Alkylation of Cyclic Amines with Arginine Modified Electrophiles

(SUPPORTING INFORMATION)

Mojmír Suchý1,2 and Robert H. E. Hudson1

1Department of Chemistry, The University of Western Ontario, London, ON, Canada, N6A 5B7
2Centre for Functional and Metabolic Mapping, Robarts Research Institute, The University of Western Ontario, London, ON, Canada, N6A 5K8
Contents

General Procedures ................................................................................................................................ S3

Experimental procedures for the preparation of compounds 9a, 9b, 10a, 10b, 11a and 11b ............ S4

\(^1\)H NMR spectra of compounds 7a, 7b, 9a, 9b, 10a, 10b, 11a and 11b ................................. S6

\(^13\)C NMR spectra of compounds 9a and 9b .................................................................................. S10

High resolution (ESI) mass spectra of compounds 7a, 7b, 10a, 10b, 11a and 11b ......................... S11
General experimental procedures:

Chemicals:
All amino acids (naturally occurring \( L \) isomers) and reagents were commercially available, unless otherwise stated. All solvents were HPLC grade and used as such, except for water (18.2 M\( \Omega \cdot \text{cm}^{-1} \) Millipore water). Organic extracts were dried with Na\(_2\)SO\(_4\) and solvents were removed under reduced pressure in a rotary evaporator. Aqueous solutions were lyophilized.

Chromatography:
Flash column chromatography (FCC) was carried out using silica gel, mesh size 230 – 400 Å. Thin layer chromatography (TLC) was carried out on Al backed silica gel plates, compounds were visualized by UV light or I\(_2\) vapors.

Instrumental methods:
HPLC analysis and purification was done using a Delta-Pak C18 300Å column (particle size 15\( \mu \)m; 8 × 100 mm Radial-Pak cartridge). Mobile phase: Method A: 90% H\(_2\)O/10% MeCN – 50% H\(_2\)O/50% MeCN over 9 min (compounds 7\( a \), 7\( b \), 11\( a \) and 11\( b \)); Method B: 90% H\(_2\)O/10% MeCN – 59% H\(_2\)O/41% MeCN over 7 min, fractions containing an impure material were concentrated and subjected to the purification using method Method C: 99% H\(_2\)O/1% MeCN – 89% H\(_2\)O/11% MeCN over 11 min (compounds 10\( a \) and 10\( b \)). Linear gradient and flow rate 3 ml/min was used in all the methods listed above. Ultra performance liquid chromatography (UPLC) was performed using a BEH C18 column (particle size 1.7\( \mu \)m; 2.1 id × 50 mm) and high resolution mass spectroscopy (HRMS) was performed with an electron spray ionization (ESI) source and time-of-flight TOF detector. Mobile phase: Method D: 100% H\(_2\)O – 25% H\(_2\)O/75% MeCN over 3 min, linear gradient, flow rate 0.25 ml/min. NMR spectra were recorded on 400 MHz spectrometer; for \(^1\)H (400 MHz), \( \delta \) values were referenced as follows DMSO-D\(_6\) (2.49 ppm) for \(^{13}\)C (100 MHz) DMSO-D\(_6\) (39.5 ppm).
Alkylation of piperidine (9) with N-chloroacetyl-Gly-Arg(NO$_2$)-OMe (15a) and N-chloroacetyl-Arg(NO$_2$)-OMe (17a)

K$_2$CO$_3$ (138 mg, 1 mmol) was added to separate solutions of piperidine (5, 49 μl, 0.5 mmol) in MeCN (2 ml). The electrophiles were added as follows: N-chloroacetyl-Gly-Arg(NO$_2$)-OMe (3a, 183 mg, 0.5 mmol) and N-chloroacetyl-Arg(NO$_2$)-OMe (4a, 155 mg, 0.5 mmol). The mixtures were stirred for 5 h at 60 °C. The solvent was evaporated and the mixtures were partitioned between water (30 ml) and EtOAc (4 × 20 ml). Combined organic extracts were dried and were concentrated, the residues were purified by FCC as follows: N-piperidyl-acetyl-Gly-Arg(NO$_2$)-OMe (9a), 25 g SiO$_2$, CH$_2$Cl$_2$/MeOH/NH$_4$OH – 80:20:1; N-piperidyl-acetyl-Arg(NO$_2$)-OMe (9b), 25 g SiO$_2$, hexanes/acetone – 1:2; Evaporation of eluates afforded the products as colorless oils.

N-piperidyl-acetyl-Gly-Arg(NO$_2$)-OMe (9a, 81 mg, 39%); $^1$H NMR (DMSO-D$_6$) δ 8.53 (br s, D$_2$O exch., 1H); 8.36 (br d, D$_2$O exch., J = 8 Hz, 2H); 7.86 (br s, D$_2$O exch., 2H); 4.28 (m, 1H); 3.81 (dd, $J_1$ = 17, 6 Hz, 1H); 3.76 (dd, $J_2$ = 17, 6 Hz 1H); 3.63 (s, 3H); 3.14 (m, 2H); 2.88 (m, 2H); 2.39 (m, 4H); 1.73 (m, 1H); 1.52 (m, 7H); 1.38 (m, 2H); $^{13}$C NMR (DMSO-D$_6$) δ 172.4, 169.8, 169.0, 159.3, 61.9, 54.2, 52.0, 51.6, 41.3, 28.3, 25.5, 23.5. HRMS (ESI) m/z: found 416.2274 [M+H]$^+$ (416.2258 calcd for C$_{16}$H$_{30}$N$_7$O$_6$).

N-piperidyl-acetyl-Arg(NO$_2$)-OMe (9b, 127 mg, 71%); $^1$H NMR (DMSO-D$_6$) δ 8.53 (br s, D$_2$O exch., 1H); 7.96 (br d, D$_2$O exch., J = 8 Hz, 2H); 7.84 (br s, D$_2$O exch., 1H); 4.32 (m, 1H); 3.63 (s, 3H); 3.14 (m, 2H); 2.93 (d, $J_1$ = 15.5, 1H); 2.86 (d, $J_2$ = 15, 1H); 2.38 (m, 4H); 1.73 (m, 2H); 1.52 (m, 6H); 1.38 (m, 2H); $^{13}$C NMR (DMSO-D$_6$) δ 172.3, 169.8, 159.3, 61.8, 54.1, 52.0, 51.1, 40.1, 29.6, 28.1, 25.5, 23.5. HRMS (ESI) m/z: found 359.2029 [M+H]$^+$ (359.2043 calcd for C$_{14}$H$_{27}$N$_6$O$_5$).

Alkylation of piperazine (6) with N-chloroacetyl-Gly-Arg(NO$_2$)-OMe (3a) and N-chloroacetyl-Arg(NO$_2$)-OMe (4a)

N-chloroacetyl-Gly-Arg(NO$_2$)-OMe (3a, 129 mg, 0.35 mmol) and N-chloroacetyl-Arg(NO$_2$)-OMe (4a, 109 mg, 0.35 mmol) were added to separate solutions of piperazine (6, 15 mg, 0.18 mmol) and DIPEA (61 μl, 0.35 mmol) in MeCN (1.8 ml) and DMF (200 μl). The mixtures were stirred for 24 h at 70 °C, MeCN was evaporated, the residues were dissolved in MeOH/water (1.5 ml each) and were subjected to semi-preparative HPLC purification. The fractions containing the products were concentrated to afford the products as colorless solids.

N,N’-piperazyl-diacetyl-Gly-Arg(NO$_2$)-OMe (10a, 57 mg, 44%); HPLC, Method B, $t_R$ 3.0 min followed by Method C, $t_R$ 13.0 min; $^1$H NMR (DMSO-D$_6$) δ 8.66 (br s, D$_2$O exch., 3H); 8.49 (br m, 3H); 7.97 (br
s, D$_2$O exch., 4H); 4.27 (m, 2H); 3.84 (m, 8H); 3.62 (s, 6H); 3.30 (m, 8H); 3.14 (m, 4H); 1.73 (m, 2H); 1.62 (m, 2H); 1.51 (m, 4H). HRMS (ESI) m/z: found 747.3467 [M+H]$^+$ (747.3498 calcd for C$_{26}$H$_{47}$N$_{14}$O$_{12}$).

$N,N'$-piperazyl-diacetyl-Arg(NO$_2$)-OMe (10b, 62 mg, 56%); HPLC, Method B, $t_R$ 3.1 min followed by Method C, $t_R$ 13.0 min; $^1$H NMR (DMSO-D$_6$) δ 8.66 (br s, D$_2$O exch., 2H); 8.52 (br s, 2H); 7.93 (br s, D$_2$O exch., 4H); 4.30 (m, 2H); 3.62 (s, 6H); 3.10 (m, 12H); 1.75 (m, 2H); 1.63 (m, 2H); 1.49 (m, 4H). HRMS (ESI) m/z: found 633.3095 [M+H]$^+$ (633.3069 calcd for C$_{22}$H$_{41}$N$_{12}$O$_{10}$).

### Alkylation of cyclam (8) with N-chloroacetyl-Gly-Arg(NO$_2$)-OMe (3a) and $N$-choloroacetyl-Arg(NO$_2$)-OMe (4a)

$N$-chloroacetyl-Gly-Arg(NO$_2$)-OMe (3a, 220 mg, 0.6 mmol) and $N$-chloroacetyl-Arg(NO$_2$)-OMe (4a, 186 mg, 0.6 mmol) were added to separate solutions of cyclam (8, 30 mg, 0.15 mmol) and DIPEA (105 μl, 0.6 mmol) in MeCN (1.8 ml) and DMF (200 μl). The mixtures were stirred for 24 h at 70 °C, MeCN was evaporated, the residues were dissolved in MeOH/water (1.5 ml each) and were subjected to semi-preparative HPLC purification. The fractions containing the trialkylated products were concentrated to afford the products as colorless solids.

Triacetyl-Gly-Arg(NO$_2$)-OMe cyclam (11a, 170 mg, 75%); HPLC, Method A, $t_R$ 4.8 min; $^1$H NMR (DMSO-D$_6$) 7.93-8.87 (br m, D$_2$O exch., 16H); 4.24 (m, 3H); 3.85 (m, 6H); 3.62 (s, 9H); 2.65-3.42 (br m, 32H); 1.51-1.75 (br m, 12H). HRMS (ESI) m/z: found 1191.5909 [M+H]$^+$ (1191.5943 calcd for C$_{43}$H$_{79}$N$_{22}$O$_{18}$).

Triacetyl-Arg(NO$_2$)-OMe cyclam (11b, 124 mg, 64%); HPLC, Method A, $t_R$ 5.0 min; $^1$H NMR (DMSO-D$_6$) 7.95-9.13 (br m, D$_2$O exch., 13H); 4.26 (m, 3H); 3.65 (s, 9H); 2.64-3.39 (br m, 32H); 1.52-1.79 (br m, 12H). HRMS (ESI) m/z: found 1020.5345 [M+H]$^+$ (1020.5299 calcd for C$_{37}$H$_{70}$N$_{19}$O$_{15}$).
Figure S1: $^1$H NMR spectrum (DMSO-d$_6$) of NOTAM-Gly-Arg(NO$_2$)-OMe (7a)

Figure S2: $^1$H NMR spectrum (DMSO-d$_6$) of NOTAM-Arg(NO$_2$)-OMe (7b)
Figure S3: $^1$H NMR spectrum (DMSO-d$_6$) of $N$-piperidyl-acetyl-Gly-Arg(NO$_2$)-OMe (9a)

Figure S4: $^1$H NMR spectrum (DMSO-d$_6$) of $N$-piperidyl-acetyl-Arg(NO$_2$)-OMe (9b)
Figure S5: $^1$H NMR spectrum (DMSO-$d_6$) of $N,N'$-piperazyl-diacetyl-Gly-Arg(NO$_2$)-OMe (10a)

Figure S6: $^1$H NMR spectrum (DMSO-$d_6$) of $N,N'$-piperazyl-diacetyl-Arg(NO$_2$)-OMe (10b)
Figure S7: $^1$H NMR spectrum (DMSO-$d_6$) of triacetyl-Gly-Arg(NO$_2$)-OMe cyclam (11a)

Figure S8: $^1$H NMR spectrum (DMSO-$d_6$) of triacetyl-Arg(NO$_2$)-OMe cyclam (11b)
**Figure S9:** $^{13}$C NMR spectrum (DMSO-d$_6$) of $N$-piperidyl-acetyl-Gly-Arg(NO$_2$)-OMe (9a)

**Figure S10:** $^{13}$C NMR spectrum (DMSO-d$_6$) of $N$-piperidyl-acetyl-Arg(NO$_2$)-OMe (9b)
Figure S11: HR-ESI-MS spectrum of NOTAM-Gly-Arg(NO₂)-OMe (7a, MW 1120.5) showing a charge state envelope (1120.5, M⁺ and 560.7, M²⁺)

Figure S12: Expanded view of M⁺ species in the HR-ESI-MS spectrum of NOTAM-Gly-Arg(NO₂)-OMe (7a, MW 1120.5) used for the elemental composition analysis
**Figure S13:** HR-ESI-MS spectrum of NOTAM-Arg(NO$_2$)-OMe (7b, MW 949.3) showing a charge state envelope (949.3, M$^+$ and 475.2, M$^{2+}$)

**Figure S14:** Expanded view of M$^+$ species in the HR-ESI-MS spectrum of NOTAM-Arg(NO$_2$)-OMe (7b, MW 949.4) used for the elemental composition analysis
Figure S15: HR-ESI-MS spectrum of \(N,N'\)-piperazyl-diacetyl-Gly-Arg(NO\(_2\))-OMe (10a, MW 747.3) showing a charge state envelope (747.3, \(M^+\) and 374.2, \(M^{2+}\))

Figure S16: Expanded view of \(M^+\) species in the HR-ESI-MS spectrum of \(N,N'\)-piperazyl-diacetyl-Gly-Arg(NO\(_2\))-OMe (10a, MW 747.3) used for the elemental composition analysis
Figure S17: HR-ESI-MS spectrum of $N,N'$-piperazyl-diacetyl-Arg(NO$_2$)-OMe (10b, MW 633.3) showing a charge state envelope (633.3, $M^+$ and 317.1, $M^{2+}$)

Figure S18: Expanded view of $M^+$ species in the HR-ESI-MS spectrum of $N,N'$-piperazyl-diacetyl-Arg(NO$_2$)-OMe (10b, MW 633.3) used for the elemental composition analysis
Figure S19: HR-ESI-MS spectrum of triacetyl-Gly-Arg(NO\textsubscript{2})-OMe cyclam (11a, MW 1191.6) showing a charge state envelope (1191.6, M\textsuperscript{+} and 596.8, M\textsuperscript{2+})

Figure S20: Expanded view of M\textsuperscript{+} species in the HR-ESI-MS spectrum of triacetyl-Gly-Arg(NO\textsubscript{2})-OMe cyclam (11a, MW 1191.6) used for the elemental composition analysis
**Figure S21:** HR-ESI-MS spectrum of triacetyl-Arg(NO$_2$)-OMe cyclam (11b, MW 1020.5) showing a charge state envelope (1020.5, M$^+$ and 510.7, M$^{2+}$).

**Figure S22:** Expanded view of M$^+$ species in the HR-ESI-MS spectrum of triacetyl-Gly-Arg(NO$_2$)-OMe cyclam (11b, MW 1020.5) used for the elemental composition analysis.