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Trifluoroacetyl as an Orthogonal Protecting Group for Guanidines

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Received June 24, 2003

The trifluoroacetyl moiety has been used as a new protecting group for guanidine functionality. The protecting group is easily cleaved under mild basic conditions and is complementary to the Boc, Cbz, and Ddpe protecting groups. The protecting group can be applied to peptide synthesis in solution as well as on a solid phase as it is orthogonal to a Boc and Cbz strategy and semiorthogonal to an Fmoc strategy.

Introduction

The guanidine moiety, often found in natural and pharmaceutical compounds,[1] plays an essential role in biological systems[2] and is often used in molecular recognition.[3] Consequently, procedures that allow the generation of highly substituted guanidines in good yields and the selective removal of the guanidine protecting group under mild conditions are of great interest in medicinal chemistry, and much effort has been directed toward developing efficient synthetic routes for the preparation of these compounds.[4]

In our research we have developed tweezer receptors[5] with a guanidinium "head" group as peptide receptors. We have prepared both split-and-mix libraries of such receptors[3h] and individual tweezer compounds,[3d] using solid-phase and solution methodology, but to date, in all cases, the choice of protecting group for the guanidine moiety has been the tosyl group (Figure 1). Cleavage of the tosyl group, however, requires harsh conditions (hydrofluoric acid), which presents practical difficulties and has limited the scope of the chemistry.

Investigations were therefore performed to find alternative protecting groups, which are orthogonal to protecting groups used in peptide synthesis. Our attention has focused on the trifluoroacetyl group, which is a well-known protecting group for amine functionality and is easily cleaved[6] but, to date, has not been used as a protecting group for guanidines.[7] The scope and limitation of the trifluoroacetyl group as a protecting group for guanidines has therefore been investigated, and the experimental details are reported herein.

Results and Discussion

Various methods exist for the synthesis of guanidines from different starting materials and reagents.[4] One of the common methods involves the guanidinylation of a thiourea. In the protocol we have followed the thiourea was activated as its thiouronium salt before guanidinylation.[3h,8]

To gauge the applicability of the trifluoroacetamide guanidinylation procedure, thioureas **3a**−**f** with different protecting groups were prepared, and conversion to the corresponding guanidines was investigated. Thioureas **3a**−**f** were synthesized from the corresponding amine **1** and isothiocyanate **2** (Scheme 1).

Alkylation of all the thioureas **3a**−**f** with methyl iodide and counterion exchange gave the thiouronium hexafluorophosphate, which was guanidinylated in the presence of DBU and trifluoroacetamide to give the protected guanidine **4**.

Guanidinylation of thiouroniums proceeded with very good yields for **4a**−**d** and **4f** (Table 1, entries 1−4 and 6), whereas a moderate yield was obtained in the case of **4e** (Table 1, entry 5). The reduced yield of **4e** is due to

(1) (a) Greenhill, J. V.; Lue, L. In *Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier Science: New York, 1993; Vol. 30, Chapter 5. (b) Yamamoto, Y., Kojima, S. In *Synthesis and Chemistry of Guanidine Derivatives*; Patai, S., Rappoport, Z., Eds.; John Wiley & Sons, Ltd.: New York, 1991; Vol. 2, pp 485−526.

(2) Hannon, C. L.; Anslyn, E. V. *Bioorganic Chemistry Frontiers*; Springer: Berlin, 1993; Vol. 3, pp 193−255.

(3) For a review see: (a) Fitzmaurice, R. J.; Kyne, G. M.; Douheret, D.; Kilburn, J. D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 841−864. See also, for example: (b) Galán, A.; Andreu, D.; Echavarren, A.; Prados, P.; de Mendoza, J. *J. Am. Chem. Soc.* **1992**, *114*, 1511−1512. (c) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609−1646. (d) Davis, M.; Bonnat, M.; Guillier, F.; Kilburn, J. D.; Bradley, M. *J. Org. Chem.* **1998**, *63*, 8696−8703. (e) Linton, B.; Hamilton, A. D. *Tetrahedron* **1999**, *55*, 6027−6038. (f) Schneider, S. E.; O'Neil, S.; Anslyn, E. V. *J. Am. Chem. Soc.* **2000**, *122*, 542−543. (g) Cabell, L. A.; Best, M. D.; Lavigne, J. J.; Schneider, S. E.; Perreault, D. M.; Monahan, M. K.; Anslyn, E. V. *J. Chem. Soc., Perkin Trans. 2* **2001**, 315−323 and references therein. (h) Jensen, K. B.; Braxmeier, T. M.; Demarcus, M.; Kilburn, J. D. *Chem.—Eur. J.* **2002**, *8*, 1300−1309.

(4) (a) Orner, B. P.; Hamilton, A. D. *J. Inclusion Phenom. Macrocyclic Chem.* **2001**, *41*, 141−147, (b) Manimala, J. C.; Anslyn, E. V. *Tetrahedron Lett.* **2002**, *43*, 565−567 and references therein.

(5) For original work on tweezer receptors, see: (a) Chen, C.-W.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 4921−4922. (b) Zimmerman, S. C.; Wu, W.; Zeng, Z. *J. Am. Chem. Soc.* **1991**, *113*, 196−201.

(6) Boger, D. L.; Yohannes, D. *J. Org. Chem.* **1989**, *54*, 2498−2502.

(7) Reaction of guanidino-α-amino acids (e.g., arginine) with trifluoroacetic anhydride has been described: Klein, C.; Schulz, G.; Steglich, W. *Liebigs Ann. Chem.* **1983**, 1623−1637.

(8) See, for example: (a) Kurzmeier, H.; Schmidtchen, F. P. *J. Org. Chem.* **1990**, *55*, 3749−3755. (b) Kneeland, D. M.; Ariga, K.; Lynch, V. M.; Huang, C.-Y.; Anslyn, E. V. *J. Am. Chem. Soc.* **1993**, *115*, 10042−10055. (c) Bonnat, M.; Bradley, M.; Kilburn, J. D. *Tetrahedron Lett.* **1996**, *37*, 5409−5412.

10.1021/jo0348874 CCC: $25.00
Published on Web 10/24/2003

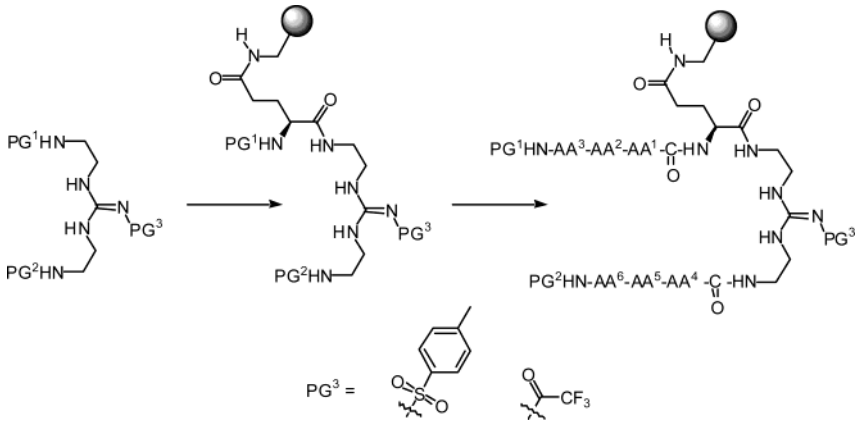
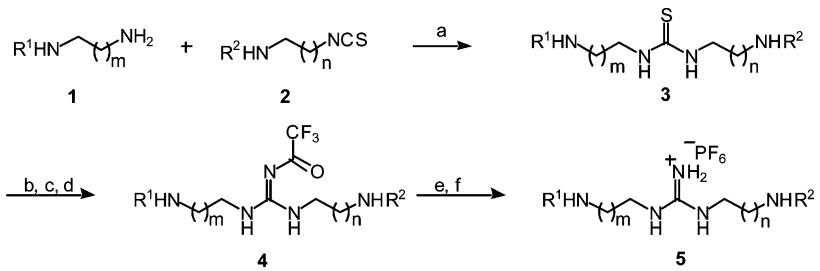


FIGURE 1. Synthesis of tweezer receptors.

SCHEME 1[a]

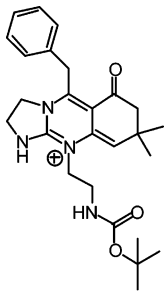


[a] Reagents and conditions: (a) MeOH/CH_2Cl_2, 20 °C; (b) CH_3I, acetone; (c) NH_4PF_6, MeOH/CH_2Cl_2; (d) CF_3CONH_2, DBU, toluene/$CHCl_3$, reflux; (e) K_2CO_3, CH_3OH/H_2O; (f) NH_4PF_6, CH_3OH.

TABLE 1. Synthesis of Trifluoroacetylguanidines 5

entry	3/4/5	R^1	R^2	m	n	yield of **3** (%)	yield of **4** (%)	yield of **5** (%)
1	**a**	Boc	Boc	2	2	75	86	90
2	**b**	Boc	Boc	1	1	75	84	88
3	**c**	Boc	Aloc	1	1	72	92	83
4	**d**	Cbz	Boc	2	1	72	85	90
5	**e**	Ddpe	Boc	1	1	69	45	75
6	**f**	Ddpe	Boc	2	1	78	75	76

both the partial cleavage of the Ddpe protecting group[9] (R^1) and the formation of a cyclic byproduct with presumed structure **6**, formed via nucleophilic attack of the Ddpe nitrogen onto the thiocarbonyl.



6

The corresponding byproduct is not formed when the longer propylene spacer is used to separate the Ddpe and the guanidine moieties, and consequently **4f** is formed in a much better yield, although some cleavage of the Ddpe protecting group is observed. The guanidinylation was not compatible with the Fmoc group which was cleaved under the basic conditions used, and Fmoc-protected variants of **4** could not be prepared directly by this route. However, treatment of **4c** with 9-fluorenyloxycarbonyl chloride and tributyltin hydride in the presence of a catalytic amount of Pd(0)[10] gave guanidine **4g** in 72% yield (Scheme 3).

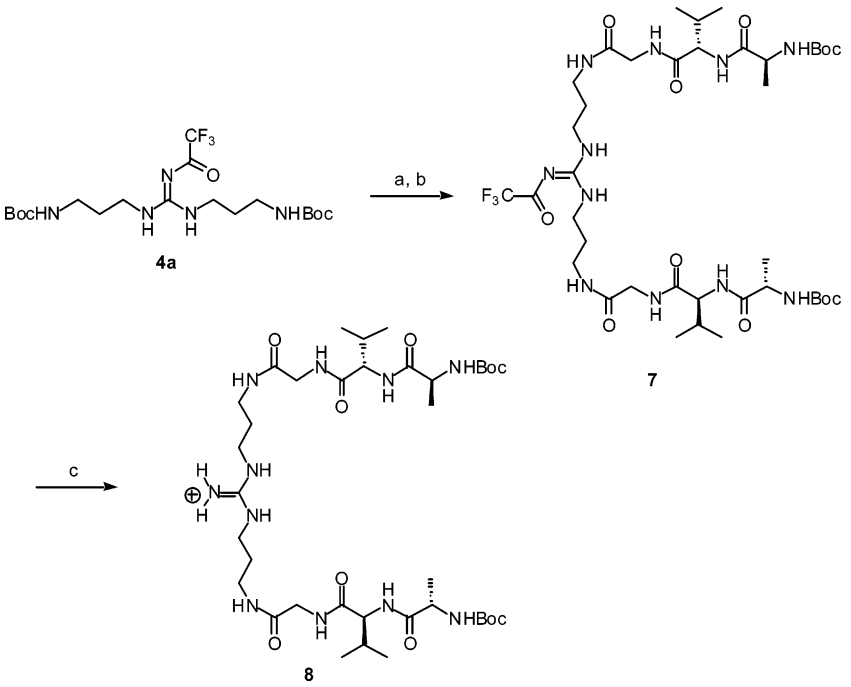
The resulting guanidines **4** could in each case be converted to the corresponding guanidinium hexafluorophosphate **5** (Scheme 1) when treated with potassium carbonate in a mixture of MeOH and water, without loss of any of the accompanying protecting groups, except, again, in the case of the Fmoc group. However, all of the amine protecting groups, including the Fmoc protecting group, could be cleaved with standard methods [(Aloc) $Pd(PPh)_3$, tributyltin hydride (1.2 equiv);[10] (Boc) 20% TFA, CH_2Cl_2; (Ddpe) aqueous hydrazine (5%);[11] (Fmoc) piperidine, CH_2Cl_2), giving quantitative yields of the

(9) Chhabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan, W. C. *Tetrahedron Lett.* **1998**, *39*, 1603−1606.

(10) Roos, E. C.; Bernabe, P.; Hiemstra, H.; Speckamp, W. N.; Kaptein B.; Boesten, W. H. J. *J. Org. Chem.* **1995**, *60*, 1733−1740.

(11) Kellam, B.; Chan, W. C.; Chhabra, S. R.; Bycroft, B. W. *Tetrahedron Lett.* **1997**, *38*, 5391−5394.

SCHEME 2[a]



[a] Reagents and conditions: (a) 30% CF_3CO_2H, CH_2Cl_2; (b) *N*-Boc-Gly-L-Val-L-Ala-OH, EDC, HOBt, DIPEA, DMF (45%); (c) K_2CO_3, $MeOH/H_2O$.

correspondent free amines and without affecting the trifluoroacetyl protecting group.

To show the utility of the new protecting group in peptide chemistry, we chose to synthesize two tweezers, in which the guanidine has been incorporated. The peptide arms of the tweezer could be synthesized either in solution using a Boc-coupling strategy (Scheme 2) or on a solid phase using an Fmoc-coupling strategy (Scheme 3). For the peptide coupling in solution, guanidine **4a** was used as starting material. After Boc deprotection of **4a** and coupling with *N*-Boc-Gly-L-Val-L-Ala-OH using EDC/HOBt, the guanidine derivative **7** was obtained in 45% yield. Cleavage of the trifluoroacetyl protecting group afforded the hydrogen carbonate salt of the guanidinium tweezer **8** as a white powder in quantitative yield.

To prepare a guanidinium tweezer on a solid phase, the ester derivative **9** was synthesized in two steps from the guanidine derivative **4g**. After Boc deprotection of **4g** and coupling with *N*-Fmoc-L-Glu(O^{t}Bu)-OH using Py-BOP/HOBt, the guanidine derivative **9** was obtained in 80% yield. Deprotection of the *tert*-butyl group provided **10**, suitably functionalized with a carboxylic acid moiety to allow attachment to a solid support. Coupling of acid **10** to Rink amide resin and Fmoc cleavage were followed by sequential coupling of Fmoc-Gly and Fmoc-Val. The resin was treated with potassium carbonate in a mixture of MeOH and water to remove the trifluoroacetyl group, and finally, cleavage from the Rink amide resin gave the guanidinium tweezer **11** as a white TFA salt without notable impurities.

In summary, a new protecting group for guanidines is described. The protecting group is orthogonal to acid-cleavable protecting groups as well as Cbz and Ddpe, and is easily cleaved under mild basic conditions. The utility of the protecting group for the guanidine moiety is shown with examples from peptide synthesis in solution as well as on a solid support. We believe this protecting group may find widespread use in the synthesis of both simple and highly complex guanidine-containing compounds.

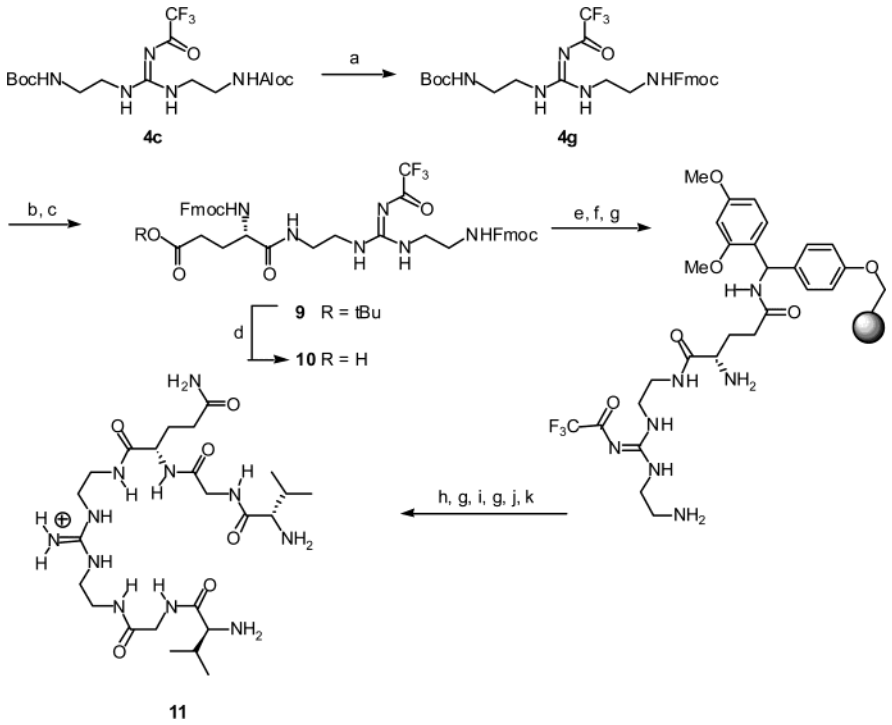
Experimental Section

2-{1-[(2-Aminoethyl)amino]-2-phenylethylidene}-5,5-dimethyl-1,3-cyclohexanedione (1e). 2-(1-Hydroxy-2-phenylethylidene)-5,5-dimethylcyclohexane-1,3-dione[9] (2.3 g, 8.9 mmol) in CH_2Cl_2 (200 mL) was added dropwise to a solution of ethylenediamine (2.25 g, 37.4 mmol) and TFA (70 μL, 0.91 mmol) in CH_2Cl_2 (10 mL), over a period of 10 h, with vigorous stirring. The stirring was continued for a further 36 h at room temperature. The solution was washed with a 2 M solution of sodium carbonate (2 × 100 mL) and water (2 × 100 mL). The organic layer was dried over magnesium sulfate and the solvent evaporated under reduced pressure to afford **1b** as a pale yellow oil (2.70 g, 100%): IR ν_{max} (film) = 2961 (w), 2927 (w), 2858 (w), 2364 (w), 2339.96 (w), 1647 (s), 1556 (s), 1496 (m), 1449 (m), 1404 (m), 1036 (m) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (s, 6H), 1.50 (br s, 2H), 2.38 (m, 4H), 2.89 (m, 2H), 3.39 (q, J = 5.7 Hz, 2H), 4.60 (s, 2H), 7.20 (m, 5H), 12.32 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5, 30.2, 35.4, 40.8, 45.9, 53.5, 111.8, 126.7, 128.1, 128.9, 136.1, 174.0, 197.6; MS (ES^+) m/z (rel intens) = 601 (13) $[2M + H]^+$.

Procedure A for the Synthesis of Thioureas 3c, 3d, 3e, and 3f. Monoprotected diamine **1** (7.6 mmol) was added to isothiocyanate **2** (6.9 mmol) in a 1:1 solvent mixture of MeOH (10 mL) and CH_2Cl_2 (10 mL). After the mixture was stirred at room temperature for 48 h, the solvent was removed under reduced pressure. The crude product was purified by FC on silica gel (eluting with $EtOAc/CH_2Cl_2$, 50:50 for **3c** and **3f**, 30:70 for **3d**, and EtOAc/petroleum ether, 80:20 for **3e**).

{2-[3-(2-Vinyloxycarbonylaminoethyl)thioureido]ethyl}-carbamic Acid *tert*-Butyl Ester (3c). This compound was synthesized according to procedure A on a 6.9 mmol scale: yield 1.72 g (72%); pale yellow oil; R_f = 0.16 ($EtOAc/CH_2Cl_2$, 50:50); IR (neat) ν_{max} = 3300 (m), 2964 (m), 2930 (m), 2883 (w) 1669 (s), 1521 (s), 1245 (s), 1160 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 3.31 (q, J = 5.7 Hz, 2H), 3.42 (q,

SCHEME 3[a]



[a] Reagents and conditions: (a) FmocCl, $Pd(PPh_3)_4$, Bu_3SnH, CH_2Cl_2 (72%); (b) 20% CF_3CO_2H, CH_2Cl_2; (c) Fmoc-L-Glu(O^{t}Bu)-OH, PyBOP, HOBt, DIPEA, CH_2Cl_2 (80%); (d) 60% CF_3CO_2H, CH_2Cl_2; (e) Fmoc-deprotected Rink amide resin (sub 0.45 mmol/g), HOBt, PyBOP, DIPEA, DMF/CH_2Cl_2; (f) acetic anhydride, DMAP, CH_2Cl_2; (g) 20% piperidine in DMF; (h) Fmoc-Gly-OH, DIC, HOBt, DIPEA, DMF; (i) Fmoc-L-Val-OH, DIC, HOBt, DIPEA, DMF; (j) K_2CO_3, MeOH/H_2O; (k) 15−30% CF_3CO_2H, TIS, CH_2Cl_2.

J = 5.7 Hz, 2H), 3.54 (br s, 2H), 3.62 (br s, 2H), 4.57 (d, J = 5 Hz), 5.09 (br s, 1H), 5.22 (dd, J = 10.6, 1.3 Hz, 1H), 5.30 (dd, J = 17.1, 1.3 Hz, 1H), 5.45 (br s, 1H), 5.90 (m, 1H), 6.83 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 39.7, 40.3, 44.9, 45.2, 65.9, 80.4, 117.9, 132.7, 157.3, 182.3; MS (ES^+) m/z (rel intens) = 347 (5) $[M + H]^+$, 693 (5) $[2M + H]^+$, 715 (27) $[2M + Na]^+$. All structural assignments were in agreement with MS and ^{1}H and ^{13}C NMR data available from the literature.[3h]

{3-[3-(2-*tert*-Butoxycarbonylaminoethyl)thioureido]-propyl}carbamic Acid Benzyl Ester (3d). This coumpound was synthesized according to procedure A on a 2.53 mmol scale: yield 750 mg (72%); white foam; R_f = 0.19 (EtOAc/CH_2Cl_2, 30:70); IR (neat) ν_{max} = 3302 (m), 2961 (m), 2932 (m), 2883 (w) 1679 (s), 1521 (s), 1245 (s), 1161 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (s, 9H), 1.70 (quint, J = 6 Hz, 2H), 3.19 (m, 4H), 3.45 (br s, 4H), 5.02 (s, 3H), 5.32 (br s, 1H), 6.76 (br s, 2H), 7.27 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.8, 29.8, 38.2, 40.2, 41.5, 67.2, 80.6, 128.4, 128.6, 128.9, 136.9, 157.5, 157.6, 182.3; MS (ES^+) m/z (rel intens) = 411 (20) $[M + H]^+$, 433 (100) $[M + Na]^+$, 449 (40) $[M + K]^+$, 843 (25) $[2M + Na]^+$.

[2-(3-{2-[1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-2-phenylethylamino]ethyl}thioureido)ethyl]carbamic Acid *tert*-Butyl Ester (3e). This compound was synthesized according to procedure A on a 8.3 mmol scale: yield 3.0 g (69%); white foam; R_f = 0.18 (EtOAc/petroleum ether, 80:20); IR (neat) ν_{max} = 3118 (m), 2909 (w), 2250 (w), 1659 (s), 1596 (s), 1475 (s), 1406 (s), 1304 (m), 1185 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (s, 6H), 1.43 (s, 9H), 2.40 (s, 4H), 3.29 (m, 2H), 3.54 (br s, 2H), 3.70 (br s, 4H), 4.59 (s, 2H), 7.19 (m, 5H), 12.46 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 28.5, 30.2, 35.4, 39.1, 42.5, 44.0, 53.0, 81.0, 108.5, 127.7, 128.3, 128.9, 141.9, 158.9, 166.1, 179.3, 198.4; MS (ES^+) m/z (rel intens) = 503 (79) $[M + H]^+$, 1005 (20) $[2M + H]^+$.

[2-(3-{3-[1-(2,6-Dioxocyclohexilidene)-2-phenylethyl-amino]propyl}thioureido)ethyl]carbamic Acid *tert*-Butyl Ester (3f). This compound was synthesized according to procedure A on a 1 mmol scale: yield 400 mg (78%); white foam; R_f = 0.20 (EtOAc/CH_2Cl_2, 50:50); IR (neat) ν_{max} = 3288 (m), 2954 (m), 2931 (m), 2869 (w) 1688 (m), 1630 (m), 1565 (s), 1496 (s), 1450 (s), 1341 (s), 1272 (s), 1248 (s), 1163 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (s, 6H), 1.36 (s, 9H), 1.82 (quint, J = 7 Hz, 2H), 2.34 (s, 4H), 3.19 (t, J = 6 Hz, 2H), 3.38 (q, J = 7 Hz, 2H), 3.43 (br s, 4H), 4.50 (s, 2H), 7.15 (m, 5H), 13.53 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.1, 28.2, 28.5, 29.9, 35.0, 38.3, 39.7, 40.8, 52.8, 80.2, 108.0, 126.5, 128.0, 128.8, 136.2, 157.9, 174.3, 182.5, 197.8; MS (ES^+) m/z (rel intens) = 517 (80) $[M + H]^+$.

Procedure B for the Synthesis of Thioureas 3a and 3b. 1-(*tert*-Butyloxycarbonyl)propyldiamine (1.2 g, 6.9 mmol) was dissolved in chloroform (50 mL), and triethylamine (0.9 mL, 6.8 mmol) was added to the solution, which was cooled to 0 °C. Thiophosgene (264 μL, 3.4 mmol) was added dropwise and the solution allowed to stir at room temperature for 36 h. The solvents were evaporated under reduced pressure to give a yellow oil. Further purification by column chromatography (EtOAc/CH_2Cl_2, 25:75) afforded the pure products as white foams.

{3-[3-(3-*tert*-Butoxycarbonylaminopropyl)thioureido]-propyl}carbamic Acid *tert*-Butyl Ester (3a). This compound was synthesized according to procedure B on a 3.4 mmol scale: yield 1.00 g (75%); R_f = 0.28 (EtOAc/CH_2Cl_2, 25:75); IR (neat) ν_{max} = 3295 (m), 2978 (m), 2935 (m), 1686 (m), 1625 (s), 1520 (s), 1251 (s), 1163 (s), cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (s, 18H, $(CH_3)_3C$), 1.68 (quint, J = 6 Hz, 4H, $CH_2CH_2CH_2$), 3.13 (q, J = 6 Hz, 4H, CH_2NHCO), 3.49 (br s, 4H, CH_2NHCS), 4.87 (br s, 2H, NHBoc), 6.70 (br s, 2H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.4, 28.7, 36.3, 39.8, 78.7, 155.9, 180.5; MS (ES^+) m/z (rel intens) = 391 (100) $[M + H]^+$, 413 (20) $[M + Na]^+$.

{3-[3-(3-*tert*-Butoxycarbonylaminoethyl)thioureido]-ethyl}carbamic Acid tert-Butyl Ester (3b). This compound was synthesized according to procedure B on a 3.0 mmol scale: yield 821 mg (75%); R_f = 0.25 (EtOAc/CH_2Cl_2, 25:75); IR (neat) ν_{max} = 3334 (m), 3256 (m), 2974 (m), 2935 (m), 1676

(m), 1624 (s), 1532 (s), 1437 (m), 1365 (m), 1275 (m), 1237 (s), 1160 (s), cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.37 (s, 18H), 3.26 (q, $J = 6$ Hz, 4H), 3.48 (br s, 4H), 5.09 (br s, 2H), 6.82 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.8, 36.3, 40.1, 80.5, 157.5, 182.5; MS (ES^+) *m/z* (rel intens) = 363 (18) $[M + H]^+$, 385 (100) $[M + Na]^+$.

General Procedure for the Synthesis of Guanidine 4. Methyl iodide (200 μL, 2.1 mmol) was added to a stirred solution of thiourea **3** (1.3 mmol) in acetone (20 mL), and the reaction mixture was stirred for 18 h at room temperature. The solvent and all volatile compounds were removed under reduced pressure to give a slightly yellow foam. The foam was redissolved in a 1:1 mixture of CH_2Cl_2 (15 mL) and MeOH (15 mL). Ammonium hexafluorophosphate (424 mg, 2.6 mmol) was added and the resulting solution stirred for 18 h at room temperature. The solvents were evaporated, and the yellow oily residue was redissolved in CH_2Cl_2 (100 mL) and washed with water (80 mL). The organic solution was dried over magnesium sulfate and the solvent removed under reduced pressure to afford the methylthiouronium hexafluorophosphate as a white foam in quantitative yield. The hexafluorophosphate was redissolved in a 4:1 mixture of toluene (20 mL) and chloroform (5 mL), and neat DBU (450 μL, 3.0 mmol) and trifluoroacetamide (542 mg, 4.8 mmol) were added. The mixture was refluxed for 8−18 h under vigorous stirring. The solvents were removed under reduced pressure to give an oil. The crude product was purified by FC on silica gel using the eluent mixture EtOAc/CH_2Cl_2. All the NMR spectra show that the trifluoroacetylguanidines exist in solution as more than one isomer/conformer; the major conformers are in all cases the ones containing a hydrogen bond between the CO of the trifluoroacetyl and one of the guanidine NH groups. Thus, the symmetrical compounds **4a** and **4b** have only one major form, but the unsymmetrical ones show two major isomers. ^{13}C NMR spectra are therefore very complex; signals for the major isomers/conformers are reported.

{3-[*N′*-(3-tert-Butoxycarbonylaminopropyl)-*N″*-(2,2,2-trifluoroacetyl)guanidino]propyl}carbamic Acid *tert*-Butyl Ester (4a). This compound was synthesized according to the general procedure on a 1.3 mmol scale: yield 524 mg (86%); white hygroscopic foam; $R_f = 0.28$ (EtOAc/CH_2Cl_2, 40:60); IR (neat) $\nu_{max} = 3328$ (m), 2975 (m), 2939 (m), 1692 (m), 1629 (s), 1520 (s), 1438 (m), 1371 (m), 1257 (m), 1163 (s), 1171 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 1.34 (s, 18H), 1.55 (br s, 2H), 1.67 (br s, 2H), 3.03 (m, 4H), 3.15 (m, 2H), 3.35 (m, 2H), 5.37 (br s, 1H), 5.46 (br s, 1H), 6.37 (br s, 1H), 9.29 (br s, 1H); ^{13}C NMR (100 MHz, CD_3CN) δ 28.3, 29.6, 30.5, 37.3, 38.1, 38.4, 39.1, 79.1, 117.0 (q, $J = 285$ Hz), 156.1, 156.2, 160.2, 165.3 (q, $J = 35$ Hz); MS (ES^+) *m/z* (rel intens) = 470 (100) $[M + H]^+$, 492 (35) $[M + Na]^+$, 939 (20) $[2M + Na]^+$, 961 (15) $[2M + Na]^+$. Anal. Calcd for $C_{19}H_{34}F_3N_5O_5$: C, 48.61; H, 7.30; N, 14.92. Found: C, 48.38; H, 7.41; N, 14.80.

{3-[*N′*-(3-tert-Butoxycarbonylaminoethyl)-*N″*-(2,2,2-trifluoroacetyl)guanidino]ethyl}carbamic Acid *tert*-Butyl Ester (4b). This compound was synthesized according to the general procedure on a 0.56 mmol scale: yield 207 mg (84%); white hygroscopic foam; $R_f = 0.16$ (EtOAc/CH_2Cl_2, 40:60); IR (neat) $\nu_{max} = 3328$ (m), 2975 (m), 2939 (m), 1692 (m), 1629 (s), 1520 (s), 1438 (m), 1371 (m), 1257 (m), 1163 (s), 1171 (s), 1139 (m), cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 1.43 (s, 18H), 3.10−3.20 (m, 6H), 3.30−3.40 (m, 2H), 5.50 (br s, 1H), 5.60 (br s, 1H), 6.60 (br s, 1H), 9.29 (br s, 1H); ^{13}C NMR (100 MHz, (CD_3CN) δ ^{13}C NMR (100 MHz, CD_3CN) δ 27.3, 38.2, 39.9, 40.5, 41.8, 78.1, 78.8, 116.8 (q, $J = 285$ Hz), 156.4, 160.9, 165.2 (q, $J = 32$ Hz); MS (ES^+) *m/z* (rel intens) = 442 (100) $[M + H]^+$, 464 (30) $[M + Na]^+$, 883 (10) $[2M + H]^+$, 905 (10) $[2M + Na]^+$. Anal. Calcd for $C_{17}H_{30}F_3N_5O_5$: C, 46.25; H, 6.85; N, 15.86. Found: C, 45.98; H, 7.03; N, 15.60.

{2-[*N′*-(2-Allyloxycarbonylaminoethyl)-*N″*-(2,2,2-trifluoroacetyl)guanidino]ethyl}carbamic Acid *tert*-Butyl Ester (4c). This compound was synthesized according to the general procedure on a 0.19 mmol scale: yield 74 mg (92%); white hygroscopic foam; $R_f = 0.16$ (EtOAc/CH_2Cl_2, 40:60); IR (neat) $\nu_{max} = 3326$ (m), 2978 (m), 1690 (m), 1630 (s), 1520 (s), 1438 (m), 1370 (m), 1257 (m), 1139 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 3.26 (br s, 6H), 3.47 (br s, 2H), 4.54 (d, $J = 5.5$ Hz, 2H), 5.20 (d, $J = 8.0$ Hz, 1H), 5.30 (d, $J = 17.6$ Hz, 1H), 5.68 (br s), 5.93 (m, 1H), 6.07 (br s), 6.47 (br s), 6.64 (br s), 6.96 (br s), 9.32 (br s, N*H*CNR); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.3, 38.1, 38.6, 38.9, 39.9, 40.2, 40.9, 41.3, 41.7, 65.1, 79.1, 79.2, 115.8 (q, $J = 285$ Hz), 116.5, 133.0, 156.7, 157.0, 160.7, 165.2 (q, $J = 35.0$ Hz); MS (ES^+) *m/z* (rel intens) 426 (95) $[M + H]^+$, 448 (100) $[M + Na]^+$. Anal. Calcd for $C_{16}H_{26}F_3N_5O_5$: C, 45.17; H, 6.16; N, 16.45. Found: C, 45.22; H, 6.30; N, 16.32.

{3-[*N′*-(2-tert-Butoxycarbonylaminoethyl)-*N″*-(2,2,2-trifluoroacetyl)guanidino]propyl}carbamic Acid Benzyl Ester (4d). This compound was synthesized according to to the general procedure on a 1.22 mmol scale: yield 510 mg (85%); white hygroscopic foam; $R_f = 0.28$ (EtOAc/CH_2Cl_2, 30:70); IR (neat) $\nu_{max} = 3329$ (m), 2973 (m), 2939 (m), 1690 (m), 1629 (s), 1438 (m), 1371 (m), 1257 (m), 1163 (s), 1171 (s), 1139 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 1.42 (s, 9H), 1.60−1.90 (m, 4H), 3.10−3.60 (m, 8H), 5.09 (s, 2H), 5.69 (br s,), 5.81 (br s), 5.89 (br s), 7.36 (m, 5H), 6.67 (br s), 9.42 (br s); ^{13}C NMR (100 MHz, CD_3CN) δ 27.2, 28.4, 29.1, 37.0, 37.5, 37.7, 38.1, 38.3, 39.8, 40.4, 41.8, 65.6, 78.3, 78.8, 117.0 (q, $J = 286$ Hz), 127.4, 127.5, 128.1, 137.1, 156.4, 160.5, 165.1 (q, $J = 35.0$ Hz); MS (ES^+) *m/z* (rel intens) = 490 (100) $[M + H]^+$, 512 (35) $[M + Na]^+$, 979 (10) $[2M + H]^+$, 1001 (5) $[2M + Na]^+$. Anal. Calcd for $C_{21}H_{30}F_3N_5O_5$: C, 51.53; H, 6.18; N, 14.31. Found: C, 51.29; H, 6.43; N, 14.08.

{2-[*N′*-{3-[1-(2,6-Dioxocyclohexylidene)-2-phenylethylamino]ethyl}-*N″*-(2,2,2-trifluoroacetyl)guanidino]ethyl}carbamic Acid *tert*-Butyl Ester (4e). This compound was synthesized according to the general procedure on a 0.12 mmol scale: yield 31 mg (45%); white hygroscopic foam; $R_f = 0.25$ (EtOAc/CH_2Cl_2, 75:25); IR (neat) $\nu_{max} = 3636$ (m), 3568 (m), 2982 (w), 1625 (m), 1566 (s), 1520 (s), 1367 (m), 1190 (m), 1140 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.05 (s, 6H), 1.46 (s, 9H), 2.41 (s, 4H), 3.00−3.80 (m, 8H), 4.52 (s, 2H), 5.12 (br s, 1H), 7.55 (br s, 1H), 7.02 (m, 2H), 7.13 (m, 1H), 7.19 (m, 2H), 9.54 (br s, 1H), 13.66 (br s, 1H); MS (ES^+) *m/z* (rel intens) = 582 (55) $[M + H]^+$, 604 (100) $[M + Na]^+$, 1185 (20) $[2M + Na]^+$; HRMS *m/z* calcd for $C_{28}H_{39}N_5O_5F_3$ $[M + H]^+$ 582.2887, found 582.2898.

{2-[*N′*-{3-[1-(2,6-Dioxocyclohexylidene)-2-phenylethylamino]propyl}-*N″*-(2,2,2-trifluoroacetyl)guanidino]ethyl}carbamic Acid *tert*-Butyl Ester (4f). This compound was synthesized according to the general procedure on a 0.72 mmol scale: yield 321 mg (75%); white hygroscopic foam; $R_f = 0.28$ (EtOAc/CH_2Cl_2, 70:30); IR (neat) $\nu_{max} = 3637$ (m), 3569 (m), 2982 (w), 1625 (m), 1566 (s), 1520 (s), 1367 (m), 1190 (m), 1140 (s), 825 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 0.98 (s, 6H), 1.42 (s, 9H), 1.82 (m, 2H), 2.37 (s, 4H), 3.10−3.40 (m, 8H), 4.60 (s, 2H), 6.93 (br s, 1H), 7.06 (br s, 1H), 7.21 (m, 2H), 7.30 (m, 1H), 7.38 (m, 2H), 7.60 (br s, 1H), 9.12 (br s, 1H); ^{13}C NMR (100 MHz, CD_3CN) δ 27.0, 27.3, 28.6, 29.3, 34.2, 38.3, 38.4, 40.2, 40.4, 52.2, 79.2, 107.2, 117.0 (q, $J = 286$ Hz), 126.1, 127.7, 128.3, 136.1, 156.1, 156.6, 160.5, 173.1; MS (ES^+) *m/z* (rel intens) = 596 (100) $[M + H]^+$, 1181 (10) $[2M + H]^+$. Anal. Calcd for $C_{29}H_{40}N_5O_5F_3$: C, 58.48; H 6.77; N 11.76. Found: C, 58.23; H, 6.96; N, 11.54.

General Procedure for the Deprotection of the Trifluoroacetamide and Exchange of the Counterion to the Hexafluorophosphate 5. Dicarbamate **4** (0.092 mmol) was dissolved in a mixture of MeOH and water (5:2, 3.5 mL), and potassium carbonate (100 mg, 0.725 mmol) was added. The mixture was stirred for 3 h at room temperature.[12] Solvents were removed under reduced pressure, and the residue was suspended in EtOAc (3 × 10 mL) and filtered. The organic

(12) Boger, D. L.; Kim, S. H.; Mori, Y.; Weng, J. H., Rogel, O.; Castle, S. L.; McAtee, J. J. *J. Am. Chem. Soc.* **2001**, *123*, 1862−1871.

layer was concentrated and dissolved in MeOH (5 mL). Ammonium hexafluorophosphate was added and the solution stirred for 3 h. The solvent was removed under reduced pressure and the residue dissolved in EtOAc (30 mL) and washed with water (10 mL). The solution was dried over magnesium sulfate and the solvent removed to give the crude product, which was purified by column chromatography (5% MeOH in CH_2Cl_2) to afford the pure product.

[Di(3-[(*tert*-butoxycarbonyl)amino]propylamino)-methylene]ammonium Hexafluorophosphate (5a). This compound was synthesized according to the general procedure on a 42 mg (0.090 mmol) scale: yield 42 mg (90%); white foam; R_f = 0.19 (5% MeOH in CH_2Cl_2); IR (neat) ν_{max} = 3411 (m), 2976 (m), 2936 (m), 1658 (s), 1635 (s), 1518 (s), 1367 (s), 1277 (m), 1252 (m), 1161 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 1.37 (s, 18H), 1.68 (quint, J = 6 Hz, 4H), 3.13 (q, J = 6 Hz, 4H), 3.49 (br s, 4H), 4.87 (br s, 2H), 6.08 (br s, 2H), 6.70 (br s, 2H); ^{13}C NMR (100 MHz, CD_3CN) δ 27.3, 28.8, 36.6, 38.6, 78.5, 155.5, 156.6; MS (ES^+) m/z (rel intens) = 374 $[M]^+$ (100). Anal. Calcd for $C_{17}H_{34}N_5O_4PF_6 \cdot 2H_2O$: C, 36.76; H, 7.26; N, 12.61. Found: C, 36.61; H, 7.31; N, 12.40.

[Di(3-[(*tert*-butoxycarbonyl)amino]ethylamino)-methylene]ammonium Hexafluorophosphate (5b). This compound was synthesized according to the general procedure on a 20 mg (0.045 mmol) scale: yield 20 mg (88%); white foam; R_f = 0.17 (5% MeOH in CH_2Cl_2); IR (neat) ν_{max} = 3409 (m), 2966 (m), 2934 (m), 1658 (s), 1635 (s), 1516 (s), 1360 (s), 1275 (m), 1252 (m), 1161 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 1.43 (s, 18H), 3.20−3.22 (m, 8H), 5.70 (br s, 2H), 6.26 (br s, 2H), 6.62 (br s, 2H); ^{13}C NMR (100 MHz, CD_3CN) δ 27.3, 38.5, 41.7, 79.1, 155.9, 157.2; MS (ES^+) m/z (rel intens) = 346 $[M]^+$ (100). Anal. Calcd for $C_{15}H_{32}N_5O_4PF_6 \cdot 2H_2O$: C, 34.16; H, 6.88; N, 13.28. Found: C, 34.25; H, 6.60; N, 13.32.

([(2-{[(Allyloxy)carbonyl)amino}ethyl)amino](2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)methylene]-ammonium Hexafluorophosphate (5c). This compound was synthesized according to the general procedure on a 20 mg (0.047 mmol) scale: yield 18 mg (83%); white foam; R_f = 0.17 (5% MeOH in CH_2Cl_2); IR (neat) ν_{max} = 3414 (m), 2975 (m), 2937 (m), 1659 (s), 1634 (s), 1517 (s), 1367 (s), 1249 (m), 1249 (m), 1159 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 1.34 (s, 9H), 3.09−3.16 (m, 4H), 3.16−3.20 (m, 4H), 4.45 (d, J = 6.0 Hz), 5.12 (dd, J = 11, 1 Hz, 1H); 5.12 (dd, J = 17, 1 Hz, 1H), 5.59 (br s, 1H), 5.80−5.90 (m, 2H), 6.15 (br s, 2H), 6.42−6.50 (m, 2H); ^{13}C NMR (100 MHz, CD_3CN) δ 27.3, 38.5, 38.9, 41.5, 41.6, 65.0, 79.2, 116.3, 133.0, 155.9, 157.1; MS (ES^+) m/z (rel intens) = 330 $[M]^+$. Anal. Calcd for $C_{14}H_{27}N_5O_4PF_6 \cdot H_2O$: C, 34.08; H, 6.13; N, 14.19. Found: C, 34.29; H, 6.06; N, 13.92.

{3-[*N*′-(2-*tert*-Butoxycarbonylaminoethyl)guanidinium hexafluorophosphate]propyl}carbamic Acid Benzyl Ester (5d). This compound was synthesized according to the general procedure on a 45 mg (0.092 mmol) scale: yield 45 mg (90%); white foam; R_f = 0.17 (5% MeOH in CH_2Cl_2); IR (neat) ν_{max} = 3417 (m), 2975 (m), 2938 (m), 1659 (s), 1633 (s), 1516 (s), 1453 (m), 1367 (m), 1247 (s), 1158 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 1.33 (s, 9H), 1.64 (quint, J = 7.0 Hz, 2H), 3.07−3.12 (m, 8H), 4.98 (s, 2H), 5.59 (br s, 1H), 5.71 (br s, 1H), 6.07 (br s, 2H), 6.40 (br s, 2H), 7.28 (m, 5H); ^{13}C NMR (100 MHz, CD_3CN) δ 27.3, 28.4, 37.2, 38.6, 41.7, 65.8, 79.2, 127.4, 127.7, 128.2, 137.0, 155.7, 156.8, 156.9; MS (ES^+) m/z (rel intens) = 394 $[M]^+$ (100). Anal. Calcd for $C_{19}H_{32}N_5O_4PF_6 \cdot H_2O$: C, 40.94; H, 6.15; N, 12.56. Found: C, 41.05; H, 6.01; N, 12.23.

[2-(*N*′-{3-[1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-2-phenylethylamino]ethyl}guanidinium hexafluorophosphate)ethyl]carbamic Acid *tert*-Butyl Ester (5e). This compound was synthesized according to the general procedure on a 20 mg (0.034 mmol) scale: yield 16 mg (75%); white foam; R_f = 0.15 (5% MeOH in CH_2Cl_2); IR (neat) ν_{max} = 2969 (m), 2884 (m), 1629 (s), 1560 (s), 1517 (s), 1449 (s), 1282 (m), 1247 (m), 1158 (s) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 1.04 (s, 6H), 1.43 (s, 9H), 2.39 (s, 4H), 3.18−3.20 (m, 4H), 3.31 (q, J = 6.0 Hz, 2H), 3.54 (q, J = 6.0 Hz, 2H), 4.58 (s, 2H), 5.77 (br s, 1H); 6.16 (br s, 2H), 6.56 (m, 2H), 7.17 (d, J = 7.5 Hz, 2H), 7.26 (t, J = 7.5 Hz, 1H), 7.33 (t, J = 7.5 Hz, 2H), 13.67 (br s, 1H); ^{13}C NMR (100 MHz, CD_3CN) δ 27.0, 27.3, 29.3, 34.1, 38.5, 40.4, 41.2, 52.1, 79.3, 107.7, 126.2, 127.7, 128.4, 136.0, 155.8, 173.5; MS (ES^+) m/z (rel intens) = 486 $[M]^+$ (100). Anal. Calcd for $C_{26}H_{40}N_5O_4PF_6 \cdot 2H_2O$: C, 46.77; H, 6.64; N, 10.49. Found: C, 46.69; H, 6.34; N, 10.23.

[2-(*N*′-{3-[1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-2-phenylethylamino]propyl}guanidinium hexafluorophosphate)ethyl]carbamic Acid *tert*-Butyl Ester (5f). This compound was synthesized according to the general procedure on a 20 mg (0.034 mmol) scale: yield 17 mg (76%); white foam; R_f = 0.16 (5% MeOH in CH_2Cl_2); IR (neat) ν_{max} = 2970 (m), 2885 (m), 1630 (s), 1558 (s), 1517 (s), 1449 (s), 1280 (m), 1245 (m), 1160 (s), 1025 (m) cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 1.02 (s, 6H), 1.40 (s, 9H), 1.81 (quint, J = 7.0 Hz, 2H), 2.35 (s, 4H), 3.09−3.17 (m, 6H), 3.41 (q, J = 6.0 Hz, 2H), 3.54 (q, J = 7.0 Hz, 2H), 4.56 (s, 2H), 5.72 (br s, 1H), 6.15 (br s, 2H), 6.45−6.55 (m, 2H), 7.16 (d, J = 7.0 Hz, 2H), 7.23 (t, J = 7.0 Hz, 1H), 7.29 (t, J = 7.5 Hz, 2H), 13.64 (br s, 1H); ^{13}C NMR (100 MHz, CD_3CN) δ 27.0, 27.3, 27.6, 29.3, 34.2, 38.6, 40.0, 41.7, 52.2, 79.3, 107.3, 126.1, 127.7, 128.3, 136.1, 155.6, 173.2; MS (ES^+) m/z (rel intens) = 500 $[M]^+$ (100). Anal. Calcd for $C_{27}H_{42}N_5O_4PF_6 \cdot H_2O$: C, 48.87; H, 6.68; N, 10.55. Found: C, 48.69; H, 6.69; N, 10.30.

{2-[*N*′-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-ethyl]-*N*″-(2,2,2-trifluoroacetyl)guanidino]ethyl}carbamic Acid *tert*-Butyl Ester (4g). 9-Fluoroenylmethyl chloroformate (184 mg, 0.7 mmol) was added to a solution of **4c** (272 mg, 0.64 mmol) in CH_2Cl_2 (10 mL) followed by the addition of a palladium tetrakis(triphenylphosphine) solution (36 mg, 32 μmol in 2 mL of CH_2Cl_2) and tributyltin hydride (206 μL, 0.77 mmol).[10] After the additions the solution was stirred for 1 h at room temperature. The solvent was removed under reduced pressure and the crude product washed with petroleum ether. Purification by column chromatography on silica gel (CH_2Cl_2/EtOAc, 50:50) afforded **4g** (258 mg, 72%) as a white solid: mp 152−154 °C; R_f = 0.38 (CH_2Cl_2/EtOAc, 50:50); ^1H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 9 H), 3.16−3.56 (br s, 8H), 4.12 (t, J = 7.0 Hz, 1H), 4.28−4.38 (br m, 2H), 5.00 (br s), 5.32 (br s), 5.37 (br s), 5.92 (br s), 7.03 (br s), 7.23 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.50 (d, J = 7.5 Hz, 2H), 7.68 (d, J = 7.5 Hz, 2H), 9.54 (br s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.7, 39.7, 40.1, 40.8, 41.2, 41.8, 42.5, 43.4, 47.6, 67.4, 81.4, 117.3 (q, J = 285 Hz), 120.4, 125.5, 127.5, 128.1, 141.7, 144.3, 157.6, 157.8, 161.7, 167.2 (q, J = 35.0 Hz); MS (ES^+) m/z (rel intens) = 564 (43) $[M + H]^+$, 586 (100) $[M + Na]^+$.

(4*S*)-4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-4-{2-[*N*′-[2-(9*H*-fluoren-9-ylmethoxycarbonylamino)ethyl]-*N*″-(2,2,2-trifluoroacetyl)guanidino]ethylcarbamoyl}-butyric Acid *tert*-Butyl Ester (9). 4g (204 mg, 0.36 mmol) was stirred in a 20% solution of TFA in CH_2Cl_2 (12 mL) at room temperature for 2 h. After addition of toluene (75 mL) the solvents were removed under reduced pressure to yield the corresponding TFA salt as an oil. A solution of *N*-Fmoc-L-Glu(O^{t}Bu)-OH (183 mg, 0.43 mmol), PyBOP (224 mg, 0.43 mmol), and HOBt (66 mg, 0.43 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 10 min and then added to a solution of the TFA salt in CH_2Cl_2 (10 mL) followed by the addition of DIPEA (0.17 mL, 0.96 mmol). After the reaction had been stirred for 18 h, the solvent was removed under reduced pressure to give a brown oil. Purification by column chromatography on silica gel (CH_2Cl_2/EtOAc, 50:50) gave **9** (250 mg, 80%) as a white solid: mp 162 °C; $[\alpha]_D^{rt} = -1.7°$ (c = 3.0 mg/mL, l = 2.0 dm, $(CH_3)_2SO$); R_f = 0.16 (CH_2Cl_2/EtOAc, 50:50); ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 1.48 (s, 9H), 1.84 (m, 1H), 1.98 (m, 1H), 2.31 (m, 2H), 3.27−3.42 (m, 8H), 4.07 (m, 1H), 4.34−4.41 (m, 6H), 7.42 (t, J = 7.5 Hz, 4H), 7.51 (t, J = 7.5 Hz, 2H), 7.52 (t, J = 7.5 Hz, 2H), 7.60−7.78 (br m), 7.82 (d, J = 7.0 Hz, 2H), 7.84 (d, J = 7.0 Hz, 2H), 7.99 (d, J = 7.7 Hz, 2H), 8.00 (d, J = 7.7 Hz, 2H), 8.17 (br s), 8.25 (br s), 9.25

(br s), 9.29 (br s); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 28.6, 32.3, 40.4, 40.8, 41.1, 41.3, 41.5, 41.9, 42.0, 47.6, 66.6, 80.6, 121.1, 126.0, 126.3, 128.0, 128.5, 141.7, 144.7, 144.9, 156.9, 157.1, 162.0, 172.5; MS (ES^+) *m/z* (rel intens) 871 (10) $[M + H]^+$, 893 (50) $[M + Na]^+$; HRMS (ES^+) *m/z* calcd for $C_{46}H_{49}F_3N_6NaO_8$ $[M + Na]^+$ 893.3456, found 893.3467.

Tweezer 8. Compound **4a** (220 mg, 0.47 mmol) was stirred in a 20% solution of TFA in DCM (20 mL) for 3 h. After addition of toluene (80 mL) the solvents were removed under reduced pressure to give a yellow oil. The residue was redissolved in a mixture of THF (5 mL) and DMF (5 mL) and cooled to 0 °C. To the solution were added *N*-Boc-Ala-Val-Gly-OH (436 mg, 1.25 mmol), HOBt (192 mg, 1.42 mmol), DIPEA (400 μL, 2.3 mmol), and EDC (240 mg, 1.25 mmol), and the mixture was stirred for 18 h (0 °C to room temperature). THF was removed and the residue diluted with DCM (100 mL) and washed with a 1 M solution of sodium hydrogen carbonate (50 mL), a 1 M solution of sodium hydrogen sulfate (50 mL), and brine (50 mL). The organic layer was dried over magnesium sulfate, and after the solvent was removed, the residue was washed with ether, dissolved in the minimum amount of CH_2Cl_2 (1 mL), and precipitated with ether (5 mL) to yield 195 mg of **7** as a cream-colored solid (45%): ^{1}H NMR (400 MHz, CD_3OD) δ 0.91 (d, $J = 6$ Hz, 12H), 1.21 (d, $J = 7$ Hz, 6H), 1.39 (s, 18H), 1.64 (m, 2H), 1.76 (m, 2H), 2.07 (m, 2H), 3.15−3.30 (m, 6H), 3.32−3.44 (m, 2H), 3.77 (m, 2H), 4.00−4.20 (m, 4H). Tweezer **7** (25 mg, 0.027 mmol) was dissolved in a mixture of MeOH and water (5:2, 3.5 mL), and potassium carbonate (100 mg, 0.725 mmol) was added. The mixture was stirred for 3 h at room temperature.[12] The solvents were then removed under reduced pressure, and the residue was extracted with EtOAc (3 × 20 mL) and chloroform (2 × 20 mL). The combined organic layer was then concentrated to afford **8** as a white solid (23 mg, 96% yield): mp 119−120 °C; $[\alpha]_D^{rt} = -15$ ($c = 3.0$ mg/mL, $l = 2.0$ dm, $(CH_3)OH$); ^{1}H NMR (400 MHz, CD_3CN) δ 0.92 (d, $J = 7$ Hz, 12H), 1.26 (d, $J = 7$ Hz, 6H), 1.39 (s, 18H), 1.70 (m, 4H), 2.10 (m, 2H), 3.00−3.25 (m, 8H), 3.74 (d, $J = 8.0$ Hz, 4H), 3.90−4.10 (m, 4H), 5.95 (br s, 2H), 6.98 (br s, 2H), 7.11 (br s, 2H), 7.23 (d, $J = 8.0$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3CN) δ 16.5, 17.2, 18.3, 27.3, 28.1, 29.6, 35.7, 38.4, 42.5, 50.4, 59.4, 79.1, 155.9, 160.8, 169.3, 171.9, 174.2; MS (ES^+) *m/z* (rel intens) = 828 (100) $[M]^+$; analytical data, HPLC 3.50 min (S5OD, gradient 60−45% MeCN, analytical).

General Procedure for Fmoc Deprotection on a Solid Phase. The Fmoc-protected resin was suspended in a solution of 20% piperidine in DMF (20 mL/g of resin) and agitated for 30−45 min. The resin was drained and washed with CH_2Cl_2 (3×), DMF (3×), and CH_2Cl_2 (3×) (10 mL of solvent/g of resin). The procedure was repeated once and the progress of the deprotection monitored by the ninhydrin test.

General Procedure for Boc Deprotection on a Solid Phase. The Boc-protected resin was suspended in a mixture of 45% CH_2Cl_2, 45% TFA, 4% EDT, 3% DMS, and 3% anisole (20 mL/g of resin) and agitated for 60−120 min. The resin was drained and washed with CH_2Cl_2 (3×), DMF (3×), a 20% solution of DIPEA in CH_2Cl_2 (3×), MeOH (3×), DMF (3×), and CH_2Cl_2 (3×) (10 mL of solvent/g of resin). The DIPEA solution wash was omitted for samples requiring prolonged storage. The progress of the deprotection was monitored by the ninhydrin test.

Preparation of Tweezer 11. The carboxylic acid **10** (46.7 mg, 57 μmol) was dissolved in a solvent mixture (DMF/CH_2Cl_2, 2:1; 3 mL) followed by addition of PyBOP (30 mg, 57 μmol) and HOBt (8.7 mg, 57 μmol). The reaction mixture was stirred for 5 min and then added to a preswollen and Fmoc-deprotected Rink amide resin (100 mg, 45 μmol of free NH_2) in DMF (2 mL) followed by addition of neat DIPEA (23 μL, 128 μmol). After agitation on a tube rotator for 24 h at room temperature, the resin was drained, washed with CH_2Cl_2 (3 × 5 mL), DMF (3 × 5 mL), and CH_2Cl_2 (3 × 5 mL), and dried. A ninhydrin test indicated the presence of free amino groups on the resin (0.17 mmol/g). A second coupling was performed with carboxylic acid **10** (18.7 mg, 23 μmol), PyBOP (15 mg, 23 μmol), HOBt (9 mg, 23 μmol), and DIPEA (9 μL, 52 μmol). The resins were agitated on a tube rotator for 24 h at room temperature, then washed with CH_2Cl_2 (3 × 5 mL), DMF (3 × 5 mL), and CH_2Cl_2 (3 × 5 mL), and dried. A second quantitative ninhydrin test indicated the presence of free amino groups on the resin (∼0.10 mmol/g). To cap remaining free amino functions, the resin was agitated on a tube rotator for a further 18 h after addition of acetic anhydride (20 μL, 0.21 mmol), DIPEA (40 μL, 0.22 mmol), and DMAP (0.4 mg, 3 μmol) in CH_2Cl_2 (5 mL). Subsequent washing with CH_2Cl_2 (3 × 5 mL), DMF (3 × 5 mL), and CH_2Cl_2 (3 × 5 mL) afforded a resin, which gave a negative ninhydrin test. Fmoc deprotection was achieved as described above in the general procedures, and subsequent washing with CH_2Cl_2 (3 × 5 mL), DMF (3 × 5 mL), and CH_2Cl_2 (3 × 5 mL) yielded a resin, which gave a positive ninhydrin test.

Quantities of reagents required were calculated on the basis of the loading of the commercially available Rink amide resin (100 mg, 45 μmol; two amino sites per guanidine unit, 90 μmol of free NH_2). All deprotection steps were monitored by ninhydrin tests. A solution of Fmoc-Gly (43 mg, 144 μmol), DIC (23 μL, 144 μmol), and HOBt (22 mg, 144 μmol) in DMF (3 mL) was stirred for 5 min and then added to the preswollen resin in DMF (2 mL) followed by addition of DIPEA (56 μL, 324 μmol). After agitation on a tube rotator for 18 h at room temperature, the resin was drained, washed with CH_2Cl_2 (3 × 5 mL), DMF (3 × 5 mL), and CH_2Cl_2 (3 × 5 mL), and dried. A ninhydrin test indicated complete coupling. An Fmoc deprotection followed by coupling of Fmoc-L-valine (49 mg, 144 μmol) provided the resin-bound trifluoroacetyl-protected tweezer after a final Fmoc deprotection. Cleavage of the trifluoroacetyl group on the guanidine was achieved by stirring the resin in a potassium carbonate solution (0.15 M; MeOH/DMF/water, 2:2:1; 5 mL) at room temperature for 3 h. Cleavage of the tweezer **11** from the Rink amide resin was performed in polypropylene filtration tubes with polyethylene frits by letting a cleavage mixture percolate slowly through the resin. A cleavage mixture of 15% TFA, 1% TIS, 1% water, and 83% CH_2Cl_2 (10 mL) was first used followed by a 30% TFA, 1% TIS, 1% water, and 68% CH_2Cl_2 cleavage solution (10 mL). After addition of toluene (4 mL), solvents were removed under reduced pressure, and a white TFA salt of **11** precipitated after addition of diethyl ether to the oily crude. The TFA salt was triturated with diethyl ether and centrifuged. The precipitate was washed once more with diethyl ether and dried to give the TFA salt of **11** in a quantitative yield (24.1 mg) without any byproducts: $[\alpha]_D^{rt} = 7.6°$ ($c = 5.0$ mg/mL, $l = 2.0$ dm, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 0.97 (d, $J = 7.0$ Hz, 12H), 1.89 (m, 1H), 2.01 (m, 1H), 2.16 (m, 2H), 2.28 (t, 2H, $J = 8.0$ Hz), 3.26−3.34 (m, 8H), 3.80 (d, 2H, $J = 6.0$ Hz), 3.91 (s, 2H), 3.96 (s, 2H), 4.23 (dd, $J = 9.5$, 4.0 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 15.9, 16.5, 26.0, 28.9, 30.1, 37.1, 39.6, 41.2, 41.3, 52.4, 57.7, 58.0, 115.4 (q, $J = 290.0$ Hz) 155.1, 162.2 (q, $J = 35.0$ Hz), 169.0, 169.9, 170.3, 172.8, 176.8; MS (TOF LD^+) *m/z* (rel intens) = 586.6 (100) $[M + H]^+$, 608.6 (20) $[M + Na]^+$; analytical data, HPLC 3.50 min (S15OD, gradient 50−60% MeCN, analytical).

Acknowledgment. We thank EPSRC (Grant GR/L98916) for funding, the EC for a Marie Curie fellowship for K.B.J., and the Leverhulme Trust for a Research Fellowship for J.D.K..We also thank Allison Carley for assistance in the preparation of some intermediates.

Supporting Information Available: General methods, materials, and abbreviations. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0348874