A straight forward synthesis of cyanoguanidines tethered dipeptidomimetics

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This work describes a simple and straight forward route for the synthesis of cyanoguanidine tethered dipeptidomimetics. The protocol is simple and uses thioureidopeptides as starting materials. $HgCl_2$ serve as an effective desulphurizing agent in affording the desired products in good yields.

Keywords: Cyanoguanidine, dipeptidomimetics, $HgCl_2$, thioureidopeptides, thiourea

Importance of guanidine and its derivatives has been a subject of rising attention. One of the interesting and widely used guanidine derivatives is cyanoguanidine which is a known cross linking agent in resin production as well as a source for the synthesis of biologically active compounds[1]. Examples of drugs that contain cyanoguanidine functionality include the antihypertensive Pinacidil and antiulcerative Cimetidine[2] (Figure 1).

Most of the cyanoguanidine derivatives were derived from corresponding thioureas. The standard methods to accomplish this transformation typically involve the conversion of thiourea to a carbodiimide[3] (*in situ* or isolated) or a methyl thioether followed by the addition of cyanamide in presence of a tertiary amine[4]. Cyanoguanidines can also be obtained by employing carbodiimides in presence of Lewis acid catalyst like $Ti(OiPr)_4$ (Ref 5). However, usage of EDC[6] results in longer reaction duration and poor to moderate yields of the products.

An important bioisosteric replacement for thiourea and urea functionality in drug discovery is the cyanoguanidine moiety. Cyanoguanidines are known to be excellent surrogates for the urea linkage and often provide an improved potency[7]. Wolin *et al.*[8] have reported the synthesis of several analogues of γ-amino acids containing cyanoguanidine moiety. In peptide chemistry, backbone modification of a peptide with urea and thiourea functionality has been delineated by our group[9]. Recently our group has also reported three examples of cyanoguanidine tethered peptidomimetics through carbodiimides synthesized *via* Staudinger reaction followed by Aza-Wittig type reaction[9c]. With the continuing interest to synthesize bioisosteric replacement of thus prepared peptidomimetics, we envisaged the incorporation of cyanoguanidine unit in place of urea or thiourea unit of the peptidomimetic backbone (Figure 2).

Results and Discussion

In the present protocol, *N*-protected thioureidopeptide prepared according to the literature procedure[9b] was used as starting material. The possible routes for the preparation of title molecules include the *S*-alkylation[10] or desulfurization[11] of corresponding thioureidopeptides followed by treatment with cyanamide source.
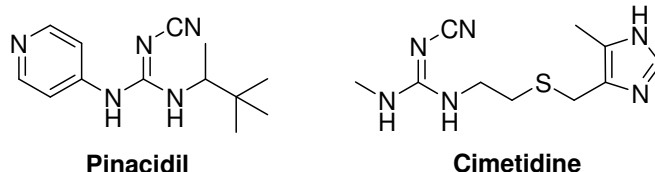

Pinacidil **Cimetidine**

Figure 1 — Antihypersensitive and antiulcerative agents

In our first attempt, we carried out the *S*-methylation of thioureidopeptide using MeI followed by the treatment with NaNHCN at ambient temperature. However, it leads to poor yield. As an alternative, we undertook the desulfurization of *N*-protected thioureidopeptide using various desulphurising agents such as DCC, EDC, Mukaiyama reagent and HgCl$_2$. Among the reagents screened, HgCl$_2$ afforded the corresponding cyanoguanidine in good yield.

In a typical experiment, Fmoc-Val-ψ[CH$_2$-NH-C(S)-NH]-Ala-OMe, **1a** was employed as a model substrate. To a solution of **1a** in DMF, was added HgCl$_2$ (0.5 mmol) and TEA (2.2 mmol). The reaction mixture was then allowed to stir for 15-20 min at RT and then a deprotonated solution of sodium cyanamide in DMF was added to the reaction mixture. After completion of the reaction, as monitored through TLC, the crude compound was isolated and purified. The formation of product was initially confirmed by recording IR of the crude sample where it exhibited a strong absorption band at 2190 cm^{-1} corresponding to the cyano group (C≡N stretch) of the product[12]. The compounds were analyzed by ^{1}H and ^{13}C NMR, and mass spectrometric analyses.

The generality of the protocol was exemplified by using various amino acids with different urethane protections such as Fmoc, Boc and Cbz (Scheme I) and the corresponding products were obtained in good yields (Table I).
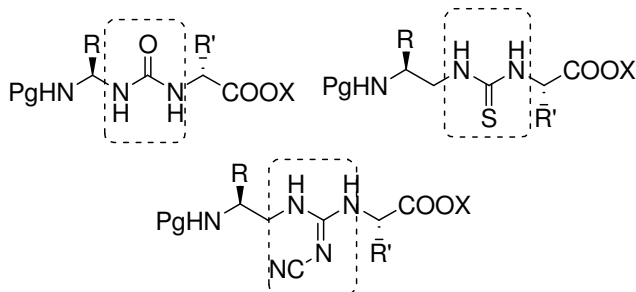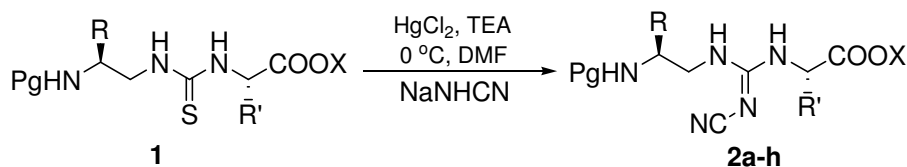

Figure 2

Experimental Section

Typical procedure for the preparation of cyanoguanidinopeptide mimics
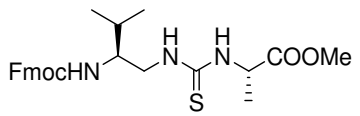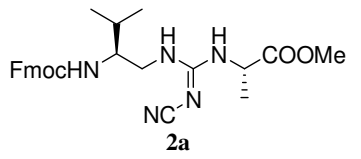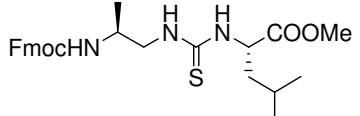
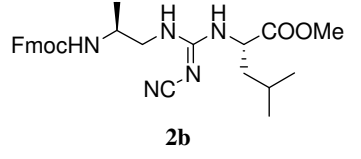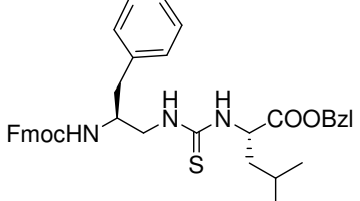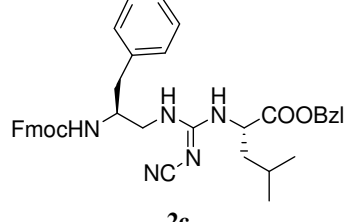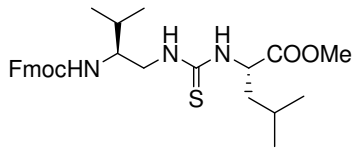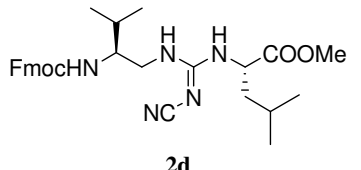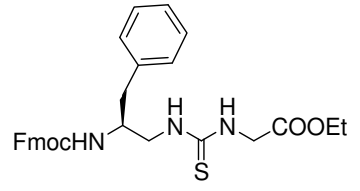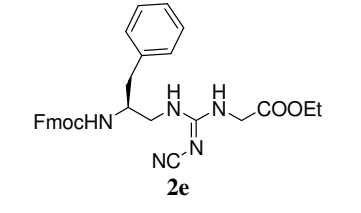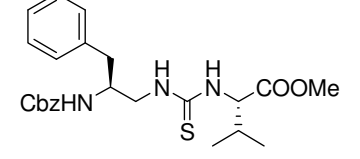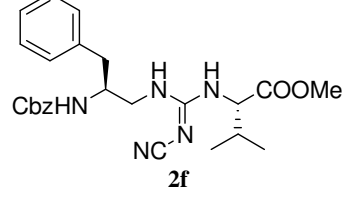To a solution of thioureidopeptide (1.0 mmol) and TEA (2.2 mmol) in DMF (5 mL) at 0°C was added HgCl$_2$ (1.1 mmol). The reaction mixture was stirred for 20 min and then a deprotonated solution of sodium cyanamide was added to the reaction mixture. The resultant mixture was then warmed to RT. When the reaction was completed as monitored through TLC, the reaction mixture was diluted with EtOAc (10 mL) and filtered through celite. The celite cake was then washed with additional EtOAc (5 mL). The filtrate was washed with water, then with brine and the organic layer was dried over anhydrous Na$_2$SO$_4$ and the solvent evaporated under reduced pressure. The crude product thus obtained was further purified on a silica gel column (EtOAc: *n*-hexane).

Characterisation data for compounds 2a-h

(*S*)-Methyl 2-((*E*)-3-((*S*)-2-(((9*H*-fluoren-9-yl)methoxy) carbonyl)-3-methylbutyl)-2-cyanoguanidino) propanoate, Fmoc-Val-ψ[CH$_2$NHC(NCN)NH]-Ala-OMe, 2a: Yield 74%. ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.96 (dd, *J* = 7.1 Hz, 6H), 1.41 (d, *J* = 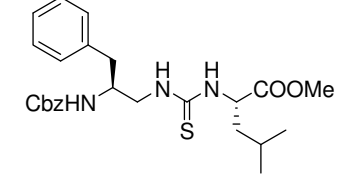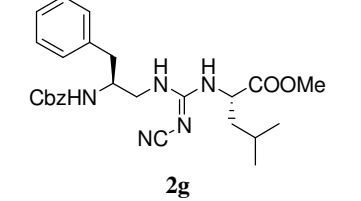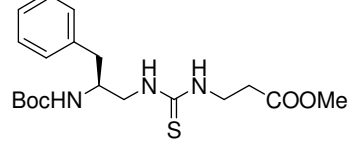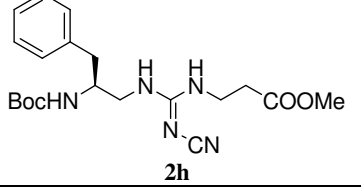7.2 Hz, 3H), 2.04-2.18 (m, 1H), 3.13 (m, 2H), 3.72 (s, 3H), 4.00-4.11 (m, 1H), 4.22 (t, *J* = 7.0 Hz, 1H), 4.26-4.50 (m, 2H), 4.50-4.66 (m, 1H), 5.44 (br s, 1H), 5.58 (d, *J* = 8.8 Hz, 1H), 6.56 (br s, 1H), 7.31 (m, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.1 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 16.8, 21.5, 29.9, 44.3, 46.8, 52.5, 59.5, 63.4, 65.7, 116.8, 126.6, 127.2, 127.8, 128.5, 141.2, 142.9, 154.2, 157.3, 170.6; HRMS: Calcd for C$_{26}$H$_{31}$N$_5$O$_4$: *m/z* 500.2274 (M$^+$+Na). Found: 500.2276.

(*S*)-Methyl 2-((*E*)-3-((*S*)-2-(((9*H*-fluoren-9-yl) methoxy)carbonyl)propyl)-2-cynoguanidino)-4-methylpentanoate, Fmoc-Ala-ψ[CH$_2$NHC(NCN)NH]-Leu-OMe, 2b: Yield 75%. ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.92 (dd, *J* = 6.8 Hz and *J* = 14.6 Hz, 6H), 1.30-1.32 (m,1H), 1.34 (d, *J* = 7.2 Hz, 3H), 1.61-1.64 (m, 2H), 3.08-3.12 (m, 2H), 3.73 (s, 3H), 3.84-4.00

Pg = Fmoc, Cbz or Boc group; **X** = Me, Bzl; **R** = **R'** = amino acid side chain.

Scheme I — Synthesis of N-protected cyanoguanidines tethered dipeptidomimetics **2a-h**

Table I — List of *N*-urethane protected cyanoguanidines tethered dipeptidomimetics **2a-h**

Entry	Reactant (1)	Product (2)	Yield (%)
1		**2a**	74
2		**2b**	75
3		**2c**	71
4		**2d**	70
5		**2e**	68
6		**2f**	67
7		**2g**	69
8		**2h**	63

(m, 1H), 4.50-4.64 (m, 3H), 5.10 (d, J = 7.9 Hz, 1H), 7.02-7.58 (m, 8H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 19.59, 19.91, 20.6, 31.1, 42.3, 44.5, 47.4, 52.4, 56.5, 62.6, 67.9, 116.6, 120.5, 125.4, 125.5, 127.6, 128.3, 136.4, 141.7, 143.9, 154.6, 156.2, 170.4; HRMS: Calcd for C$_{27}$H$_{33}$N$_5$O$_4$: m/z 514.2430 (M$^+$+Na). Found: 514.2428.

(*S*)-Benzyl-2-((*E*)-3-((*S*)-2-(((9*H*-fluoren-9-yl) methoxy)carbonyl)-3-phenylpropyl)-2-cyanoguanidino)-4-methylpentanoate, Fmoc-Phe-ψ[CH$_2$NHC(NCN) NH]-Leu-OBzl, 2c: Yield 71%. ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.82 (d, J = 5.4 Hz, 6H), 1.42 (t, J = 7.1 Hz, 2H), 1.49-1.63 (m, 1H), 2.51 (d, J = 3.9 Hz, 1H), 2.59 (d, J = 6.5 Hz, 1H), 2.65 (d, J = 6.2 Hz, 2H), 3.29 (t, J = 11.1 Hz, 1H), 3.89 (m, 1H), 4.36 (t, J = 4.9 Hz, 1H), 4.74 (d, J = 3.9 Hz, 2H), 5.22 (s, 2H), 6.01 (s, br, 1H), 6.39 (s, br, 1H), 6.92–7.75 (m, 18H and NH); ^{13}C NMR (75 MHz, CDCl$_3$): δ 22.8., 23.0, 34.3, 40.4, 42.2, 47.4, 48.0, 60.0, 65.3, 67.7, 117.5, 125.7, 126.4, 127.6, 128.2, 128.6, 128.9, 129.2, 129.8, 136.6, 139.7, 141.7, 143.1, 154.4, 156.2, 171.1; HRMS: Calcd for C$_{39}$H$_{41}$N$_5$O$_4$: m/z 666.3056 (M$^+$+Na). Found: 666.3058.

(*S*)-Methyl 2-((*E*)-3-((*S*)-2-(((9*H*-fluoren-9-yl) methoxy)carbonyl)-3-methylbutyl)-2-cyanoguani-dino)-4-methylpentanoate, Fmoc-Val-ψ[CH$_2$NHC (NCN)NH]-Leu-OMe, 2d: Yield 70%. ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.84-1.02 (m, 12H), 1.62-1.66 (m, 2H), 1.81-1.85 (m, 1H), 2.25-2.28 (m, 1H), 3.27-3.31 (m, 1H), 3.46-3.50 (m, 1H), 3.66-4.04 (m, 4H), 4.32 (d, J = 8.0 Hz, 2H), 4.34-4.37 (m, 1H), 4.70-4.74 (m, 1H), 5.17 (br s, 1H), 6.26 (br s, 1H), 7.26-7.50 (m, 8H), 7.54 (d, J = 4.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 16.9, 22.9, 25.6, 31.2, 40.4, 42.3, 47.5, 49.5, 52.3, 59.6, 66.8, 116.8, 126.6, 127.2, 127.8, 128.4, 141.2, 142.8, 155.5, 157.8, 170.5; HRMS: Calcd for C$_{29}$H$_{37}$N$_5$O$_4$: m/z 542.2743 (M$^+$+Na). Found: 542.2745.

(*S*,*E*)-Ethyl-2-(3-(2-(((9*H*-fluoren-9-yl)methoxy) carbonyl)-3-phenylpropyl)-2-cyanoguanidino)acetate, Fmoc-Phe-ψ[CH$_2$NHC(NCN)NH]-Gly-OEt, 2e: Yield 68%. ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.21 (t, J = 4.7 Hz, 3H), 2.59 (d, J = 3.2 Hz, 1H), 2.67 (d, J = 5.6 Hz, 1H), 2.75 (d, J = 5.7 Hz, 2H), 3.49 (s, 2H), 3.85 (m, 1H), 4.05 (m, 2H), 4.32 (t, J = 5.9 Hz, 1H), 4.65 (d, J = 3.1 Hz, 2H), 5.48 (s, br, 1H), 6.10 (s, br, 1H), 6.82 (s, br, 1H), 7.15–7.79 (m, 13H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 14.1, 38.4, 41.8, 47.6, 50.0, 60.2, 67.5, 116.5, 125.4, 127.0, 127.6, 128.2, 129.1, 129.6,

130.5, 130.9, 137.9, 141.8, 144.1, 153.9, 156.0, 170.1; HRMS: Calcd for C$_{30}$H$_{31}$N$_5$O$_4$: m/z 548.0785 (M$^+$+Na). Found: 548.0781.

(*S*)-Methyl 2-((*E*)-3-((*S*)-2-(benzyloxycarbonyl)-3-phenylpropyl)-2-cyanoguanidino)-3-methylbutanoate, Cbz-Phe-ψ[CH$_2$NHC(NCN)NH]-Val-OMe, 2f: Yield 67%. ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.94 (dd, J = 6.8 Hz, 6H), 1.38-1.42 (m, 1H), 3.10 (d, J = 4.8 Hz, 2H), 3.50-3.53 (m, 2H), 3.70 (s, 3H), 4.52 (t, J = 8Hz, 1H), 5.04 (s, 2H), 5.22 (br, 1H), 5.63 (q, J = 8.4 Hz, 1H), 6.92-7.31 (m, 10H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 17.2, 30.3, 34.05, 42.56, 50.78, 53.95, 57.61, 67.02, 117.1, 126.52, 128.12, 128.26, 128.69, 128.83, 129.07, 136.85, 137.79, 139.94, 154.8, 155.7, 171.2; HRMS: Calcd for C$_{25}$H$_{31}$N$_5$O$_4$: m/z 488.2274 (M$^+$+Na). Found: 488.2276.

(*S*)-Methyl 2-((*E*)-3-((*S*)-2-(benzyloxycarbonyl)-3-phenylpropyl)-2-cyanoguanidino)-4-methylpentanoate, Cbz-Phe-ψ[CH$_2$NHC(NCN)NH]-Leu-OMe, 2g: Yield 69%. ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.97 (d, J = 6.8 Hz, 6H), 1.41-1.43 (m, 2H), 1.89-1.91 (m, 1H), 2.77-2.79 (m, 2H), 3.10-3.12 (m, 1H), 3.26 (m, 1H), 3.72 (s, 3H), 4.35-4.38 (m, 1H), 4.90-4.92 (m, 1H), 5.05 (s, 2H), 6.07 (br, 1H), 6.70 (br, 1H), 7.12-7.22 (m, 10H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 18.6, 21.2, 36.3, 43.2, 45.1, 52.3, 56.7, 64.8, 67.9, 116.7, 127.1, 127.8, 128.2, 128.6, 129.4, 129.7, 137.2, 138.2, 155.4, 156.1, 170.1; HRMS: Calcd for C$_{26}$H$_{33}$N$_5$O$_4$: m/z 502.2430 (M$^+$+Na). Found: 502.2426.

(*S*,*E*)-Methyl 3-(3-(2-(tert-butoxycarbonyl)-3-phenylpropyl)-2-cyanoguanidino)propanoate, Boc-Phe-ψ[CH$_2$NHC(NCN)NH]-β-Ala-OMe, 2h: Yield 63%. ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.31 (s, 9H), 2.54 (d, J = 6.4 Hz, 2H), 2.81-2.85 (m, 2H), 3.56 (s, 3H), 3.71-3.73 (m, 4H), 3.98 (br, 1H), 5.44 (br, 1H), 6.77 (br, 2H), 7.01-7.36 (m, 5H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 22.3, 30.19, 32.57, 33.99, 39.41, 47.54, 52.37, 80.1, 116.9, 121.5, 128.2, 129.2, 137.5, 139.4, 155.6, 158.5, 171.6; HRMS: Calcd for C$_{20}$H$_{29}$N$_5$O$_4$: m/z 426.2117 (M$^+$+Na). Found: 426.2119.

Conclusion

In conclusion, herein we have described a simple and straight forward route for the synthesis of cyanoguanidine tethered dipeptidomimetics. The protocol is simple and uses thioureidopeptides as starting materials. HgCl$_2$ serves as an effective desulphurizing agent in affording the desired products in good yields. The protocol involves mild reaction

conditions amenable for amino acid chemistry. A simple work up followed by column chromatography yields the desired product with high purity.

Acknowledgement

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