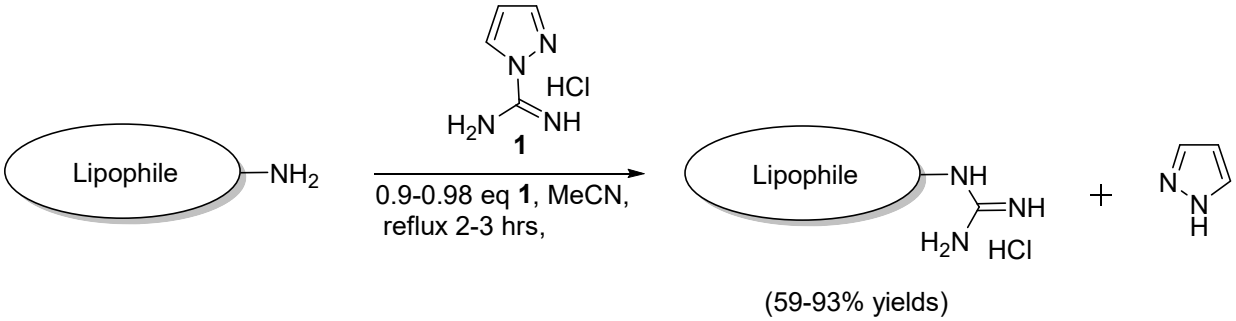
Simple generalized reaction conditions for the conversion of primary aliphatic amines to surfactant-like guanidine salts with 1*H*-pyrazole carboxamidine hydrochloride

T. A. Bakka and O. R. Gautun
Department of Chemistry, Norwegian University of Science and Technology (NTNU), NO-7491 Trondheim, Norway

GRAPHICAL ABSTRACT

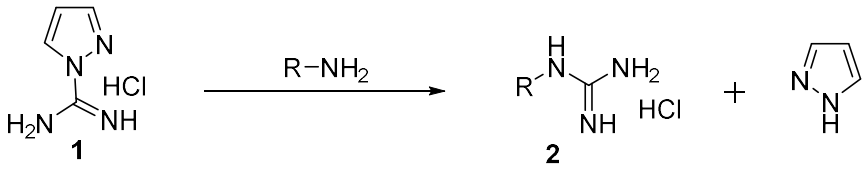


Abstract *Improved reaction conditions for the electrophilic reaction between a free aliphatic amine and 1H-pyrazole carboxamidine have been discovered. The often hard to work with surfactant-like guanidine salts were obtained in decent yields with short reaction times, minimal work up and high level of purity.*

Keywords 1*H*-pyrazole carboxamidine; guanidine synthesis; surfactant synthesis.

INTRODUCTION

1*H*-Pyrazole carboxamidine hydrochloride (**1**) is a commonly used electrophilic guanylation reagent for primary amines.[1] This guanylation agent was originally presented by Bernatowicz *et al.* in 1992 as a means for guanyl introduction in peptide synthesis, thus circumventing the usage of the sometimes problematic protected arginines.[2] Bernatowicz reported that **1** showed better reactivity and solubility characteristics than other guanylation compounds at the time.[3-8] The reaction of **1** with a primary amine is shown in scheme 1. A literature search shows that many have employed this method in the synthesis of a variety of compounds.[9] Where the most common conditions are an amine, an organic (Hünig's base or TEA) or inorganic base, and **1** in equimolar amounts in a solvent (most often DMF). Removal of base and solvents with high boiling points may be troublesome in the work-up. Thus, many of the procedures reported employ chromatography and other techniques in the purification step. And as guanidines may be troublesome to work with due to their heavily polar nature, a minimum of work-up is highly preferred.



Scheme 1: Reaction between 1*H*-pyrazole carboxamidine (**1**) hydrochloride and a primary aliphatic amine, leading to formation of the guanylated product **2** and 1*H*-pyrazole as a byproduct.

The reaction conditions presented here renders complete conversion in a matter of hours, requires minimal work-up, and needs no chromatographic purification.

RESULTS AND DISCUSSION

In our search for simple versatile conversions of amines in order to expand a compound library of 1,2,3-triazoles for antimicrobial screening, we have found simple and efficient conditions for converting amines to guanidines utilizing the already reported reagent **1**. We have found that for many amines addition of base does not necessarily decrease reaction time nor increase yield. In addition, by running the reactions in acetonitrile at reflux we achieved rapid full conversion and chromatographically pure compounds (**2e**-**2g**) with minimal work-up as depicted in the experimental section. The conditions yielding optimal results have been: amine and **1** in a 1:0.9 – 1:0.99 ratio in acetonitrile (1 mL/100 mg amine) followed by 1-3 hours of reflux, leading to complete conversion of **1**. These reaction conditions were easily scalable to a preparative level (>10 g), which in turn was performed to make 13 g of guanidine **2c** (93% yield). There have been reported a couple of similar standalone reactions performed without base in acetonitrile either at room temperature or reflux.[10-12] However, these examples required a reaction time above 16 hours. Also, there have been reported some reactions with short reaction times (0.5 hours) in a volatile solvent (EtOH), but this was performed under microwave conditions with a base additive. [13]

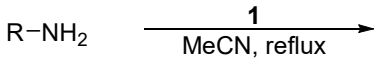
It should also be noted that we have used a slight excess of amine in order to ensure complete conversion of **1**, as there sometimes were some uncertainty regarding the purity of amines synthesized through several previous steps.

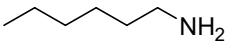
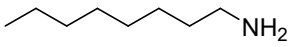
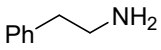
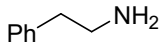
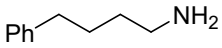
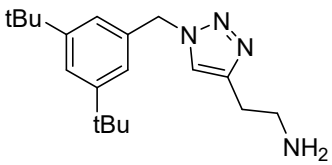
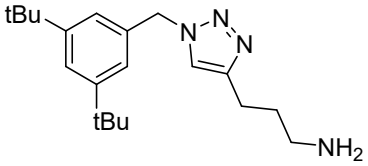
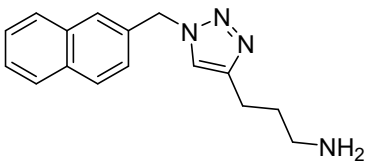
The three bottom products given in Table 1 showed a level of purity acceptable for biological testing, thus making these reaction conditions favorable for compounds intended for pharmacological testing.

SUMMARY

A simplified protocol for guanylation of primary amines is hereby reported. Seven guanidines (**2a**-**g**) were prepared, where the synthesis of **2c** also was performed on a >10 g scale. Three of these compounds (**2e**-**g**) were subjected to HPLC-analysis, giving sufficiently pure compounds (>95%) for biological testing.

Table 1 Results of exposing amines containing lipophilic groups to the improved reaction condition in order to synthesize surfactant-like guanidine salts.



		1 (eq)	Reflux (hrs)	Yield (%)
	**2a**	0.9	3	74
	**2b**	0.9	3	59
	**2c**	0.9	3	84
	**2c**	0.9	3	93[a]
	**2d**	0.9	3	70
	**2e**	0.9	1.5	63
	**2f**	0.9	3	70
	**2g**	0.98	12	76

[a]) Experiment performed in multi-gram scale

EXPERIMENTAL SECTION

All chemicals and solvents applied were of PA quality unless otherwise stated and used as received without further purification. NMR spectra were recorded on a Bruker Avance DPX400 instrument. Chromatographic analyses were performed on an Agilent 1290 chromatograph equipped with a Zorbax Eclipse C18 5 µm column (21 x 250 mm) and a diode array detector. IR analyses were performed on a Thermo Nicolet Nexus FT-IR spectrometer equipped with a Smart Endurance reflection cell. Accurate mass determination in positive and negative mode was performed on a "Synapt G2-S" Q-TOF instrument from Waters™. Samples were ionized by the use of ASAP probe (APCI) or ESI probe.

General procedure for guanylation of primary aliphatic amines:

Amine and **1** (0.9-0.99 eq) was added to MeCN (1 mL/100 mg amine) on a 50 – 100 mg scale and refluxed for 2-3 hours. After complete disappearance of **1** (by TLC w/ 70:30:3 $CHCl_3$:MeOH:NH_4OH) the reaction mixture was cooled down to room temperature. Futhermore, depending on whether the product precipitated out or not, the following was done:

- **A)** If the product precipitated by cooling the reaction mixture to room temperature; the supernatant was removed and the precipitate was washed with MeCN and dried under reduced pressure.
- **B)** If the product did not precipitate by cooling the reaction mixture to room temperature; Et_2O was added to induce precipitation. The precipitate was then washed with Et_2O and dried under reduced pressure.

Synthesis of 2a:

General procedure B lead to **2a** as a viscous oil (131 mg, 0.72 mmol, 74% yield). ^{1}H NMR (400 MHz, *d4*-MeOH): δ = 3.19 (t, 2H, *J* = 7.0 Hz, -**CH$_2$**-NH-), 1.62 (p, 2H, *J* = 6.9 Hz, -**CH$_2$**-CH_2-NH-), 1.42 – 1.29 (m, 6H, 3 x **CH$_2$**), 0.96 (t, 3H, *J* = 6.9 Hz, **CH$_3$**) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 157.2 (NH-**C**=NH), 41.1 (-**CH$_2$**-NH-), 31.1, (-**CH$_2$**-CH_2-NH-), 28.5, 26.0, 22.2, 12.9 (-**CH$_3$**) ppm. NMR spectra is similar to spectra of the tosylate salt of the same compound synthesized by Katritzky *et al.*[14]

Synthesis of 2b:

General procedure B lead to **2b** as an oil (94 mg, 0.45 mmol, 59% yield). ^{1}H NMR (400 MHz, *d4*-MeOH): δ = 3.16 (t, 2H, *J* = 7.0 Hz, -**CH$_2$**-NH-), 1.58 (p, 2H, *J* = 7.1 Hz, -**CH$_2$**-CH_2-NH-), 1.43 – 1.25 (m, 10H, 5 x **CH$_2$**), 0.91 (t, 3H, *J* = 7.0 Hz, **CH$_3$**) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 157.2 (NH-**C**=NH), 41.1 (-**CH$_2$**-NH-), 31.5 (-**CH$_2$**-CH_2-NH-), 28.9, 28.9, 28.5, 26.3, 22.3, 13.0 (-**CH$_3$**) ppm. Proton NMR spectra coincided with previously reported spectra for the neutral *n*-octyl guanidine in *d6*-DMSO.[15]

Synthesis of 2c:

1) General procedure B lead to **2c** as a light yellow sticky wax (137 mg, 0.69 mmol, 84% yield).
2) General procedure B[a] lead to **2c** as an off-white solid (13 g, 65 mmol, 93% yield).

^{1}H NMR (400 MHz, *d4*-MeOH): δ = 7.36 – 7.22 (m, 5H, **Ph**-), 3.46 (t, 2H, *J* = 7.1 Hz, -NH-**CH$_2$**-), 2.88 (t, 2H, *J* = 7.1 Hz, Ph-**CH$_2$**-) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 157.1 (NH-**C**=NH), 137.9 (CH-**C**-CH), 128.4 (2x**CH**), 128.3 (2x**CH**), 126.4 (**CH**), 42.3 (-**CH$_2$**-NH-), 34.5 (C-**CH$_2$**-) ppm. Spectra is highly similar to spectra of the sulfate salt in D_2O reported in a patent by the university of Strathclyde.[16]

[a]) Preparative scale work-up: The reaction mixture was partially evaporated and added Et_2O (200 mL). The ether phase was decanted and the residual oil was washed with Et_2O (3 x 200 mL), before it was added one last portion of Et_2O (200 mL) and left for 48 hours. The hard crystalline precipitate was then crushed and stirred with Et_2O (3 x 100 mL) for 10 minutes each time. Decanting and drying afforded **2c** as an off-white solid.

Synthesis of 2d:

General procedure B lead to **2d** as a white solid (105 mg, 0.46 mmol, 70% yield). ^{1}H NMR (400 MHz, *d4*-MeOH): δ = 7.30 – 7.22 (m, 2H, **Ph**), 7.21 – 7.12 (m, 3H, **Ph**), 3.19 (t, 2H, *J* = 6.6 Hz, -**CH$_2$**-NH-), 2.66 (t, 2H, *J* = 7.7, -**CH$_2$**-Ph), 1.69 (p, 2H, *J* = 7.7, -**CH$_2$**-CH$_2$-Ph), 1.60 (p, 2H, *J* = 6.7 Hz, -**CH$_2$**-CH$_2$-NH-) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 157.2 (NH-**C**=NH), 141.8 (CH-**C**-CH), 128.0 (2x**CH**), 128.0 (2x**CH**), 125.5 (**CH**), 40.9 (-**CH$_2$**-NH-), 34.9 (C-**CH$_2$**-CH$_2$-), 28.2 (C-CH$_2$-**CH$_2$**-), 28.0 (-**CH$_2$**-CH$_2$-NH) ppm. Spectra is highly similar to spectra of the neutral compound synthesized by Tsubokura *et al*. [17]

Synthesis of 2e:

General procedure A lead to **2e** as a white solid (71 mg, 0.18 mmol, 70% yield, mp: 221 – 222.4 °C). Purity confirmed by HPLC with a C18 Zorbax Eclipse reverse phase column and 5:3 MeOH:H_2O + 0.1% TFA as the eluent system (21.86 min, 0.75 mL/min, 99% pure). ^{1}H NMR (400 MHz, *d4*-MeOH): δ = 7.82 (s, 1H, -N-**CH**=C-), 7.45 (s, 1H, **Ar**), 7.22 (2xs, 2H, **Ar**), 5.56 (s, 2H, Ar-**CH$_2$**-N-), 3.51 (t, 2H, *J* = 6.7 Hz, -**CH$_2$**-NH-), 2.98 (t, 2H, *J* = 6.7 Hz, -**CH$_2$**-CH$_2$-NH), 1.32 (s, 18H, 2xtBu) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 157.2 (NH-**C**=NH), 151.5 (2xCH-**C**-(tBu)-CH), 144.2 (CH-**C**-CH$_2$-CH$_2$), 134.6 (**C**-CH$_2$-N-), 122.6 (**CH**-C-CH$_2$-CH$_2$), 122.2 (C-**CH**-C), 122.1 (2x C-**CH**-C), 54.1 (Ar-**CH$_2$**-N-), 40.5 (-**CH$_2$**-NH-), 34.3 (2x -**C**-(CH$_3$)$_3$), 30.4 (6x**CH$_3$**), 24.6 (-**CH$_2$**-CH$_2$-NH) ppm. IR: 3396 (bs), 3233 (bs), 3110 (bs), 1684 (s), 1637 (s), 1621 (s), 1600 (s), 1475 (s), 1364 (s), 882 (s), 814 (s), 781 (s), 711 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 357.2767 (Calcd. $C_{20}H_{33}N_6$, 357.2761, $[M+H]^+$).

Synthesis of 2f:

General procedure A lead to **2f** as a white solid (58 mg, 0.14 mmol, 70% yield, mp: 219.0 – 220.1 °C). Purity confirmed by HPLC with a C18 Zorbax Eclipse reverse phase column and 5:3 MeOH:H_2O + 0.1% TFA as the eluent system (32.91 min, 0.75 mL/min, 99% pure). ^{1}H NMR (400 MHz, *d4*-MeOH): δ =

7.75 (s, 1H, -N-**CH**=C-), 7.42 (s, 1H, **Ar**), 7.17 (s, 2H, **Ar**), 5.54 (s, 2H, Ar-**CH_2**-N-), 3.22 (t, 2H, *J* = 7.3 Hz, -**CH_2**-NH-), 2.76 (t, 2H, *J* = 7.3 Hz, -**CH_2**-CH_2-CH_2-NH), 1.93 (p, 2H, *J* = 7.3 Hz, -CH_2-**CH_2**-CH_2-NH) 1.32 (s, 18H, 2 x tBu) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 157.2 (NH-**C**=NH), 151.4 (2xCH-**C**-(tBu)-CH), 146.8 (CH-**C**-CH_2-CH_2-), 134.8 (-**C**-CH_2-N-), 122.1 (C-**CH**-C), 122.0 (2 x C-**CH**-C), 121.9 (**CH**-C-CH_2-CH_2), 54.0 (Ar-**CH_2**-N-), 40.3 (-**CH_2**-NH-), 34.3 (2x -**C**-$(CH_3)_3$), 30.4 (6 x **CH_3**), 28.2 (-CH_2-**CH_2**-CH_2-NH), 21.7 (-**CH_2**-CH_2-CH_2-NH) ppm. IR: 3328 (m), 3110 (bs), 2949 (s), 1677 (s), 1639 (s), 1601 (s), 1476 (s), 1361 (s), 1060 (s), 854 (s), 791 (s), 709 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 371.2924 (Calcd. $C_{21}H_{35}N_6$, 371.2918, $[M+H]^+$).

Synthesis of 2g:

General procedure A lead to **2g** as a white solid (98 mg, 0.28 mmol, 76% yield, mp: 162.2 – 164.2 °C). TLC analysis after 12 hours showed full conversion, it was then left over night. Purity confirmed by HPLC with a C18 Zorbax Eclipse reverse phase column and 1:1 MeOH:H_2O + 0.1% TFA as the eluent system (11.07 min, 0.75 mL/min, 98% pure). ^{1}H NMR (400 MHz, *d4*-MeOH): δ = 7.91 – 7.83 (m, 5H, **Ar** + -N-**CH**=C-), 7.55 – 7.50 (m, 2H, **Ar**), 7.40 (dd, 1H, *J* = 1.6, 8.4 Hz, **Ar**), 5.72 (s, 2H, Ar-**CH_2**-), 3.22 (t, 2H, *J* = 7.1 Hz, -CH_2-**CH_2**-NH-), 2.76 (t, 2H, *J* = 7.5 Hz, -**CH_2**-CH_2-CH_2-NH-), 1.93 (p, 2H, *J* = 7.3 Hz, -**CH_2**-CH_2-NH-) ppm. ^{13}C NMR (100 MHz, *d4*-MeOH): 157.3 (NH-**C**=NH), 146.9 (CH-**C**-CH_2-CH_2-), 133.4, 133.2, 132.8, 128.5, 127.6, 127.4, 126.3, 126.2, 125.1, 122.2 (**CH**-C-CH_2-CH_2), 53.7 (Ar-**CH_2**-N-), 40.3 (-**CH_2**-NH-), 28.1 (-CH_2-**CH_2**-CH_2-NH), 21.8 (-**CH_2**-CH_2-CH_2-NH) ppm. IR: 3451 (bs), 3134 (bs), 2951 (m), 1680 (s), 1648 (s), 1617 (s), 1466 (s), 1342 (s), 1060 (s), 794 (s), 783 (s), 769 (s) cm^{-1}. HRMS (APCI/ASAP, m/z): 309.1830 (Calcd. $C_{17}H_{21}N_6$, 309.1822, $[M+H]^+$).

SUPPLEMENTARY INFORMATION

Supplementary information can be accessed on the publisher`s website. Supplementary data contains ^{1}H NMR spectra of previously synthesized compounds and full characterization spectra (^{1}H NMR, ^{13}C NMR and, 2D-techniques) for new compounds.

REFERENCES

(1) Katritzky, A. R.; Rogovoy, B. V. *Arkivoc* **2005**, *4*, 49.
(2) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497.
(3) Davis, T. L. *Org. Synth.* **1927**, *7*.
(4) Cosand, W. L.; Merrifield, R. B. *Proc. Natl. Acad. Sci. U. S. A.* **1977**, *74*, 2771.
(5) Kämpf, A. *Chem. Berichte* **1904**, *37*, 1681.
(6) Arndt, F.; Rosenau, B. *Chem. Berichte* **1917**, *50*, 1248.
(7) Braun, C. E. *J. Am. Chem. Soc.* **1933**, *55*, 1280.
(8) Bannard, R. A. B.; Casselman, A. A.; Cockburn, W. F.; Brown, G. M. *Can. J. Chem.* **1958**, *36*, 1541.
(9) Reaxys database reaction search, august 2016.
(10) Bennett, C. N. H., Kurt D.; Harrison, S. D.; Longo, K. A.; MacDougald, O. A.; Wagman, A. S., GSK-3 inhibitors, US patent No. US20050054663, **2005**.

(11) Amgen Inc, Aminopyrimidine compounds and methods of use, US patent number WO20060661172 A1, **2006.**
(12) Nuss, J. M. H.; Stephen D.; Ring, D. B.; Boyce, R. S.; Johnson, K; Pfister, K. B.; Ramurthy, S.; Seely, L.; Wagman, A. S.; Desai, M.; Levine, B. H., Inhibitors of glycogen synthase kinase 3, US patent number US20020156087 A1, **2002**.
(13) Nielsen, M. C.; Larsen, A. F.; Abdikadir, F. H.; Ulven, T. *Eur. J. Med. Chem.* **2014**, *72*, 119.
(14) Katritzky, A. R.; Parris, R. L.; Allin, S. M.; Steel, P. J. *Synth. Comm.* **1995**, *25*, 1173.
(15) Solodenko, W.; Bröker, P.; Messinger, J.; Schön, U.; Kirschning, A. *Synthesis* **2006**, *2006*, 461.
(16) Waigh, R. D.; Harvey, A. L.; Mooney, M. H.; Coxon, G., Weight reducing compounds, US patent number WO2009087395, **2009**.
(17) Tsubokura, K.; Iwata, T.; Taichi, M.; Kurbangalieva, A.; Fukase, K.; Nakao, Y.; Tanaka, K. *Synlett* **2014**, *25*, 1302.