	65%	**3a**[b]	61%
2	**1b**		(Phe)	**2b**[b]	72%	**3b**	98%
3	**1c**		(Trp)	**2c**	65%	**3c**	97%
4	**1d**		(Val)	**2d**	77%	**3d**	66%
5	**1e**		(Leu)	**2e**	71%	**3e**	72%

[a] (a) *N*,*N*′-Bis(*tert*-butoxycarbonyl)thiourea, 2-chloro-1-methylpyridiunium iodide (Mukaiyama's reagent), DMF, DIPEA, 5 h. (b) 4 equiv of LiOH, 6:1 acetone/H_2O (**3a**,**b**) or 3:1 MeCN/H_2O (**3c**−**e**), 2−5 h. [b]Syntheses of **2b**[7d] and **3a**[7b] have been reported previously.

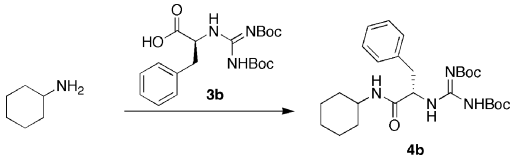
potential of chiral α-guanidino acids in medicinal chemistry and molecular recognition.

Molecules containing α-guanidino acid subunits are generally synthesized via coupling of a nucleophile with a suitably N-protected α-amino acid, followed by N-deprotection, N-guanylation, and subsequent guanidine deprotection to generate the α-guanidino acid-containing molecule. While this approach takes advantage of commercially available protected α-amino acids, it has the significant disadvantage of requiring multistep manipulation after the initial amide bond formation. In addition, this approach is frequently characterized by very low reported yields.[5a,d] Importantly, epimerization at the α-position has not been explicitly examined. In the synthesis of molecules containing multiple α-guanidino acid subunits for biomolecular recognition, the significant late-stage manipulation required by standard approaches is particularly unattractive.[7f] We therefore sought to employ a more convergent approach, in which protected α-guanidino acids were directly coupled to a nucleophile of interest, eliminating the first deprotection step and the potentially problematic polyguanylation of a complex molecule.

Coupling of *N*,*N*′-bis(Boc)-protected α-guanidino acids to amines, followed by acidic deprotection, would allow rapid access to complex molecules containing α-guanidino acid subunits. Surprisingly, despite the interest in guanidiniums, the protected α-guanidino acid building blocks **3b**−**e** (Table 1) required for this approach have never been reported.[7] Therefore, we sought to synthesize the protected α-guanidino acids **3a**−**e** to examine a convergent coupling strategy for the synthesis of functionally rich guanidiniums.

Guanylation of the α-amino group of a series of α-amino acid methyl esters, following the method used by Lipton to synthesize **2b**, proceeded readily to generate **2a**−**e**.[7d,10] Saponification with LiOH generated the protected α-guanidino acids **3a**−**e**. These reactions proceeded cleanly and in good yield, providing ready access to protected α-guanidino acid building blocks with chemically diverse α-substituents, including aromatic groups and sterically demanding hydrophobic functional groups.

TABLE 2. Examination of Racemization under Different Coupling Conditions



3b **4b**

entry	coupling reagent	solvent	time, min	yield	% ee
1	DCC	CH_2Cl_2	60	95%	84%
2	DCC/HOBt	CH_2Cl_2	60	78%	86%
3	DCC	DMF	60	40%	97%
4	EDCI/HOBt	CH_2Cl_2	120	81%	84%
5	EDCI/HOBt	DMF	120	84%	single isomer

The coupling of the protected α-guanidino acids to an amine was examined. To date, the only reported amide coupling reactions of bis(Boc)-protected-α-guanidino acids that have proceeded in reasonable yield have utilized the glycine derivative.[7g,11] To determine the ability of bis(Boc)-protected-α-guanidino acids to couple with amines to form amide bonds, we examined the coupling of **3b** with cyclohexylamine under various amide coupling conditions (Table 2). To assess the possibility of racemization in the guanylation, saponification, or amide coupling steps, *ent*-**3b** was synthesized from (*R*)-phenylalanine methyl ester (*ent*-**1b**). Coupling of *ent*-**3b** to cyclohexylamine generated *ent*-**4b**. Phenylalanine-derived guanidino acids were chosen on the basis of the greater susceptibility to racemization of phenylalanine compared to other canonical amino acids.

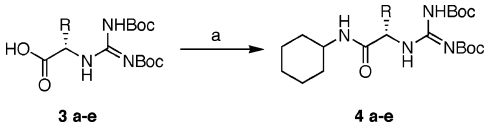
Although most conditions proceeded with good chemical yields, significant racemization was observed when coupling reactions were conducted in CH_2Cl_2 (Table 2). In contrast, chiral HPLC analysis of **4b** and *ent*-**4b** revealed that both **4b** and *ent*-**4b** exhibited high enantiopurity (no evidence of the enantiomer) when employing the optimized conditions of EDCI/HOBt activation and coupling in DMF (Table 2 and Supporting Information). These results indicate that the guanylation, saponification, and amide coupling reactions all proceeded with minimal racemization, suggesting generality in the coupling of protected α-guanidino acids with amines.

Using the optimized coupling conditions, **3a**−**e** were coupled with cyclohexylamine (Table 3). Coupling of the bis(Boc)-

(10) Synthesis of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea: Iwanowicz, E. J.; Poss, M. A.; Lin, J. *Synth. Commun.* **1993**, *23*, 1443−1445. The NMR spectra of **2a**−**e** showed the presence of a minor isomeric product, presumably the (Z) Boc imine. The observation of one guanidino proton doublet and one singlet in each isomer argues against the alternative tautomeric isomer as one of the products. Similar isomerism was observed in **3a**−**e**, **4a**−**e**, and **5**, but was not observed in **6**.

(11) (a) Pons, J.-F.; Fauchère, J.-L.; Lamaty, F.; Molla, A.; Lazaro, R. *Eur. J. Org. Chem.* **1998**, 853−859. (b) Addicks, E.; Mazitschek, R.; Giannis, A. *ChemBioChem* **2002**, *3*, 1078−1088. (c) Osterkamp, F.; Ziemer, B.; Koert, U.; Wiesner, M.; Raddatz, P.; Goodman, S. L. *Chem.*−*Eur. J.* **2000**, *6*, 666−683. Osterkamp et al. reported that the attempted coupling of the glycine derivative failed, producing only the creatinine-like side product.

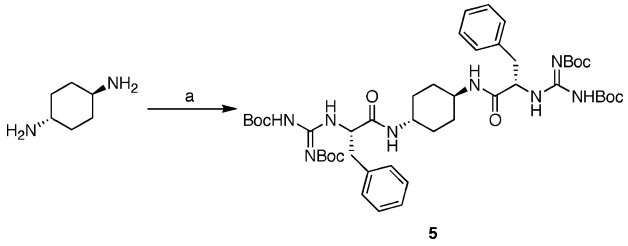
TABLE 3. Coupling of Cyclohexylamine and Bis(Boc)-α-guanidino Acids[a]



entry	substrate	guanidino acid	product	yield (%)
1	**3a**	Gly	**4a**	61%
2	**3b**	Phe	**4b**	84%
3	**3c**	Trp	**4c**	60%
4	**3d**	Val	**4d**	71%
5	**3e**	Leu	**4e**	65%

[a] (a) Cyclohexylamine, EDCI, HOBt, DIPEA, DMF, 2 h.

SCHEME 1. Coupling of a Protected α-Guanidino Acid to a Diamine[a]



[a] (a) **3b** (2.5 equiv), DCC, DIPEA, MeCN, 0 °C → RT, 2 h, 56%.

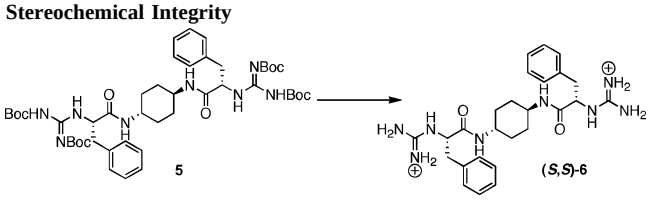
protected α-guanidino acids to cyclohexylamine proceeded readily under the optimized, mild amide coupling conditions, generating the protected products **4a**−**e** in good yields at room temperature.[12] The syntheses of **4b**−**e** are the first reported examples of amide coupling reactions proceeding in good yield using a non-glycine bis(Boc)-α-guanidino acid.

Arginine residues are broadly employed in biological recognition. However, the linear alkyl side chain attached to the guanidinium of arginine likely contributes only minimally toward target specificity in most cases. The introduction of functional groups adjacent to the guanidinium group of arginine mimetics can result in very significant increases in affinity and specificity of the interaction of guanidiniums.[1−5] To examine the utility of α-guanidino acids in the synthesis of molecules containing multiple α-functionalized guanidiniums, the coupling of two protected α-guanidino acid monomers to a single molecule was examined. **5** was synthesized by coupling *trans*-1,4-diaminocyclohexane to the protected phenylalanine α-guanidino acid **3b** (Scheme 1). The coupling reaction proceeded readily under amide coupling conditions chosen for optimal solubility of the starting material and product. **5** is the first reported example of the coupling of two bis(Boc)-α-guanidino acid monomers to a single molecule.

Stereochemistry at the α-position critically affects function for molecules containing α-guanidino acids.[5] However, the effect of deprotection conditions on stereochemical integrity has not been reported, despite the potentially greater susceptibility to racemization for protected α-guanidino acid derivatives compared to protected α-amino acids, owing to extended

(12) These results are in contrast to those of Wen et al., who observed that the coupling of highly sterically hindered α-*N*,*N*′-bis(Boc)-guanidino-, *β*-*N*-Fmoc-amino acids to amines did not proceed: Wen, K.; Han, H. S.; Hoffman, T. Z.; Janda, K. D.; Orgel, L. E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 689−691.

TABLE 4. Effect of Guanidine Deprotection Conditions on Stereochemical Integrity



entry	reagent	solvent	time, min	diastereomer ratio (*S*,*S*)+(*R*,*R*):(*S*,*R*)
1	50% TFA	CH_2Cl_2	60	50:50
2	90% TFA	H_2O	60	50:50
3	0.35 M $SnCl_4$	EtOAc	180	60:40
4	1 M HCl	EtOAc	60	81:19
5	0.5 M HCl	EtOAc	60	94:6

conjugation and multiple electron-withdrawing protecting groups on the guanidine. Examination of the effects of deprotection conditions on epimerization is thus generally applicable for all applications employing α-guanidino acids.

When Boc deprotection of **5** to generate **6** was conducted using standard Boc deprotection conditions of 50% TFA/CH_2Cl_2 or 90% TFA/H_2O, two isomeric products **6** were observed (dr 50:50) (Table 4). The diastereomers of **6** were readily resolved by reverse phase HPLC and were distinguishable by ^{1}H NMR (Supporting Information). Even under mild Lewis acid-mediated deprotection with $SnCl_4$,[13] significant epimerization was observed. In contrast, predomainantly a single diastereomer of **6** was observed (dr 94:6, indicating epimerization at 3% of α-positions) when conducting the Boc deprotection under mild conditions (0.5 M HCl in EtOAc). These results further indicate that the guanylation, saponification, amide coupling, and Boc deprotection steps all proceeded with minimal racemization or epimerization (Supporting Information).

In order to determine the generality of this approach to synthesize chiral guanidiniums as a single stereoisomer, the effect of deprotection conditions was further examined. Notably, epimerization occurred only in the protected molecule **5**, not in the deprotected bis-guanidinium **6**: subjection of purified (*S*,*R*)-**6** to either 50% TFA/CH_2Cl_2 or 90% TFA/H_2O resulted in no evidence of epimerization (Supporting Information). In total, these results indicate that Boc-protected chiral α-substituted guanidines are readily deprotected under mildly acidic conditions and should be amenable to incorporation in complex molecular libraries.

We have described and demonstrated the feasibility of a general approach to the convergent synthesis of compounds containing chiral α-substituted guanidiniums derived from α-amino acids. The syntheses are practical and straightforward and, due to the wide availability of chemically and structurally diverse α-amino acids, should be readily applicable to the preparation of molecular libraries with complex functionality. Coupling of α-guanidino acids to a diamine and acidic deprotection under mild conditions proceeded readily with retention of stereochemistry, providing a route to rapidly generate diverse and functionally complex chiral polyguanidiniums.

Experimental Section

Representative Synthesis of Protected Guanylated Amino Acid Esters: Synthesis of 2e. Following the approach of Lipton,[7d] to a solution of L-leucine methyl ester (1.27 g, 7 mmol) (**1e**) in

(13) Miel, H.; Rault, S. *Tetrahedron Lett.* **1997**, *38*, 7865−7866.

anhydrous DMF (2 mL) were added diisopropylethylamine (3.9 mL, 21 mmol) and *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea[10] (1.95 g, 7 mmol). A slurry of 2-chloro-1-methylpyridinium iodide (Mukaiyama's reagent) (1.8 g, 7 mmol) in anhydrous DMF (10 mL) was added dropwise via syringe to the reaction mixture, and the reaction was stirred at room temperature until product formation ceased (5 h). The reaction mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 × 80 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was evaporated to give a crude oil. The crude mixture was purified (SiO_2, 70:30 hexanes/EtOAc) to give the protected guanylated α-amino acid methyl ester **2e** (2.01 g, 71%): ^{1}H (400 MHz, $CDCl_3$) δ 11.36 (s, 1H), 8.60 (d, J = 7.7 Hz, 1H), 4.46 (m, 1H), 3.71 (s, 3H), 1.91−1.86 (m, 1H), 1.78−1.65 (m, 2H), 1.50 (s, 9H), 1.48 (s, 9H), 0.98 (d, J = 6.1 Hz, 3H), 0.94 (d, J = 6.5 Hz, 3H); ^{13}C ($CDCl_3$) δ 172.7, 161.9, 157.2, 153.1, 83.5, 80.0, 52.5, 39.6, 30.1, 28.5, 28.4, 24.9; HRMS calculated mass = 387.2447 (M + H), observed mass = 388.2458 (M + H); IR (CH_3OH, cm^{-1}) 1724, 1642, 1614, 1561, 1416, 1366, 1318, 1283, 1155, 810; specific rotation $[\alpha]_D^{25} = -21.0$.

Representative Synthesis of Protected Guanidino Acids: Synthesis of 3e. To the protected guanylated amino acid methyl ester **2e** (1.5 g, 4 mmol) was added a solution of LiOH (0.480 g, 20 mmol, 5 equiv) in 3:1 MeCN/H_2O (10 mL). The reaction was allowed to stir at room temperature for 2 h. The reaction mixture was cooled to 0 °C and quenched by dropwise addition of a 0.5 N solution of HCl with continuous stirring. When the pH of the reaction mixture was between pH 4 and pH 6, the solution was allowed to warm to room temperature and extracted with EtOAc (3 × 25 mL). The combined EtOAc layers were dried (Na_2SO_4) and the solvent was evaporated to give the protected guanylated α-amino acid **3e** as a white solid (1.01 g, 72%) after purification (SiO_2, 60:40 hexanes/EtOAc): ^{1}H (400 MHz, $CDCl_3$) δ 11.34 ((s, 1H), 9.30 (br s, 1H), 8.61 (d, J = 4.4 Hz, 1H), 4.51−4.43 (m, 1H), 1.90−1.85 (m, 1H), 1.78−1.63 (m, 2H), 1.49 (s, 9H), 1.47 (s, 9H), 0.97 (d, J = 6.3 Hz, 3H), 0.92 (d, J = 6.1 Hz, 3H); ^{13}C ($CDCl_3$) δ 172.7, 161.9, 157.2, 153.2, 84.8, 80.9, 53.6, 32.1, 28.4, 28.1, 22.3, 22.1; HRMS calculated mass = 374.2291 (M + H), observed mass = 374.2300 (M + H); IR (CH_3OH, cm^{-1}) 3424, 3281, 2935, 1744, 1709, 1655, 1568, 1133, 1020; specific rotation $[\alpha]_D^{25} = -44.0$.

Representative Amide Coupling Reaction of Cyclohexylamine with a Protected Guanidino Acid: Synthesis of 4e. To a stirred solution of the Boc-protected α-guanidino acid **3e** (0.516 g, 0.8 mmol) were added 1-ethyl-3-(3′-dimaminopropyl)carbodiimide (EDCI) (0.153 g, 0.8 mmol) and N-hydroxybenzotriazole (HOBt) (0.108 g, 0.8 mmol) at room temperature and allowed to stir for 15 min, followed by slow addition of cyclohexylamine (0.092 mL, 0.079 g, 0.8 mmol) over 5 min and the subsequent addition of DIPEA (0.260 mL, 0.208 g, 1.6 mmol). Stirring was continued for 2 h at room temperature. The reaction mixture was quenched by addition of water and extracted with EtOAc (3 × 30 mL). The combined EtOAc layers were washed with a saturated brine solution and dried (Na_2SO_4), and the solvent was evaporated to give the product **4e** as a colorless oil (0.24 g, 65%) after purification (SiO_2, 60:40 hexanes/EtOAc): ^{1}H (400 MHz, $CDCl_3$) δ 11.35 (s, 1H), 8.69 (d, J = 7.7 Hz, 1H), 6.50 (d, J = 8.2 Hz, 1H), 4.15−4.09 (m, 1H), 3.83−3.72 (m, 1H), 2.28−2.19 (m, 1H), 1.91−1.80 (m, 2H), 1.71−1.62 (m, 4H), 1.51−1.47 (m, 20H), 1.41−1.14 (m, 6H), 1.03−0.95 (m, 6H); ^{13}C ($CDCl_3$) δ 169.8, 163.5, 156.6, 153.2, 83.8, 79.7, 79.6, 60.8, 48.2, 33.3, 33.0, 29.7, 28.6, 28.5, 26.0, 24.8, 19.8, 19.0; HRMS calculated mass = 455.3233 (M + H), observed mass = 455.3252 (M + H); IR (CH_3OH, cm^{-1}) 3751, 3681, 2934, 1708, 1655, 1568, 1519, 1401, 1318, 1129, 1052; specific rotation $[\alpha]_D^{25} = -46.0$.

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Supporting Information Available: Experimental procedures, characterization data, HPLC chromatograms, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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