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The Guanidinium Group in Molecular Recognition: Design and Synthetic Approaches

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Abstract

The guanidinium group is found in many natural products and has been extensively incorporated into various drug designs as well as artificial receptor structures. In this paper we critically review the various synthetic routes to guanidiniums and describe a novel approach that allows the mild formation of multi-substituted derivatives.

The guanidine group in natural products, pharmaceuticals, and supramolecular chemistry

The guanidine functionality is found widely in natural products, pharmaceutically active compounds and in molecules used for supramolecular studies [1, 2]. Many natural metabolites found in a variety of organisms contain guanidines [3, 4]. The bisguanidine, TAN-1057 D (**1**), was isolated from *Flexibacter* sp. PK-74 bacteria and shows potent activity, rivaling that of vancomycin, against β-lactam-resistant, Gram-positive bacteria [5]. The amino acid, capreomycidine (**2**), is a biosynthetic precursor to the antibiotic, capreomycin [6]. The N, N'-disubstituted guanidine within this compound is incorporated in a six-membered ring. More recently, complex guanidine-containing natural products have been isolated and subjected to synthetic studies. For example, the tricyclic guanidine, ptilomycalin A (**3**), was isolated from sponges harvested from both the Caribbean and the Red Seas, and has cytotoxic, antifungal, antimicrobial, and antiviral activities [3, 7–10].

A number of pharmaceutically active drugs contain guanidines. The N, N'-bis-substituted guanidine, RPR 120033 (**4**) is a sub-micromolar antagonist of the glycoprotein C5a, a receptor involved in the recruitment of leukocytes to sites of inflammation [11]. Compound **5**, synthesized by Parke-Davis, contains two guanidines, and is active as a tyrosine kinase inhibitor [12]. A cyano, tris-substituted guanidine developed by Boehringer Ingelheim, Therbogrel (**6**), demonstrates activity similar to that of aspirin, as a combined thromboxane A_2 receptor/thromboxane A_2 synthase inhibitor and has been shown to disrupt plaques on the interior of blood vessels [13].

Because of the anion-binding and hydrogen bond donating properties of the guanidinium group, a number of guanidine-containing molecules have been designed for supramolecular studies [14]. The tetraguanidine, **7** was designed by Kagechika, and binds in the minor groove of DNA with sequence specificity and with low micromolar affinity [15]. The design provides five π-stacked aromatic rings flanked by charged guanidinium groups to allow phosphate and hydrogen bond complementarity and to provide van der Waal's interactions with the narrow DNA minor groove. De Mendoza's receptor, **8**, was designed to bind zwitterionic aromatic amino acids. The naphthalene interacts with the sidechain, the crown ether binds to the ammonium, and the bicyclic guanidinium recognizes the carboxylate of the amino acid [16]. Receptor, **9**, was designed in our laboratory to catalyze the hydrolysis of phosphodiesters. The guanidinium groups bind the phosphate tightly, while the trialkylamine acts as a general base to activate the internal alcohol for displacement of the spectroscopically monitored nitrophenol [17, 18]. Using **10**, we were able to explore the rapid dynamics of host guest complexation. The guanidinium groups were used to bind the carboxylates of a fluorophore, and fluorescence anisotropy measurements determined that the complex varies from planarity by as much as 30° [19]. Bruice created the guanidinium linked oligonucleoside (**11**) for incorporation into antisense oligodeoxyribonucleotides. The guanidinium changes the net charge of the oligomer and thereby increases its binding activity. The synthesis was designed to allow easy implementation of conventional solid phase DNA synthesis methodology [20].

Tactics for guanidinium preparation

The synthesis of guanidines is complicated because the product is electron rich and strongly basic. For this reason, many syntheses provide products that are protected with easily removed, non-polar, electron withdrawing groups. One straightforward strategy for providing substituted guanidines is to alkylate the intact, protected guanidine. Vaidyanathan developed methodology for the alkylation of bis-

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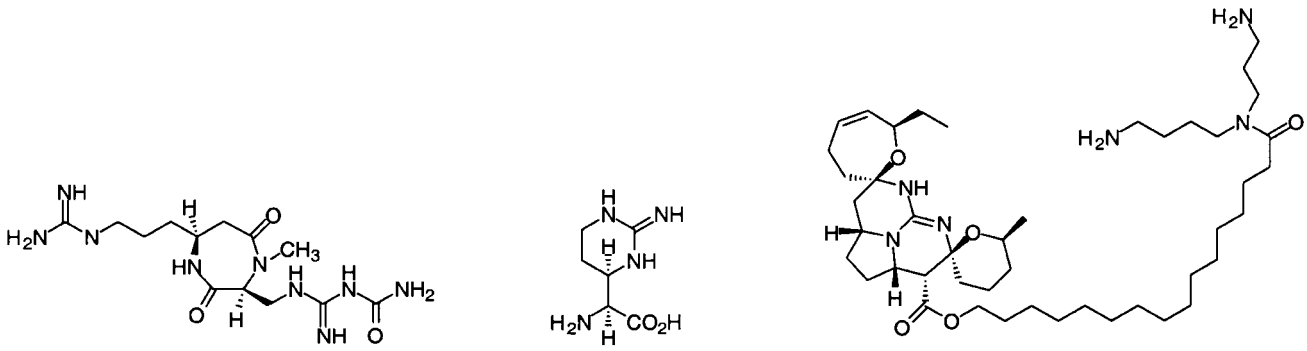
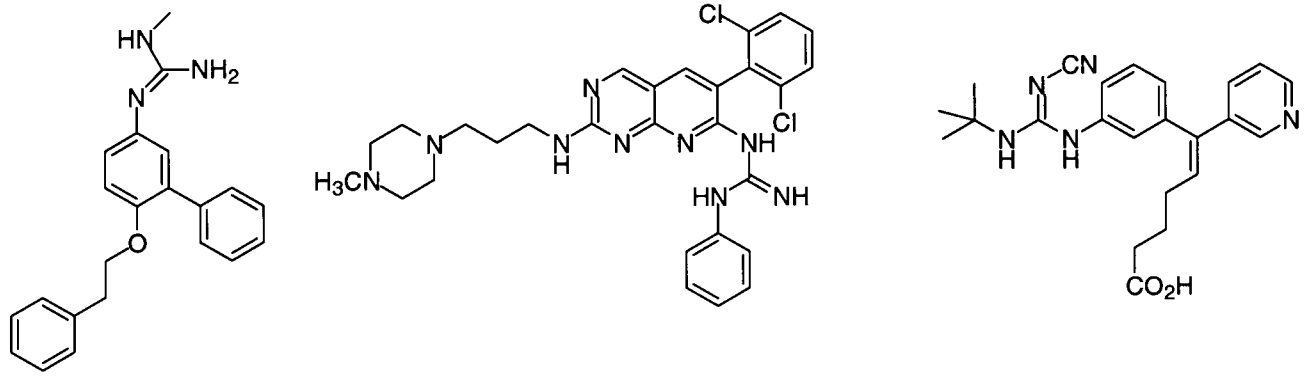


1 2 3

Figure 1. Guanidine-containing natural products. (**1**) TAN-1057 D, (**2**) Capreomycidine, (**3**) Ptilomycalin A.



4 5 6

Figure 2. Guanidine-containing pharmaceuticals. (**4**) RPR 120033, (**5**) a Parke-Davis tyrosine kinase inhibitor, (**6**) Terbogrel.

Boc guanidine with alkyl bromides (Equation **1**) [21], while Kozikowski [22] (Equation **2**) and Goodman [23] (Equation **3**) both used a Mitsunobu method for the alkylation of bisurethane and trisurethane guanidine, respectively. The electron withdrawing protective groups in these cases serve the dual role of not only increasing solubility in organic solvents, but also activating the appended nitrogen for deprotonation.

More common methods for guanidinylation involve the attack of an amine on various activated guanidinylating reagents. Barvian has reported that N, N'-bis-aryl substituted ureas, when treated with Burgess' Reagent, and then reacted with 2-aminopyridine, yields the guanidine, presumably through carbodiimide formation (Equation **4**) [12]. Barton subjected various ureas to phosgene to form the Vilsmeier salt and then treated them with amines to provide guanidines. This method was able to generate the hindered N, N, N', N', N''-pentaisopropyl guanidine (Equation **5**) [24]. Goodman developed a method involving triflicguanidines (Equation **6**) that is one of the most versatile and effective available, and Bernatowicz, from Bristol-Meyers Squibb, used a guanylpyrazole as a guanidinylating reagent (Equation **7**) [25].

Although thiourea derivatives often generate noxious-smelling byproducts, the most common guanidinylating methods involve their use. The general design strategies of these syntheses have been to provide non-polar protective groups in order to ease purification and activate attack by the incoming amine, and to increase the leaving group propensity of the sulfur. The conditions of Maryanoff (Equation **8**) rely on the attack of sulfonic acids derived from N-alkyl substituted thioureas to generate guanidines [26], while Ratcliffe's (Equation **9**) design of a guanidinylating reagent increases electrophilicity by incorporating two electron withdrawing Boc groups and dinitrothiophenol as the leaving group [27]. Cody (Equation **10**) used S-methylisothioureas protected with aryl sulfonates [28], in the presence of mercury salts, to synthesize guanidines, and Cammidge (Equation **11**) used bis-Boc-isothioureas with mercuric chloride [29–32]. Lipton (Equation **12**) developed methodology using Mukaiyama's Reagent to form a carbodiimide from bis-Boc-thiourea which was subsequently treated with amines [33]. Poss (Equation **13**) has used Boc-protected thioureas to react with amines in the presence of the water-soluble carbodiimide, EDCI, under very mild conditions without the production of an offensive byproduct [34–35].

Strategies for the synthesis of guanidinium groups

A general multi-step methodology to form substituted guanidines from commercially available starting materials has

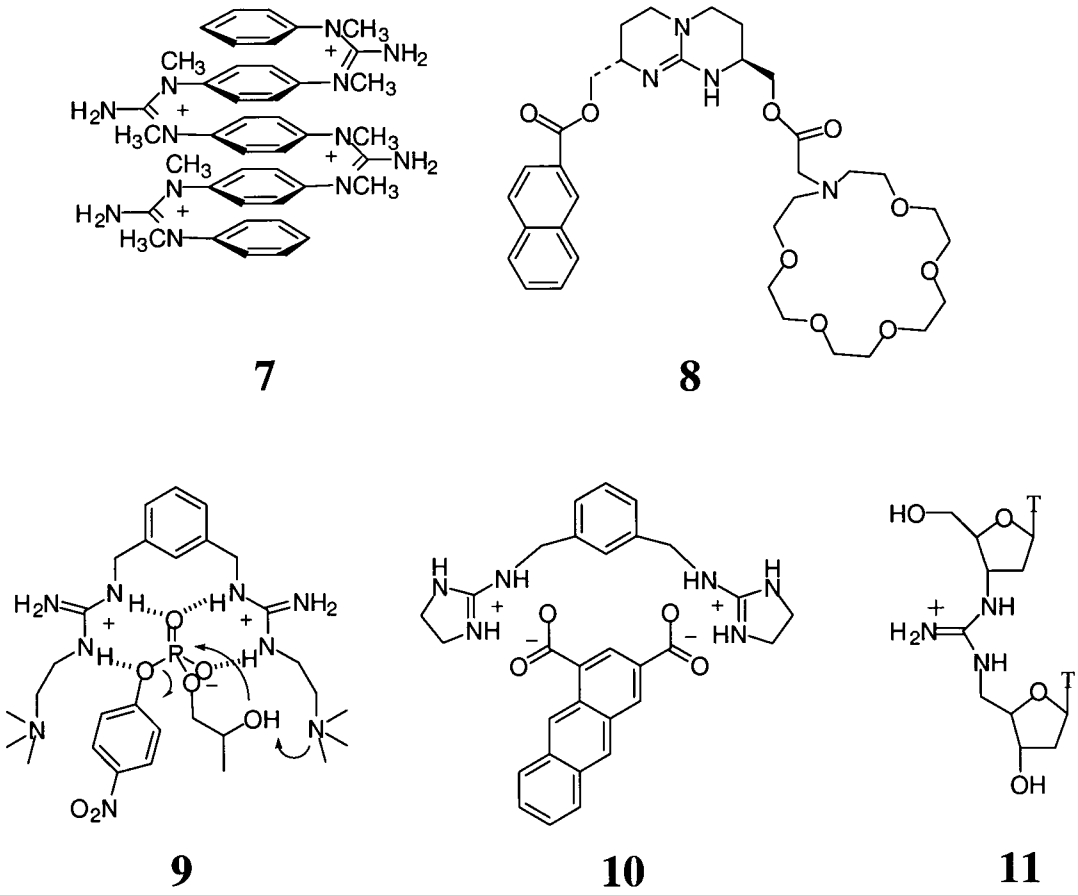


Figure 3. Guanidine-containing molecules used for supramolecular studies. Molecules designed by: (**7**) Kagechika, (**8**) de Mendoza, (**9** and **10**) Hamilton, and (**11**) Bruice.

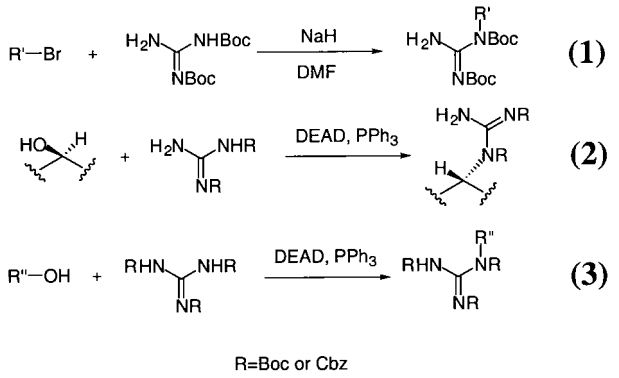


Figure 4. Guanidinylation tactics involving the alkylation of protected guanidine. Methods developed by: (Equation **1**) Vaidyanathan, (Equation **2**) Kozikowski, and (Equation **3**) Goodman.

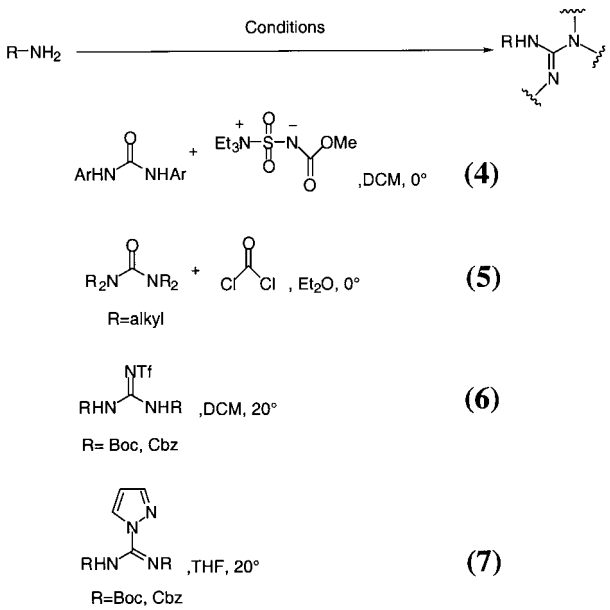


Figure 5. Guanidinylation conditions for the transformation of an amine to a guanidine. Methods developed by (Equation **4**) Barvian, (Equation **5**) Barton, (Equation **6**) Goodman, and (Equation **7**) Bernatowicz.

been the goal of many groups. A number of methods have been developed with varying generality and utility.

Rasmussen explored a method of guanidinylation which was initiated by attack of aryl amines on benzoylisothiocyanate and subsequent hydrolysis of the resulting thiourea (Equation **14**) [36, 37]. Methylation afforded the *S*-methyl isothiourea and treatment with an amine in refluxing *t*-butanol resulted in a guanidinylated product. Thiourea formation and hydrolysis were explored by using a wide range of anilines and aminopyridines. Yields of the thiourea formation step range from quantitative for electron rich anilines to ~75% for nitroanilines and the aminopyridines.

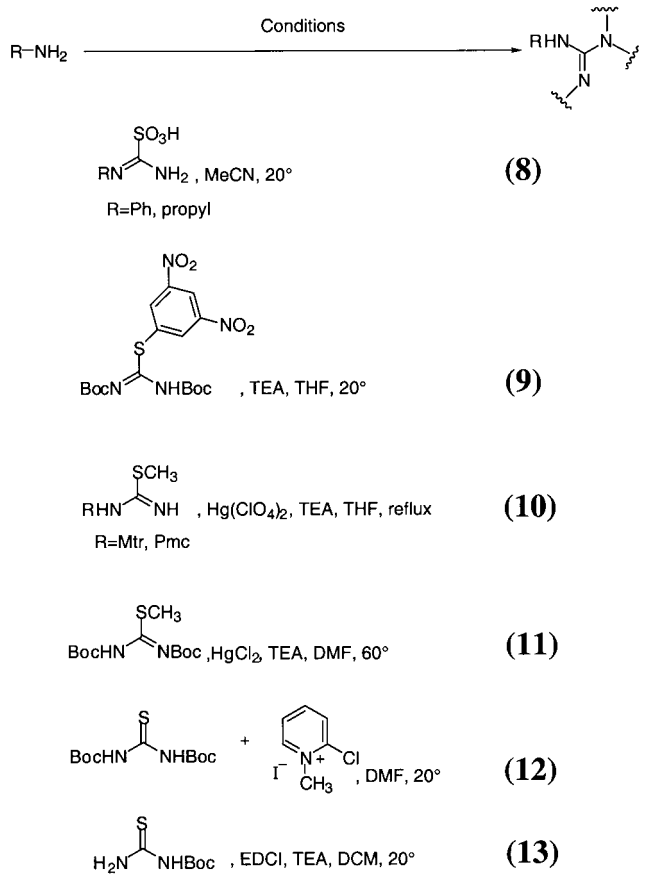


Figure 6. Guanidinylation methods involving thiourea derivatives. Conditions optimized by: (Equation **8**) Maryanoff, (Equation **9**) Ratcliffe, (Equation **10**) Cody, (Equation (**11**) Cammidge, (Equation **12**) Lipton, and (Equation **13**) Poss.

Atwal and coworkers synthesized cyanoguanidines through the reaction of arylisothiocyanates with sodium cyanamide to afford the cyanothiourea. Treatment with an amine and the mild reagent, EDCI, resulted in the desired cyanoguanidines (Equation **15**) [35]. The thioureas derived from electron poor isothiocyanates showed few advantages over electro-neutral compounds, however the use of bulky amines reduced the yield.

Poss' method is similar to that of Rasmussen, however, the resulting product is protected and thus can be more easily incorporated into a complex synthesis (Equation **16**) [34]. Poss also used EDCI guanidinylation which is milder than Rasmussen's refluxing *t*-butanol condition and does not involve the production of methanethiol. This method begins with the treatment of benzoyl-isothiocyanate with an amine and hydrolysis of the resulting amide. Protection of the thiourea with a Boc group is effected on the less sterically congested nitrogen. This mono-Boc-thiourea is then treated with EDCI and an amine to yield the mono-Boc-bis-substituted guanidine. The urethane not only serves to act as a protective group for the product, but its electron-withdrawing properties increase the rate of the guanidinylation. The reaction proceeds in high yield even with β-branched amines.

Cammige has recently demonstrated a method that gives bis- protected, bis-substituted guanidines (Equation **17**) [29]. The synthesis begins with the alkylation of bis-Boc-*S*-methylisothiourea. Guanidinylation in the presence of an amine and mercuric chloride is facilitated by the presence of two electron withdrawing protective groups, and the alkylated sulfur leaving group. This strategy gives an easily purified, non-polar product and allows incorporation of less reactive amines such as nitroanilines and diisopropylamine.

A new approach to guanidinium synthesis

Recently we have developed a method for the general synthesis of *N*-urethane protected, N', N''-substituted guanidines (Scheme 1) [38]. Our strategy involves a mild guanidinylation step and protective groups that can be removed under different conditions, i.e., ethyl, benzyl, trichlorodimethylethyl, fluorenylmethyl, and phenyl carbamates. In contrast to Poss' strategy, we incorporate the carbamate from the beginning and do not have a deprotection/protection step in the synthesis. Commercially available chloroformates are regioselectively substituted by treatment with potassium thiocyanate. The resulting isothiocyanate is treated with an amine to give the protected thiourea. This species is then subjected to guanidinylation with EDCI and a second amine to afford the carbamate protected guanidine.

We have investigated the effect of different amines on thiourea and guanidine formation and of thiourea substitution on the guanidinylation. The results are collected in Table 1. The clear limitation is that thioureas derived from the secondary amines **C** and **D** failed to form any detectable guanidine product. This could be due to either steric hinderence or the lack of a reactive proton which could inhibit carbodiimide formation. Many of these target guanidines could be synthesized, however, by the guanidinylation of amines **C** and **D** with thioureas **12A**, **12B**, **12E**, **12G**. These results suggest that the guanidinylation is more sensitive to bulky substitution on the thiourea than on the nucleophile, as further seen in guanidinylations between thioureas derived from *t*−butylamine, (**12B**), and benzylamine, (**12A**) with aliphatic amines, **A–D**. The reaction of bulky **12B** is more sluggish for all three amines than that with the less sterically demanding **12A**.

Investigation of the guanidinylation of these two aliphatic thioureas with anilines **E–G**, suggests that the steric limitations affecting the reactivity of the thiourea can be overcome. The bulky **12B** reacts in a higher yield than the smaller **12A** with methylaniline, **E**, and methoxyaniline, **F** and in a lower yield with nitroaniline, **G**. These data suggest that something other than steric effects is controlling this reaction.

Trends in the apparent electronic effects governing the guanidinylation reaction are less clear than with the steric effects. The relatively electronically neutral aniline, **E**, appears to be the most generally reactive for the aliphatic thioureas, **12A** and **12B**. The electron rich **F** is less reactive with **12A** than with bulky **12B** and the electron poor **G** is less reactive with **12B**. Of the three amines, **F** is the least reactive with **12A** and **G** the second least. Conversely, **G** is the least reactive with **12B**.

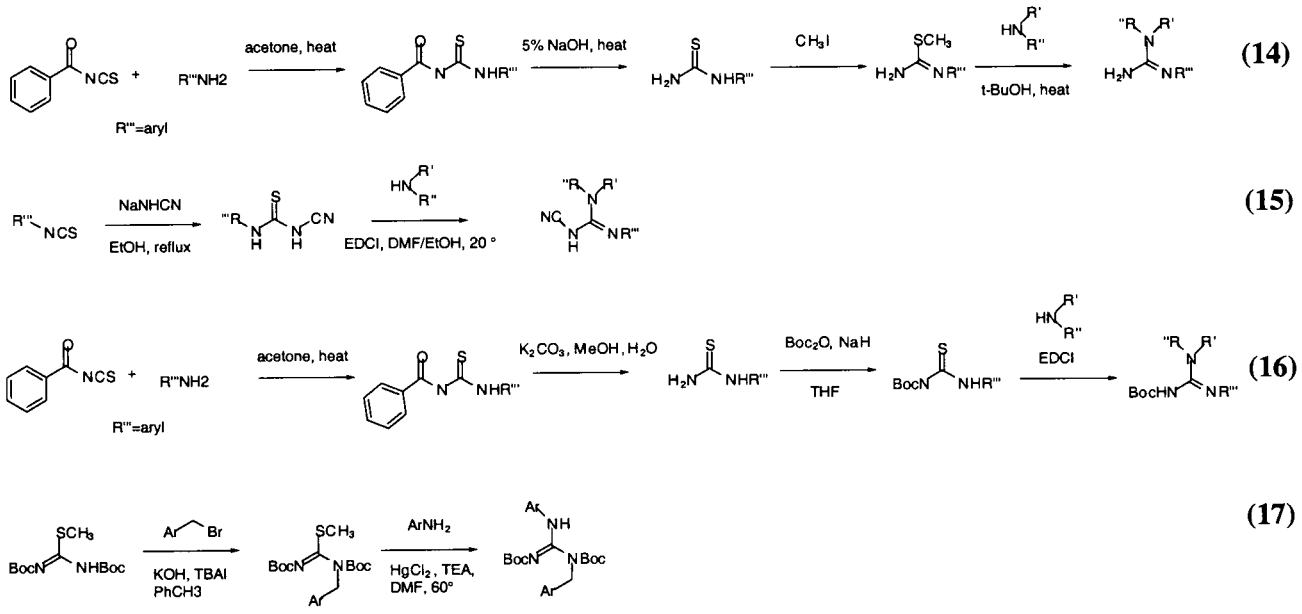


Figure 7. General strategies for guanidinylation. Methods developed by (Equation **14**) Rasmussen, (Equation **15**) Atwal, (Equation **16**) Poss, and (Equation **17**) Cammige.

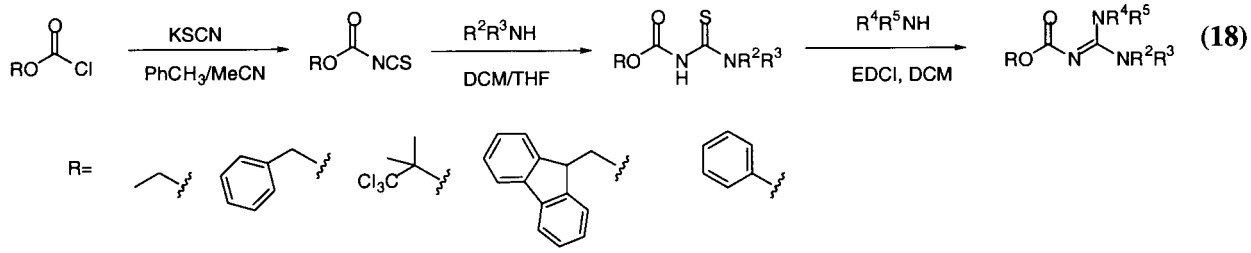


Figure 8. Our general strategy for guanidinylation.

Comparisons between the guanidinylations of the anilinic thioureas, **12E–12G**, and the aliphatic amines, **A–D**, also demonstrate complicated electronic constraints. The reactivity of the various amines for the thioureas follow the trends: **A**: **12E** > **12F** > **12G**; **B**: **12F** > **12E** > **12G**; **C**: **12E** = **12G** > **12F**; **D**: **12F** > **12E** > **12G**. The reactivity of these thioureas for the aliphatic amines is as follows: **12E**: **A** = **C** > **B** > **D**; **12F**: **B** > **A** > **D** > **C**; **12G**: **C** > **A** > **B** > **D**.

The electronic affect between the anilines (**E–G**) and the aniline thioureas (**12E–12G**) is more obvious. Electron poor thioureas and amines are both bad substrates for the reaction and combinations of the two, as exhibited by the guanidinylation between **G** and **12G**, are especially disfavored. Interestingly, the reaction between an electron rich amine, **F**, and an electron rich thiourea, **12F**, also results in a poor yield. The switch in electronic preference suggests competition between two mechanisms and explains the complicated trends in the earlier data.

A possible mechanism (Scheme 2) involves nucleophilic attack by the thiourea on EDCI, which favors electron rich thioureas, and either (1) the leaving of the EDCI-sulfur adduct to form the carbodiimide (favored by electron rich substituents) and subsequent attack by the amine or (2) direct attack by the amine on the thiourea-EDCI adduct (favored by electron poor thioureas). The rate limiting steps possibly switch between one that is expedited by electron withdrawal on the thiourea and one that is facilitated by electron donation. Electronic considerations may be the deciding factor in favor of one mechanism over the other, however this requires additional studies.

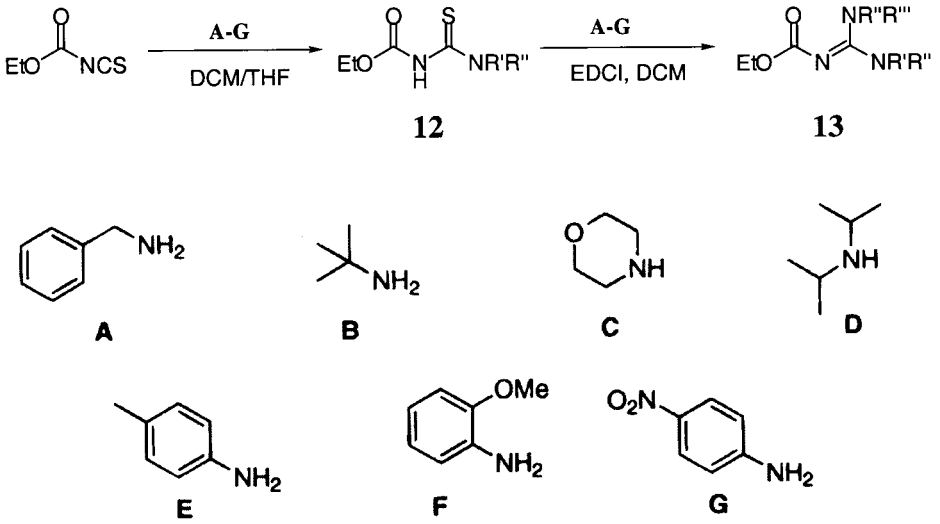
Conclusion

The guanidine functionality is found in many natural products and pharmaceuticals, and is used in a variety of supramolecular designs. Its widespread use emphasizes its importance in molecular recognition across the spectrum of organic, biological and medicinal chemistries. We have reviewed current methodologies for the synthesis of this functional group, emphasizing procedures with high generality and utility. Furthermore, we have disclosed a recent method from our laboratory, proposed a possible mechanism, and suggested future investigations.

General procedure for the preparation of carbamoyl thioureas

A solution of ethoxycarbamoyl isothiocyanate (1.0 mmol) in 50 mL dichloromethane was cooled to 0 °C and amines **A–**

Table 1. Reaction of ethoxycarbonyl isothiocyanate with various amines to form thioureas and guanidines. All yields are isolated yields



Amine	% Yield of thiourea (**12**)	% Yield of guanidine (**13**)						
		A	**B**	**C**	**D**	**E**	**F**	**G**
A	99	99	99	95	76	92	82	85
B	99	99	74	59	55	99	99	65
C	99	0	0	0	0	0	0	0
D	99	0	0	0	0	0	0	0
E	92	99	87	99	81	95	97	34
F	77	96	99	61	84	98	67	39
G	72	85	76	99	42	35	34	0

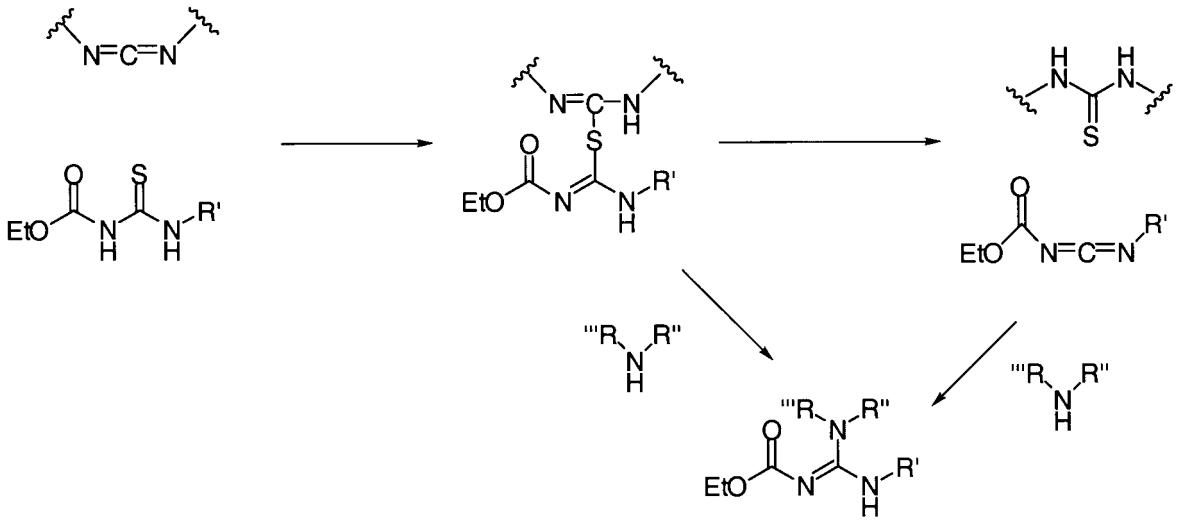


Figure 9. Possible guanidinylation mechanisms.

G (1.0 mmol) were added. The ice bath was removed and the solution was stirred for four hours under nitrogen. The solution was washed with 1% HCl, water, brine and dried with Na_2SO_4. Solvent was removed under reduced pressure and the product was purified by column chromatography.

General procedure for the preparation of carbamoyl guanidines

Carbamoyl thiourea, **12** (1.0 mmol), alkyl amine, **A–G**, (1.5 mmol), and diisopropylethylamine (1.0 mmol) were added to 10 mL anhydrous dichloromethane and cooled to 0 °C. EDCI (1.5–2.0 mmol) was added and the solution was stirred under nitrogen. After one hour the ice bath was removed and the solution was stirred for an additional 10 hours at room

temperature. In cases where TLC indicated unreacted starting material, addition of more amine and EDCI resulted in increased yields. The reaction mixture was washed with 1% HCl, water, brine and dried with Na_2SO_4. The residue that remained after removal of solvent under reduced pressure was purified by silica chromatography.

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