Reagents for Efficient Conversion of Amines to Protected Guanidines

Tomasz Gers, Danuta Kunce, Paweł Markowski, Jan Izdebski*

Laboratory of Peptides, Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland
Fax +48(22)8225996; E-mail: izdebski@chem.uw.edu.pl

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Abstract: Two new guanidinylation reagents, *N*,*N*′-bis(*ortho*-chloro-Cbz)-*S*-methylisothiourea and *N*,*N*′-bis(*ortho*-bromo-Cbz)-*S*-methylisothiourea, were compared with the already known *N*,*N*′-bis(Boc)-*S*-methylisothiourea and *N*,*N*′-bis(Cbz)-*S*-methylisothiourea. The new reagents proved to be superior to the known reagents. The reactions with all the new reagents were accelerated by addition of DMAP. *N*,*N*′-Bis(*ortho*-chloro-Cbz)- and *N*,*N*′-bis(*ortho*-bromo-Cbz)guanidines are stable when treated with trifluoroacetic acid and can be converted to guanidines by hydrogenolysis.

Key words: guanidine, guanidinylation, guanylation, isothiourea, protecting groups

The guanidino group has attracted considerable attention recently, since it is found in a wide array of natural and synthetic biologically active compounds.[1] Exploration of new areas of biochemistry reveals the crucial role of the guanidino function in many different physiological processes. Its positive charge resulting from protonation in wide range of pH values creates a base for specific intermolecular interactions comprising key-steps of many biological reactions including enzyme-mediated processes and interaction of hormones with their receptor.[2] Thus, the growing importance of developing simple and efficient methods of obtaining various guanidine derivatives arises directly from a broad range of their present and future application in the field of medicinal chemistry.[2]

Since it has been demonstrated that the introduction of the guanidine group instead of an existing amino group significantly increases the potency and/or selectivity of biologically active compounds,[3–6] a continuous interest has been shown in the transformation of amines to corresponding guanidines. A number of reagents can be used for this reaction. The most frequently used are *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea,[7–10] *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea[11–15] and *N*,*N*′-bis(benzyloxycarbonyl)-*S*-methylisothiourea.[16–20] In most cases, the reactions were promoted by $HgCl_2$. The application of $HgCl_2$ increases the yield but the formation of the insoluble mercuric sulfide precipitate makes this procedure not applicable to solid phase guanidinylation.[21] To avoid this shortcoming, the Mukayama reagent (2-chloro-*N*-methylpyridinium iodide) was suggested as a replacement for mercuric chloride.[22] A subsequent report by the same authors revealed, however, some limitations of this method.[23] To increase the yield, new reagents have been developed in recent years: 1*H*-pyrazol-1-*N*,*N*′-bis(*tert*-butoxycarbonyl)carboxyamide[24,25] and 4-nitro-1*H*-pyrazole-1-*N*,*N*′-bis(*tert*-butoxycarbonyl)carboxyamide.[23] The latter was more effective due to its more electronegative leaving group. A similar approach to obtain *N*,*N*′-bis-*tert*(butoxycarbonyl)-*N*-methylguanidines used *N*,*N*′-bis-(*tert*-butoxycarbonyl)-*N*-methyl-*S*-2,4-dinitrophenylisothiourea in which the leaving group is the electronegative thio-2,4-dinitrophenyl.[26] The introduction of a new class of guanidinylation reagents has also been reported: *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*′′-triflylguanidine and *N*,*N*′-bis(benzyloxycarbonyl)-*N*′′-triflylguanidine have been proved to guanidinylate primary and secondary amines including aminoglucosides[3,27,28] in high yield. However, it was reported recently that benzyloxycarbonyl protected triflylguanidyne reagent was not effective, while with *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylthioisourea in combination with $HgCl_2$ the desired product was obtained.[14]

The application of reagents containing two urethane-type protecting groups is beneficial for several reasons. They are more reactive than those without these protective groups or those containing only one of them. Two electron-withdrawing groups in positions conjugated with the reaction center increase the electrophilicity of the reagent. The product is usually soluble in organic solvents and the unreacted amine can be removed by washing with an acidic aqueous solution. The protecting groups can be easily removed just after guanidinylation or at the end of a multistep synthesis. *tert*-Butoxycarbonyl group can be removed by treatment with 50% trifluoroacetic acid in dichloromethane. The benzyloxycarbonyl group is more suitable for synthesis of acid sensitive products since it can be cleaved under neutral conditions by hydrogenolysis.[17]

For an ongoing project aimed at solid-phase synthesis of analogues of natural peptides containing the guanidino instead of an amino group,[5] we needed to select a guanidinylation reagent and to find suitable reaction conditions for this conversion. We decided to compare the already known reagents, *N*,*N*′-bis(Boc)-*S*-methylisothiourea (**1**) and *N*,*N*′-bis(Cbz)-*S*-methylisothiourea (**2**) with new reagents, especially prepared for this purpose: *N*,*N*′-bis(*ortho*-chloro-Cbz)-*S*-methylisothiourea (**3**) and *N*,*N*′-bis(*ortho*-bromo-Cbz)-*S*-methylisothiourea (**4**) (Figure 1).

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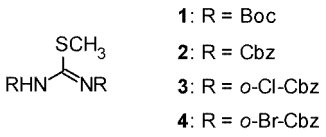
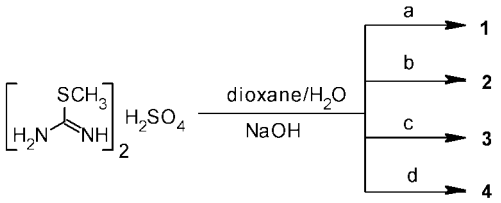


Figure 1 Known **1**,**2** and new **3**,**4** guanidinylation reagents

We expected that *ortho*-Br-Cbz and *ortho*-Cl-Cbz groups could increase the reactivity of these reagents due to the electron-attracting effect of bromine and chlorine atoms.

The guanidinylation reagents (Figure 1) were obtained by acylation of *S*-methylisothiourea as shown in Scheme 1. A new procedure for the preparation of **1** was described by us recently,[5] and here we present a further improvement of that method. To prepare **2** we used dibenzyl dicarbonate (Cbz_2O, Method 1) and benzyloxycarbonylsuccinimide (Cbz-OSu, Method 2). In both cases the yield was good and the product was identical with that obtained by acylation using benzyloxycarbonyl chloride.[16,29] Reagents **3** and **4** were obtained using *ortho*-chloro-benzyloxycarbonylsuccinimide [(*o*-Cl-Cbz)-OSu] and *ortho*-bromo-benzyloxycarbonylsuccinimide [(*o*-Br-Cbz)-OSu], respectively. The newly prepared reagents are stable crystalline compounds that can be stored for a long time without any signs of decomposition.

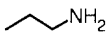
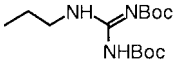
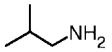
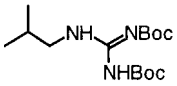
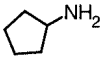
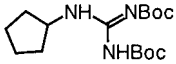
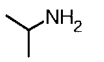
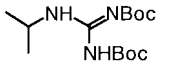
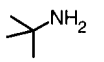
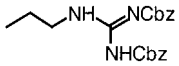
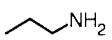
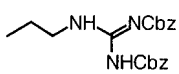
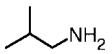
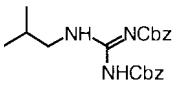
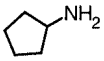
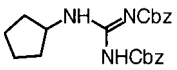
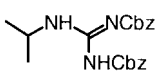
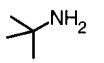
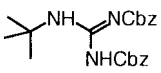


Scheme 1 Preparation of protected isothiourea derivatives **1**–**4**
Reagents and conditions: a) Boc_2O, 24 h, 82%; b) Cbz_2O, 18 h, 85% (Method 1) or Cbz-OSu, 24 h, 83% (Method 2); c) (*o*-Cl-Cbz)-OSu, 24 h, 74%, d) (*o*-Br-Cbz)-OSu, 24 h, 70%

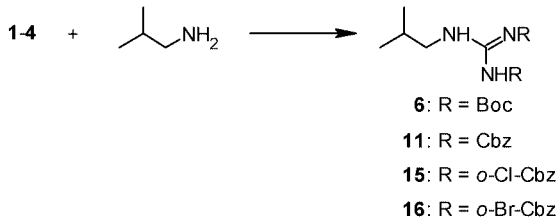
Since only a few examples of the application of **1**[11,15] and **2**[16] without the presence of $HgCl_2$ were reported in the literature, we decided, at the early stage of our studies, to use these reagents for the transformation of some amines to guanidines. Reactions of a series of structurally diverse amines with these reagents were carried out and the results are summarized in Table 1.

The course of the reaction was monitored by TLC. One molar excess of amine was used and the time of total consumption of the reagent was determined. The homogeneous product was isolated by washing with aqueous citric acid. It can be seen that primary amines react at room temperature to form protected guanidines with a reasonable yield. However, the rate of reaction ranged substantially. The reaction of *tert*-butylamine was very slow and unchanged reagents were present still after 48 hours. These results indicate that in some cases rate of the reaction is not sufficient to obtain a product within a reasonable time, when for some reason it is not advisable to use more drastic conditions. This prompted us to synthesize new more promising reagents **3** and **4**. To compare all these reagents a reaction was carried out with the same amine, isobutylamine (Scheme 2). The results are summarized in Table 2. It can be seen that the electron-withdrawing effect of the protecting groups plays an important role in the reactivity of the reagents. The reaction time of 20 hours for the reaction carried out in dichloromethane was reduced to 15 minutes when **1** was replaced by **3** or **4** (entry iii, see also entries i, v and vi). We also observed an increased reaction rate when a catalytic amount of 4-(*N*,*N*-dimetyhylamino)pyridine (DMAP) was added (entries ii and iv).

Table 1 Conversion of Amines to Guanidines[a]

Reagent	Amine	Time (min)[b]	Product	Yield (%)[c]
1		10	 **5**	83
1		20	 **6**	94
1		30	 **7**	89
1		80	 **8**	85
1		48 h	 **9**	21[d]
2		20	 **10**	85
2		30	 **11**	74
2		45	 **12**	99
2		20 h	 **13**	75
2		48 h	 **14**	54[d]

[a] Reactions were conducted using **1** (in DMF) and **2** (in CH_2Cl_2).
[b] Unless otherwise indicated.
[c] Yields of isolated products; see experimental for details.
[d] Unchanged reagent remained in solution.



Scheme 2 Guanidinylation of isobutylamine. Conditions and reaction times are given in Table 2.

To show differences in reactivity, experiments in which the concentration of the reactants was 10 times lower are also given (entries v and vi). A substantial decrease in the reaction rate was observed in dichloromethane but it was also noticeable in DMF. This confirms the observation,[27] that the rate of reaction of **1** was not measurable at 10 mM concentration.

To demonstrate the possibility of obtaining free guanidines, *N*,*N*′-bis(*o*-bromo-benzyloxycarbonyl)-*N*″-isobutylguanidine (**15**) was hydrogenated in the presence of palladium catalyst. The reaction was rapid and complete. The prolonged treatment of **15** with trifluoroacetic acid revealed the stability of this compound under these conditions.

In conclusion, we have presented here a facile method for the preparation of *o*-Cl- and *o*-Br-benzyloxycarbonyl-protected *S*-methylisothioureas. The obtained reagents are stable and react more rapidly than **1** and **2** to form protected guanidines. The products are stable when treated for a prolonged time with trifluoroacetic acid. The protecting groups can be removed by hydrogenolysis. This allows the use of these reagents in a multistep synthesis, which includes treatment with acid after introducing the guanidino function. These reagents also dispense with the treatment of acid sensitive products with trifluoroacetic acid, which is needed when **1** is used for guanidinylation, since deprotection is achieved by catalytic hydrogenolysis.

All chemicals were purchased from commercial sources and used without further treatment unless otherwise indicated. *o*-Cl-Cbz-OSu and *o*-Br-Cbz-OSu were obtained from Senn Chemicals. Melting points were uncorrected. NMR spectra were obtained for ^{1}H at 200 MHz or 500 MHz and for ^{13}C at 50 MHz or 125 MHz. Chemical shifts are reported in ppm values relative to TMS as internal standard. TLC was performed with precoated silica plates (Kieselgel 60 F_{254}, Merck) using the eluent specified. The structures of known compounds were confirmed by comparison of NMR data and mp values with those published. Elemental analyses for new compounds were performed with an elemental analyzer, Perkin-Elmer 2400 CHNS/O.

***N*,*N*′-Bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (1)**

S-Methylisothiourea sulfate (2.78 g, 10 mmol) was dissolved in a mixture of H_2O (30 mL) and dioxane (30 mL) followed by addition of 1 M aq NaOH solution (20 mL, 20 mmol) and di-*tert*-butyl dicarbonate (11.62 g, 50 mmol, 1.3 equiv). The reaction mixture was vigorously stirred overnight at r.t. The precipitate formed was filtered and washed with a small amount of H_2O. The filtrate was concentrated under reduced pressure to approximately half the volume and the solid was separated by filtration. The solids were combined and suspended in H_2O (200 mL) at approximately 50 °C, shaken and filtered. It was then dried under reduced pressure at r.t. over P_2O_5 to give **1** (5.57 g, 96%) as a white solid; mp 127 °C (Lit.[11] mp 122–123 °C).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.51 (s, 9 H), 1.52 (s, 9 H), 2.40 (s, 3 H), 11.62 (br s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.40, 28.04, 80.97, 83.23, 150.76, 160.78, 171.44.

***N*,*N*′-Bis(benzyloxycarbonyl)-*S*-methylisothiourea (2)**

Method 1: *S*-Methylisothiourea sulfate (0.67 g, 2.5 mmol) was dissolved in 5 M aq NaOH solution (10 mL, 5 mmol) and dibenzyl dicarbonate (2.86 g, 10 mmol) was added, followed by dioxane (15 mL). The reaction mixture was stirred overnight, concentrated to approximately half the volume and CH_2Cl_2 was added (100 mL). The organic layer was separated, washed with H_2O (3 × 20 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give a viscous oil, which crystallized on standing. The crude product was recrystallized from *i*-PrOH. Product **2** (1.38 g, 85%) was obtained as white crystals; mp 62–64 °C (Lit.[16] mp 56.6–57.5 °C).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.41 (s, 3 H), 5.18 (br s, 4 H), 7.36 (m, 10 H), 11.85 (br s, 1 H).

Table 2 Guanidinylation of Isobutylamine in DMF and CH_2Cl_2 Using Reagents **1**–**4**

Entry	Solvent	Catalyst[a]	Concentration[b]	Time (min[c]) for Total Consumption of the Reagent			
				1	**2**	**3**	**4**
i	DMF	absent	0.25 M	20	30	8	8
ii	DMF	present	0.25 M	10	15	7	7
iii	CH_2Cl_2	absent	0.25 M	20 h	20	15	15
iv	CH_2Cl_2	present	0.25 M	7 h	10	10	4
v	DMF	absent	0.025 M	6.5 h	45	35	25
vi	CH_2Cl_2	absent	0.025 M	72 h	16 h	7 h	6.5 h

[a] 0.1 Equiv of DMAP.
[b] Calculated for guanidinylating reagent.
[c] Unless otherwise indicated.

^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.56, 67.98, 68.35, 128.27, 128.49, 128.61, 128.72, 134.54, 135.72, 151.48, 161.01, 172.84.

Method 2: *S*-Methylisothiourea sulfate (0.67 g, 2.5 mmol) was dissolved in 5 M aq NaOH solution (10 mL, 5 mmol) and benzyloxycarbonylsuccinimide (2.49 g, 10 mmol), followed by dioxane (15 mL) were added. The reaction mixture was stirred for 2 d, concentrated to approximately half the volume and CH_2Cl_2 was added (100 mL). The organic layer was separated, washed with H_2O (3 × 20 mL), dried ($MgSO_4$) and evaporated under reduced pressure to afford a viscous oil which crystallized on standing. The crude product was recrystallized from *i*-PrOH. Product **2** (1.35 g, 83%) was obtained as white crystals. Analytical data are identical with those obtained in Method 1.

N,*N*′-Bis(*ortho*-chlorobenzyloxycarbonyl)-*S*-methylisothiourea (3)

S-Methylisothiourea sulfate (3.02 g, 10.86 mmol) was dissolved in H_2O (32 mL) and dioxane (28 mL) and 1 M aq solution of NaOH (22 mL, 22 mmol) was added, followed by (*ortho*-chlorobenzyloxy)carbonyloxysuccinimide (12.33 g, 43.47 mmol, 2 equiv). The reaction mixture was stirred at r.t. for 24 h, then concentrated under reduced pressure to approximately 30% volume. The residue was washed with EtOAc (3 × 50 mL), the organic layer was washed with H_2O (30 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The oily residue, which solidified on standing, was recrystallized from EtOH yielding **3** (6.3 g, 74%); mp 71–72 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.43 (s, 3 H), 5.31 (s, 2 H), 5.32 (s, 2 H), 7.25–7.28 (m, 8 H), 11,88 (br s, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.68, 65.19, 65.63, 126.89, 126.99, 129.41, 129.42, 129.48, 129.66, 130.09, 130.34, 132.32, 133.27, 133.52, 133.95, 151.35, 160.86, 173.17.

Anal. Calcd for $C_{18}H_{16}Cl_2N_2O_4S$: C, 50.60; H, 3.77; N, 6.56; Cl, 16.59; S, 7.50. Found: C 50.55; H, 3.70; N, 6.48; Cl, 16.62; S, 7.36.

N,*N*′-Bis(*ortho*-bromobenzyloxycarbonyl)-*S*-methylisothiourea (4)

S-Methylisothiourea sulfate (1.51 g, 5.43 mmol) was dissolved in H_2O (16 ml) and dioxane (14 mL) and 1 M aq solution of NaOH (11 mL, 11 mmol) were added, followed by (*ortho*-bromobenzyloxy)carbonyloxysuccinimide (14.27 g, 43.5 mmol, 2 equiv). The reaction mixture was stirred at r.t. for 24 h, then concentrated under reduced pressure, the residue was dissolved in EtOAc (150 mL) and washed with 10% aq citric acid (3 × 30 ml), aq sat. $NHCO_3$ solution (3 × 30 mL), and H_2O (3 × 30 mL), dried ($MgSO_4$) and evaporated. The solid residue was recrystallized from EtOH yielding product **4** (7.42 g, 70%); mp 101–103 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.45 (s, 3 H), 5.29 (s, 2 H), 5.31 (s, 2 H), 7.15–7.61 (m, 8 H), 11.89 (s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.71, 67.43, 67.84, 122.93, 123.70, 127.54, 127.64, 129.34, 129.61, 130.28, 130.35, 132.77, 132.98, 134.01, 135.10, 151.35, 160.85, 173.25.

Anal. Calcd for $C_{18}H_{16}Br_2N_2O_4S$: C, 41.88; H, 3.12; N, 5.43; Br, 30.96. Found: C, 42.01; H, 3.01; N, 5.41; Br, 30.99.

Protected Guanidines 5–16; General Procedure

To a solution of one of the isothiourea derivatives **1**–**4** (0.5 mmol) in DMF or CH_2Cl_2 (2 mL) was added an amine (1 mmol) (and optionally 0.05 mmol of DMAP), and the reaction mixture was stirred at r.t. until TLC (silica gel plates, hexane–EtOAc, 9:1, detection by UV light λ = 254 nm) showed complete consumption of the isothiourea derivative. The solvent was then evaporated under reduced pressure, and the residue was dissolved in EtOAc (50 mL). The solution was washed successively with 10% aq citric acid (3 × 10 mL), aq sat. $NaHCO_3$ solution (3 × 10 mL) and H_2O (3 × 10 mL). The organic phase was then dried ($MgSO_4$) and evaporated under reduced pressure to dryness to give the pure product. Reaction times and yields of isolated products (except **15** and **16**) are summarized in Table 1.

N,*N*′-Bis(Boc)-*N*″-propylguanidine (5)

White solid; mp 87–89 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.93–1.00 (t, 3 H, *J* = 7.5 Hz), 1.50 (s, 9 H), 1.51 (s, 9 H), 1.54–1.65 (m, 1 H), 3.34–3.44 (dt, 2 H, *J* = 7.1, 5.4 Hz), 8.33 (s, 1 H), 11.52 (s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 11.38, 22.25, 28.07, 28.31, 42.58, 79.18, 82.96, 153.34, 156.16, 163.67.

Anal. Calcd for $C_{14}H_{27}N_3O_4$: C, 55.79; H, 9.03; N, 13.94. Found: C, 56.01; H, 8.76; N, 13.80.

N,*N*′-Bis(Boc)-*N*″-isobutylguanidine (6)

White solid; mp 100–101 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.95–0.98 (d, 6 H, *J* = 6.8 Hz), 1.49 (s, 9 H), 1.51 (s, 9 H), 1.75–1.96 (m, 1 H), 3.22–3.28 (dd, 2 H, *J* = 7.0, 5.2 Hz), 8.41 (br s, 1 H), 11.52 (s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.21, 27.87, 28.09, 28.33, 48.29, 79.21, 82.99, 153.39, 156.30, 163.70.

Anal. Calcd for $C_{15}H_{29}N_3O_4$: C, 57.10; H, 9.27; N, 13.33. Found: C, 57.17; H, 9.21; N, 13.34.

N,*N*′-Bis(Boc)-*N*″-cyclopentylguanidine (7)

White solid; mp 144–145 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.49 (s, 9 H), 1.51 (s, 9 H), 1.28–2.06 (m, 8 H), 4.39–4.53 (m, 1 H), 8.34–8.37 (d, 1 H, *J* = 7.2 Hz), 11.52 (br s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.08, 23.63, 28.12, 28.29, 33.16, 51.99, 79.08, 82.89, 153.55, 163.83.

Anal. Calcd for $C_{16}H_{29}N_3O_4$: C, 58.69; H, 8.93; N, 12.83. Found: C, 58.71; H, 8.86; N, 12.89.

N,*N*′-Bis(Boc)-*N*″-isopropylguanidine (8)

White solid; mp 120–121 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.18–1.21 (d, 6 H, *J* = 6.4 Hz), 1.49 (s, 9 H), 1.50 (s, 9 H), 4.27–4.41 (m, 1 H), 8.22 (br s, 1 H), 11.51 (br s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.79, 28.13, 28.39, 42.38, 79.07, 82.91, 153.35, 155.30, 163.88.

Anal. Calcd for $C_{14}H_{27}N_3O_4$: C, 55.79; H, 9.03; N, 13.94. Found: C, 55.74; H, 9.04; N, 13.73.

N,*N*′-Bis(Boc)-*N*″-*tert*-butylguanidine (9)

White solid; mp 140–141 °C (Lit.[25] mp 140–141.5 °C).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.44 (s, 9 H), 1.49 (s, 18 H), 8.24 (br s, 1 H), 11.41 (br s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 21.77, 28.13, 28.39, 41.01, 79.06, 82.90, 153.35, 155.28, 163.89.

N,*N*′-Bis(Cbz)-*N*″-propylguanidine (10)

White solid; mp 63–65 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.92–0.99 (t, 3 H, *J* = 7.4 Hz), 1.51–1.69 (m, 2 H), 3.35–3.44 (dt, 2 H, *J* = 7.4, 5.4 Hz), 5.13 (s, 2 H), 5.17 (s, 2 H), 7.27–7.41 (m, 10 H), 8.31 (s, 1 H), 11.75 (s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 11.32, 22.21, 42.86, 67.14, 68.13, 127.90, 128.16, 128.40, 128.44, 128.71, 128.79, 134.66, 136.85, 153.93, 155.99, 163.56.

Anal. Calcd for $C_{20}H_{23}N_3O_4$: C, 65.03; H, 6.28; N, 11.37. Found: C, 65.13; H, 6.16; N, 11.45.

***N,N′*-Bis(Cbz)-*N″*-isobutylguanidine (11)**
White solid; mp 95–97 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.94–0.97 (d, 6 H, *J* = 6.6 Hz), 1.77–1.97 (m, 1 H), 3.24–3.30 (dd, 2 H, *J* = 6.9, 5.3 Hz), 5.13 (s, 2 H), 5.18 (s, 2 H), 7.29–7.39 (m, 10 H), 8.38–8.39 (br s, 1 H), 11.76 (br s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.10, 27.88, 48.49, 67.15, 68.13, 127.89, 128.14, 128.39, 128.45, 128.70, 128.79, 134.62, 136.84, 153.98, 156.09, 163.50.

Anal. Calcd for $C_{20}H_{25}N_3O_4$: C, 65.78; H, 6.57; N, 10.96. Found: C, 65.95; H, 6.44; N, 11.05.

***N,N′*-Bis(Cbz)-*N″*-cyclopentylguanidine (12)**
White solid; mp 95–96 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.38–2.02 (m, 8 H), 4.37–4.47 (m, 1 H), 5.13 (s, 2 H), 5.16 (s, 2 H), 7.27–7.42 (m, 10 H), 8.31–8.34 (d, 1 H, *J* = 6.8 Hz), 11.76 (s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 23.56, 32.99, 52.50, 67.12, 68.05, 127.87, 128.12, 128.39, 128.42, 128.53, 128.70, 128.77, 134.69, 136.95, 153.93, 155.41, 163.80.

Anal. Calcd for $C_{22}H_{25}N_3O_4$: C, 66.82; H, 6.37; N, 10.63. Found: C, 66.99; H, 6.33; N, 10.65.

***N,N′*-Bis(Cbz)-*N″*-isopropylguanidine (13)**
White solid; mp 116–118 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.19–1.22 (d, 6 H, *J* = 6.6 Hz), 4.25–4.36 (m, 1 H), 5.13 (s, 2 H), 5.17 (s, 2 H), 7.30–7.38 (m, 10 H), 8.20 (br s, 1 H), 11.76 (br s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 25.54, 42.98, 67.10, 68.05, 127.87, 128.11, 128.39, 128.42, 128.61, 128.70, 128.77, 128.85, 134.70, 136.93, 153.89, 155.07, 163.82.

Anal. Calcd for $C_{20}H_{23}N_3O_4$: C, 65.03; H, 6.28; N, 11.37. Found: C, 65.21; H, 6.22; N, 11.35.

***N,N′*-Bis(Cbz)-N″-*tert*-butylguanidine (14)**
White solid; mp 89–90 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.44 (s, 9 H), 5.14 (s, 2 H), 5.16 (s, 2 H), 7.25–7.40 (m, 10 H), 8.33 (br s, 1 H), 11.81 (br s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 28.78, 52.59, 67.10, 67.90, 127.71, 127.73, 128.37, 128.40, 128.67, 128.71, 134.76, 137.16, 154.03, 154.57, 163.39.

Anal. Calcd for $C_{20}H_{25}N_3O_4$: C, 65.78; H, 6.57; N, 10.96. Found: C, 65.88; H, 6.48; N, 10.84.

***N,N′*-Bis(*o*-Cl-Cbz)-*N″*-isobutylguanidine (15)**
Yield: 191 mg (84%); white solid; mp 101–103 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.96–0.99 (d, 6 H, *J* = 6.6 Hz), 1.79–1.99 (m, 1 H), 3.27–3.33 (dd, 2 H, *J* = 7.0, 5.4 Hz), 5.26 (s, 2 H), 5.31 (s, 2 H), 7.21–7.48 (m, 8 H), 8.43 (br s, 1 H), 11.71 (br s, 1 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.11, 27.91, 48.52, 64.33, 65.32, 126.78, 127.03, 128.91, 129.04, 129.31, 129.69, 129.96, 130.05, 132.35, 134.62, 153.81, 156.17, 163.59.

Anal. Calcd for $C_{21}H_{23}Cl_2N_3O_4$: C, 55.76; H, 5.13; N, 9.29; Cl, 15.68. Found: C, 55.61; H, 5.11; N, 9.28; Cl, 15.61.

***N,N′*-Bis(*o*-Br-Cbz)-*N″*-isobutylguanidine (16)**
Yield: 180 mg (67%); white solid; mp 103–105 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.97–0.98 (d, 6 H, *J* = 7.0 Hz), 1,86–1,91 (m, 1 H), 3.29–3.32 (dd, 2 H, *J* = 7.0, 5.5 Hz), 5.23 (s, 2 H), 5.29 (s, 2 H), 7.12–7.60 (m, 8 H), 8.44 (br s, 1 H), 11.83 (br s, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 20.11, 27.90, 48.51, 66.56, 67.51, 122.53, 123.37, 127.40, 127.65, 128.93, 129.12, 129.92, 130.21, 132.55, 132.95, 133.98, 136.21, 153.76, 156.17, 163.55.

Anal. Calcd for $C_{21}H_{23}Br_2N_3O_4$: C, 46.60; H, 4.28; N, 7.76; Br, 29.53. Found: C, 46.32; H, 4.12; N, 7.61; Cl, 29.45.

Treatment of 15 with TFA
Compound **15** (3.5 mg, 0.006 mmol) was treated with TFA–CH_2Cl_2 solution (50%, 0.1 mL) for 24 h at r.t. TLC revealed only the starting compound in the solution. No reaction with Sakaguchi reagent[30] was observed.

Catalytic Hydrogenolysis of 15
Compound **15** (20 mg, 0.037 mmol) was dissolved in MeOH (2 mL) and 10% Pd/C (100 mg) was added. The solution was stirred for 2 h under H_2 atmosphere. The catalyst was filtered off and the solvent removed under reduced pressure. TLC analysis of the product revealed absence of the starting material. In order to verify the cleavage of both protecting groups the product was subjected to Sakaguchi reaction. Intense red color implied the presence of a free guanidine derivative.

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