MgI_2-Chemoselective Cleavage for removal of amino acid protecting groups: a Fresh Vision for Peptide Synthesis.

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ABSTRACT:

In the field of peptide synthesis, the key to a successful access to synthetic targets lies on a pertinent combination of protecting groups. Their choice is directed by their selective removal conditions. We present here the behaviour of some of the most used protecting groups in peptide chemistry under experimental cleavage conditions, combining MgI_2 with MW irradiation, using 2-Me-THF as a green solvent. In these experimental conditions, the benzyloxycarbonyl protecting group as well as the Merrifield resin can be re-considered in peptide chemistry.

INTRODUCTION

Chemical strategies in peptide synthesis involve a combination of orthogonal protecting groups to successfully achieve the clean synthesis of various complex biomolecules, either in solution or on solid supports.[1] The major prerequisites for this success are both the chemoselective cleavage of protecting groups, the simplicity, efficacy, as well as the mild experimental conditions of their removal. Peptide synthesis requires manipulation of a wide variety of orthogonal protecting groups, principally to mask amino, carboxyl, hydroxyl, or thiol reactive groups. Mainly derived from solid phase peptide synthesis (SPPS), Fmoc/O*t*Bu and Boc/OBn are the the most recognized strategies.[1–3] Protecting groups are classified according to their nature, to the functional group they protect, and to their experimental removal conditions. We recently reported a MgI_2-assisted protocol, as an alternative to conventional deprotection methodologies, enlarging the orthogonal flexibility in protecting group strategies.[4] We have described an easy to handle procedure allowing (*i*) the selective removal of ethyl or methyl esters leaving unaffected Fmoc moieties, (*ii*) the selective removal of benzyl esters preserving benzyl ether moieties, and (*iii*) the successful release of peptides from Merrifield resins in mild conditions. Thereby, the commercially available MgI_2 proved to be an impressive tool to promote a chemoselective, racemization-free, eco-compatible, quantitative cleavage of various protecting groups.

Due to the large diversity of functional groups inherent to amino acid derivatives, we wished to study both the removal and stability of the commonly used protecting groups in peptide synthesis, to enlarge the chemical choice of amino acid protecting groups and as a consequence to synthetic strategies. In this context, we report in this study an efficient and simple MgI_2-assisted methodology combining, MW irradiation and a green solvent that might be useful to peptide chemists. The most significant results that were obtained rely on (*i*) the retention of a *S*-, a *N*-Trt or even a benzamide group, while removing a Boc or *t*-butyl ester moiety, (*ii*) the possibility of performing SPPS using the Cbz N-protecting group, (iii) the selective cleavage of a Boc protecting

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group preserving a silyl ether, (*iv*) the simple peptide cleavage from Merrifield resin keeping *N*-Tos and/or OcHx esters.

MATERIAL AND METHODS

List of abbreviations

2-MeTHF: tetrahydro-2-methylfuran

Ac: acetyl

Acm: acetaminomethyl

Bn: benzyl

Boc: *tert*-butoxycarbonyl

t-Bu: *tert*-butyl

Bz: benzamide

cHx: cyclohexyl

Cbz: benzyloxycarbonyl

DCM: dichloromethane

DIEA: diisopropylethyl amine

DMF: dimethyl formamide

Et_2O: diethyl ether

Fm: fluorenylmethyl

Fmoc: fluorenylmethyloxycarbonyl

For: formyl

HATU: dimethylamino)-N,N-dimethyl(3H-[1,2,3]triazolo[4,5-b] pyridin-3-yloxy) methaniminium hexafluorophosphate

HBTU: (1H-benzotriazol-1-yloxy) (dimethylamino)-N,N-dimethyl methaniminium hexafluorophosphate

MeOH: methanol

MW: microwave

Pbf: 2,2,4,6,7-pentamethyl dihydrobenzofuran-5-sulfonyl

Pht: phtaloyl

PG: protecting group

Pmc: 2,2,5,7,8-pentamethyl chromane-6-sulfonyl

SPPS : solid phase peptide synthesis

TBAF: tetra-*n*-butylammonium fluoride

TBDMS: *tert*-butyldimethyl silyl

TEA: trimethylamine

TFA: trifluoroacetic acid

TFMSA: trifluomethanesulfonic acid

TIS: triisopropylsilane

Tos: tosyl

Trt: trityl

X_{AA}: amino acid X

General

Solid supports (Sigma-Aldrich®, Bachem®), and suitable protected L-amino acids (IRIS Biotech®, Sigma-Aldrich®, Bachem®) were purchased from commercial sources and used without further purification as starting materials. Magnesium iodide was purchased from Sigma-Aldrich® with a 98% purity, except for solid phase peptide syntheses for which the monitoring by NMR required a 99.998% purity. All solvents were dried and freshly distilled before use.

Compound Analysis and Purification Using HPLC Systems

The synthesized compounds were purified by preparative HPLC and their purity assessed by LC-MS. For each HPLC system (analytical LC/MS or preparative), the separation of compounds was achieved by gradient elution using a linear system composed of 0.1% aqueous TFA (solvent A), acetonitrile containing 0.1% TFA (solvent B), and UV detection at 214 nm. Data are reported as retention time (r_t).

LC/MS was carried out on a Waters Alliance 2690 HPLC, coupled to a ZQ spectrometer (Manchester, UK) fitted with an electrospray source operated in the positive ionization mode (ESI+). All analyses were achieved on a C18 Chromolith Flash 25 x 4.6 mm column operated at a flow rate of $3mL.min^{-1}$. A gradient of 0 to 100% solvent B was developed over 3 min. Positive-ion electrospray mass spectra were acquired at a solvent flow rate

of 100-200 µL/min. Nitrogen was used for both the nebulizing and drying gas. The data were obtained in a scan mode ranging from 200 to 1700 m/z in 0.1 s intervals. A total of 10 scans were summed up to get the final spectrum.

RP-Preparative HPLC were performed on a PLC2020 Gilson® using a 75 x 21.2 mm Phenomenex® Luna 5µ C18(2) column. Compounds were separated using a linear gradient system (0 to 100% solvent B in 40 min) using a constant flow rate of 30 $mL.min^{-1}$ with the detector being fixed at 214 nm.

High Resolution Mass Spectra (HRMS). HRMS analyses were performed on a Micromass Q-Tof spectrometer equipped with electrospray source ionization (ESI), using phosphoric acid as an internal standard.

NMR analysis. NMR spectra were recorded at ambient temperature on a Bruker Avance AM-300 and/or AC-400 MHz spectrometer. Data are reported in *ppm* as: chemical shift (δ), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constants *J* in Hz, integration, and assignment.

General Procedure for Amino Acid Esterification

Trimethylsilyl chloride (5.00 eq) was added dropwise to a solution of PG-X_{AA}-OH (1.00 eq) in anhydrous alcohol (0.4 M) under argon atmosphere. After stirring at reflux or at room temperature (depending on the compound), the reaction mixture was concentrated *in vacuo*.

Reaction mixtures were magnetically stirred and monitored by thin layer chromatography using Merck-Kieselgel 60 F254 plates. Visualization was accomplished with UV light and exposure to a 10% solution of ninhydrin in ethanol followed by heating. When required, flash chromatography columns were performed on Isolera™ Four Biotage® using Biotage® Snap cartridges KP-Sil.

Fmoc-Asp(OcHx)-OMe

Fmoc-Asp(OcHx)-OMe was synthesized according to the general procedure from Fmoc-Asp(OcHx)-OH, in anhydrous methanol, for 15 min at room temperature. The crude material was concentrated *in vacuo* and the resulting residue was triturated in Et_2O affording Fmoc-Asp(OcHx)-OMe in 90% yield. ^{1}H NMR (DMSO, 400MHz) δ 1.16-1.46 (m, 6H), 1.61-1.75 (m, 4H), 2.66 (dd, 1H, *J* = 8.2 Hz, *J* = 16.1 Hz), 2.79 (dd, 1H, *J* = 5.9 Hz, *J* = 16.1 Hz), 3.63 (s, 3H), 4.21-4.24 (m, 1H), 4.31-4.33 (m, 2H), 4.45-4.50 (m, 1H), 4.64-4.70 (m, 1H), 7.32 (t, 2H, *J* = 7.4 Hz), 7.41 (t, 2H, *J* = 7.4 Hz), 7.69 (dd, 2H, *J* = 3.0 Hz, *J* = 7.3 Hz), 7.85-7.89 (m, 3H); ^{13}C NMR (DMSO, 100MHz) δ 23.03, 24.83, 30.90, 30.95, 36.22, 46.61, 50.47, 52.22, 65.76, 72.34, 120.14, 125.17, 127.06, 127.66, 140.77, 143.74, 143.77, 155.82, 169.17, 171.41; LC/MS r_t: 2.17; MS (ESI+): m/z 452.2 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{26}H_{29}NO_6 + H]^+$: 452.2073, found: 452.2074.

Fmoc-Cys(Acm)-OMe

Fmoc-Cys(Acm)-OMe was synthesized according to the general procedure from Fmoc-Cys(Acm)-OH, in anhydrous methanol, for 1h at room temperature. The crude material was concentrated *in vacuo* and the resulting residue was triturated in Et_2O affording Fmoc-Cys(Acm)-OMe in 95% yield.^{1}H NMR ($CDCl_3$, 400MHz) δ 1.85 (s, 3H), 2.84 (dd, 1H, *J* = 9.6 Hz, J = 13.7 Hz), 3.0 (dd, 1H, *J* = 5.0 Hz, *J* = 13.8 Hz) 3.64 (s, 3H), 4.17-4.26 (m,

3H), 4.29-4.32 (m, 3H), 7.32 (t, 2H, *J* = 7.3 Hz), 7.41 (t, 2H, *J* = 7.4 Hz), 7.72 (d, 2H, *J* = 7.4 Hz), 7.87-7.92 (m, 3H), 8.60 (t, 1H, *J* = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 100MHz) δ 22.58, 31.53, 40.32, 46.65, 52.14, 54.10, 65.83, 120.15, 125.30, 127.11, 127.69, 140.78, 143.79, 155.99, 169.55, 171.44; LC/MS r_t: 1.55; MS (ESI+): m/z 429.1 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{22}H_{24}N_2O_5S + H]^+$: 429.1484, found: 429.1487.

HCl.H-Cys(Fm)-OMe

HCl.H-Cys(Fm)-OMe was synthesized according to the general procedure from Boc-Cys(Fm)-OH, in anhydrous methanol, for 1h at room temperature. The crude material was concentrated *in vacuo* and the resulting residue was triturated in Et_2O affording HCl.H-Cys(Fm)-OMe in 78% yield. ^{1}H NMR (DMSO, 400Mz): δ 3.07 (dd, 2H, *J* = 2.3 Hz, *J* = 5.3 Hz), 3.25 (d, 2H, *J* = 6.1 Hz), 3.73 (s, 3H), 4.21 (t, 1H, *J* = 5.9 Hz), 4.30 (t, 1H, *J* = 5.5 Hz), 7.32-7.42 (m, 4H), 7.76 (dd, 2H, *J* = 3.1 Hz, *J* = 7.0 Hz), 7.87 (d, 2H, *J* = 7.4 Hz), 8.80 (s, 3H) ; ^{13}C NMR (DMSO, 100Mz): δ 32.83, 36.35, 46.85, 52.58, 53.37, 120.46, 125.47, 127.45, 127.96, 141.08, 146.07, 169.12; LC/MS r_t: 2,32; MS (ESI+): m/z 314.2 ($[M+H]^+$, HRMS (ESI+) m/z: Calcd for $[C_{18}H_{19}NO_2S + H]^+$: 314.1215, found: 314.1213.

Bz-Phe-OMe

Bz-Phe-OMe was synthesized according to the general procedure from Bz-Phe-OH, in refluxing anhydrous methanol for 30 min. The crude material was concentrated *in vacuo* and the resulting residue was triturated in Et_2O affording Bz-Phe-OMe in 93% yield. Spectral data were consistent with those previously reported in the literature.[5]

Bz-Phe-O*t*Bu

Bz-Phe-O*t*Bu was synthesized according to the general procedure described in the literature.[6] Spectral data obtained were consistent with those reported.

Boc-Ser(TBDMS)-OH

Boc-Ser(TBDMS)-OH was synthesized according to the general procedure described in the literature.[7] Spectral data were consistent with those previously reported.

Synthesis under MW irradiation

MW irradiation reactions were conducted with a Biotage® Initiator+. They were performed in 0.5-2 mL sealed reactors (Teflon septa and aluminium crimp) using a magnetic stirring bar. Temperature was measured with an IR sensor on the outer surface of the reactor. MW synthesizer operated at 2.45 GHz with continuous MW irradiation (0 to 400 W). The "fixed hold-time" parameter was activated, and the level of absorption was set to "very-high".

General Procedure for MgI_2-Cleavage of Protecting Groups under MW irradiation

Anhydrous 2-MeTHF (or THF, 2.0 mL) was added to the selected protected amino acid (0.12 mmol) and to MgI_2 (0.6 M) under argon atmosphere. The suspension in a sealed reactor, was heated at 120°C using MW irradiation, and stirred. A $Na_2S_2O_3$ aqueous solution (0.1 M) was then added, and the resulting homogeneous mixture was treated according to one of the following procedures:

(A) Directly purified by preparative HPLC;

(B) Extracted with EtOAc (x3). The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was diluted in H_2O:EtOH 5:1 and lyophilized.

All compounds were characterized by NMR, LC/MS, and HRMS. The NMR data fitted with those observed for authentic samples from commercial sources or with literature reports. In addition, isolated products were also shown to co-elute on LC/MS with authentic samples from commercial sources.

Fmoc-Asp(OcHx)-OH

Fmoc-Asp(OcHx)-OH was obtained from Fmoc-Asp(OcHx)-OMe according to the general procedure for 1h. Fmoc-Asp(OcHx)-OH was obtained following either workup A (94%) or workup B (97%). ^{1}H NMR (DMSO, 400MHz) δ 1.16-1.28 (m, 2H), 1.29-1.35 (m, 2H), 1.38-1.46 (m, 2H), 1.61-1.64 (m, 2H), 1.71-1.73 (m, 2H), 2.62 (dd, 1H, J = 8.4 Hz, J = 16.0 Hz), 2.78 (dd, 1H, J = 5.7 Hz, J = 16.0 Hz), 4.20-4.22 (m, 1H), 4.29-4.31 (m, 1H), 4.39 (td, 1H, J = 5.7 Hz, J = 8.4 Hz), 4.64-4.70 (m, 1H), 7.32 (t, 2H, J = 7.4 Hz), 7.41 (t, 2H, J = 7.5 Hz), 7.68-7.72 (m, 3H), 7.88 (d, 2H, J = 7.5 Hz); ^{13}C NMR (DMSO, 100MHz) δ 23.05, 24.87, 30.91, 30.99, 36.37, 46.63, 50.54, 65.76, 72.23, 120.16, 125.23, 127.09, 127.68, 140.76, 143.79; 143.82, 155.88, 169.45, 172.46, LC/MS r_t: 1.96; MS (ESI+): m/z 438.2 (%) ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{25}H_{27}NO_6 + H]^+$: 438.1917, found: 438.1919.

Fmoc-Cys(SS)-OH

Fmoc-Cys(SS)-OH was obtained from Fmoc-Cys(Trt)-OH according to the general procedure for 8h. Fmoc-Cys(SS)-OH was obtained following workup A (91%). ^{1}H NMR (DMSO, 400MHz) δ 2.94 (dd, 2H, J = 11.3 Hz, J = 12.1 Hz), 3.17-3.23 (m, 2H), 4.22-4.23 (m, 2H), 4.27-4.29 (m, 6H), 7.29-7.33 (m, 4H), 7.38-7.40 (m, 4H), 7.70 (d, 4H, J = 6.8 Hz), 7.76 (d, 2H, J = 8.1 Hz), 7.88 (d, 4H, J = 7.3 Hz); ^{13}C NMR (DMSO, 100MHz) δ 46.67, 53.10, 65.84, 120.19, 125.31, 127.18, 127.74, 140.78, 143.82, 156.10, 172.33; LC/MS r_t: 2.03; MS (ESI+): m/z 685.1 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{36}H_{32}N_2O_8S_2 + H]^+$: 685.1678, found: 685.1680. Spectral data were consistent with those previously reported in the literature.[8]

TFA.H-Cys(Trt)-OH

TFA.H-Cys(Trt)-OH was obtained from Boc-Cys(Trt)-OH according to the general procedure for 5 min. TFA.H-Cys(Trt)-OH was obtained following workup A (90%). ^{1}H NMR (DMSO, 400MHz) δ 2.52-2.63 (m, 2H), 3.66 (t, 1H, J = 5.9 Hz), 7.27-7.38 (m, 15H), 8.38 (b, 3H); ^{13}C NMR (DMSO, 100MHz) δ 31.56, 51.12, 66.55, 127.11, 128.29, 129.06, 143.65, 169.22 ; LC/MS r_t: 1.45; MS (ESI+): m/z 364.2 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{22}H_{21}NO_2S + Na]^+$: 386.1191, found: 386.1187.

Fmoc-His-OH

Fmoc-His-OH was obtained from Fmoc-His(Trt)-OH according to the general procedure for 2h30. Fmoc-His-OH was obtained following either workup A (93%) or B (91%). ^{1}H NMR (DMSO, 400MHz) δ 3.01 (dd, 1H, J = 10.1 Hz, J = 15.2 Hz), 3.16 (dd, 1H, J = 4.8 Hz, J = 15.1 Hz), 4.16-4.21 (m, 1H), 4.25-.27 (m, 2H), 4.29-4.37 (m, 1H), 7.28-.743 (m, 6H), 7.64 (d, 2H, J = 7.4 Hz), 7.82 (d, 1H, J = 8.4 Hz), 7.88 (d, 2H, J = 7.5 Hz), 8.99 (s, 1H); ^{13}C NMR (DMSO, 100MHz) δ 26.13, 46.61, 52.96, 65.72, 117.03, 120.18, 125.19, 127.12, 127.70, 129.68, 133.96, 140.75, 143.73, 156.00, 172.32; LC/MS r_t: 1.24; MS (ESI+): m/z 378.2 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{21}H_{19}N_3O_4 + H]^+$: 378.2341, found: 378.2342.

TFA.H-His(Trt)-OH

TFA.H-His(Trt)-OH was obtained from Boc-His(Trt)-OH according to the general procedure for 5 min. TFA.H-His(Trt)-OH was obtained following workup A (92%). ^{1}H NMR (THF d_8, 400MHz) δ 2.93 (dd, 1H, J = 8.0 Hz, J = 11.9 Hz), 3.14 (dd, 1H, J = 4.1 Hz, J = 12.0 Hz); 3.81-3.85 (m, 1H), 6.81 (b, 1H), 7.05-7.19 (m, 6H), 7.25-7.40 (m, 10H); ^{13}C NMR (THF d_8, 100MHz) δ 29.79, 56.29, 76.60, 121.10, 129.11, 130.79, 137.44, 139.62, 143.63, 173.36; LC/MS r_t: 1.25 ; MS (ESI+): m/z 398.2 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{25}H_{23}N_3O_2 + H]^+$: 398.1869, found: 398.1865.

Fmoc-Ser-OH

Fmoc-Ser-OH was obtained from Fmoc-Ser(Trt)-OMe according to the general procedure for 2h. Fmoc-Ser-OH was obtained following either workup A (95%) or B (94%). ^{1}H NMR (DMSO, 400MHz) δ 3.70 (d, 2H, J = 5.0 Hz), 4.07-4.12 (m, 1H), 4.21-4.25 (m, 1H), 4.30-4.32 (m, 1H), 7.33 (t, 2H, J = 7.4 Hz), 7.40-7.43 (m, 3H), 7.75 (dd, 2H, J = 7.3 Hz, J = 3.3 Hz), 7.88 (d, 2H, J = 7.5 Hz); ^{13}C NMR (DMSO, 100MHz) δ 46.71, 56.78, 61.45, 65.82, 120.19, 125.37, 127.18, 127.74, 140.81, 143.91, 156.18, 172.29; LC/MS r_t: 1.38; MS (ESI+): m/z 328.1 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{18}H_{17}NO_5 + H]^+$: 328.1185, found: 328.1186.

Bz-Phe-OH

Bz-Phe-OH was obtained from Bz-Phe-OMe according to the general procedure for 30 min. Bz-Phe-OH was obtained following either workup A (94%) or workup B (95%). ^{1}H NMR (DMSO, 400MHz) δ 3.10 (dd, 1H, J = 10.7 Hz, J = 13.7 Hz), 3.22 (dd, 1H, J = 4.4 Hz, J = 13.8 Hz), 4.63-4.69 (m, 1H), 7.16-7.20 (m, 1H), 7.25-7.29 (m, 2H), 7.33-7.34 (m, 2H), 7.42-7.46 (m, 2H), 7.50-7.54 (m, 1H), 7.80-7.82 (m, 2H), 8.72 (d, 1H, J = 8.2 Hz); ^{13}C NMR (DMSO, 100MHz) δ 36.34, 54.30, 126.43, 127.41, 128.26, 128.31, 129.13, 131.43, 133.99, 138.27, 166.48, 173.31; LC/MS r_t: 1.53; MS (ESI+): m/z 284.2 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{16}H_{15}NO_3 + H]^+$: 270.1130, found: 270.1129. Spectral data were consistent with those previously reported in the literature.[9]

Bz-Phe-OH was also obtained from Bz-Phe-O*t*Bu according to the general procedure for 30 min. The desired product was obtained following either workup A (98%) or workup B (95%). Data analyses were consistent with those reported above.

For-Phe-OH

For-Phe-OH was obtained from For-Phe-OMe according to the general procedure for 30 min. For-Phe-OH was obtained following workup A (97%). ^{1}H NMR (DMSO, 400MHz) δ 2.87 (dd, 1H, J = 9.2 Hz, J = 13.8 Hz), 3.08 (dd, 1H, J = 4.8 Hz, J = 13.9 Hz), 4.49-4.55 (m, 1H), 7.19-7.30 (m, 5H), 7.97 (s, 1H), 8.37 (d, 1H, J = 8.0 Hz); ^{13}C NMR (DMSO, 100MHz) δ 36.75, 52.00, 126.52, 128.23, 129.14, 137.32, 160.98, 172.58; LC/MS r_t: 0.96; MS (ESI+): m/z 194.1 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{10}H_{11}NO_3 + H]^+$: 194.1736, found:194.1735.

TFA.H-Cys(Fm)-OH

TFA.H-Cys(Fm)-OH was obtained from Boc-Cys(Fm)-OH according to the general procedure for 5 min. TFA.H-Cys(Fm)-OH was obtained following workup A (96%). ^{1}H NMR (DMSO, 400MHz) δ 3.43 (d, 2H, J = 5.0 Hz), 3.61-3.72 (m, 2H), 4.55 (t, 2H, J = 5.2 Hz), 4.65 (t, 2H, J = 5.8 Hz), 7.78 (d, 2H, J = 7.3 Hz), 7.85 (d, 2H, J = 7.3 Hz), 8.17 (t, 2H, J = 7.3 Hz), 8.31 (t, 2H, J = 7.4 Hz); ^{13}C NMR (DMSO, 100MHz) δ 33.10, 36.21, 46.66, 52.44, 120.32, 125.24, 127.38, 127.91, 140.89, 145.86, 169.88; LC/MS r_t: 1.28; MS (ESI+): m/z 300.1 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{17}H_{17}NO_2S + H]^+$: 300.1139, found: 300.1138.

Fmoc-Cys(Acm)-OH

Fmoc-Cys(Acm)-OH was obtained from Fmoc-Cys(Acm)-OMe according to the general procedure for 30 min. Fmoc-Cys(Acm)-OH was obtained following either workup A (93%) or workup B (95%). ^{1}H NMR (DMSO, 400MHz) δ 1.85 (s, 3H), 2.83 (dd, 1H, J = 9.9 Hz, J = 13.7 Hz), 3.04 (dd, 1H, J = 4.6 Hz, J = 13.7 Hz), 4.19-4.25 (m, 3H), 4.26-4.31 (m, 3H), 7.32 (t, 2H, J = 7.4 Hz), 7.41 (t, 2H, J = 7.5 Hz), 7.72-7.77 (m, 3H), 7.88 (d, 2H, J = 7.5 Hz), 8.52 (t, 1H, J = 6.3 Hz) ; ^{13}C NMR (DMSO, 100MHz) δ 22.63, 31.84, 46.68, 54.13, 65.83, 120.16, 125.35, 127.15, 127.72, 140.78, 143.85, 156.10, 169.54, 172.41; LC/MS r_t: 1.51; MS (ESI+): m/z 415.1 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{21}H_{22}N_2O_5S + H]^+$: 415.1328, found: 415.1327. Spectral data were consistent with those previously reported in the literature.[10]

General procedure to Monitor the MgI_2-Cleavage of Protecting Groups under MW irradiation by NMR

Deuterated-THF (1.0 mL) was added to the selected protected amino acid (0.06 mmol) and to MgI_2 (0.6 M) placed under argon atmosphere. The suspension was heated at 120°C using MW irradiation, and stirred. 100 µL of D_2O were then added at room temperature, and the homogeneous solution was directly analysed by ^{1}H NMR.

H-Trp(For)-OH

H-Trp(For)-OH was obtained from Boc-Trp(For)-OH according to the general procedure for 5 min. ^{1}H NMR (d_8 THF/D_2O 10/1, 300MHz) δ 1.64 (t, $^{1}/_{3}$ 6H, m, $H_{(C\underline{H}3)2,\ isobutene}$), 3.20 (dd, 1H, J = 10.7 Hz, J = 15.1 Hz, $H_{CHC\underline{H}2}$), 3.48 (dd, 1H, J = 2.6 Hz, J = 15.0 Hz, $H_{CHC\underline{H}2}$), 3.89 (dd, 1H, J = 2.8 Hz, J = 9.1 Hz, $H_{C\underline{H}CH2}$), 4.56-4.57 (m, $^{1}/_{3}$ 2H, $H_{C\underline{H}2,\ isobutene}$), 7.23-7.32 (m, 2H, $H_{\underline{Ar}}$), 7.77 (d, 1H, J = 5.8 Hz, $H_{\underline{Ar}}$), 8.05 (s, 1H, $H_{\underline{Ar}}$), 8.23 (d, 1H, J = 6.8 Hz, $H_{\underline{Ar}}$), 9.36 (s, 1H, $H_{C\underline{H}O}$); LC/MS r_t : 0.83; MS (ESI+): m/z 233.2 ($[M+H]^+$).

TFA.H-Cys(Fm)-OH

H-Cys(Fm)-OH was obtained from HCl.H-Cys(Fm)-OMe according to the general procedure for 30 min. ^{1}H NMR (d_8 THF/D_2O 10/1, 300MHz) δ 2.12 (s, 2H, H_{CH3I}), 3.00 (dd, 1H, J = 9.3 Hz, J = 13.9 Hz, H_{CH2}), 3.15 (dd, 1H, J = 4.2 Hz, J = 14.8 Hz, H_{CH2}), 3.22-3.27 (m, 2H, H_{CH2}), 3.73 (dd, 1H, J = 3.2 Hz, J = 9.1 Hz, H_{CH}), 4.14 (t, 1H, J = 6.2 Hz, H_{CH}), 7.23-7.32 (m, 4H, H_{Ar}), 7.70 (d, 2H, J = 6.9 Hz, H_{Ar}), 7.86 (d, 2H, J = 6.4 Hz, H_{Ar}); LC/MS r_t : 1.32; MS (ESI+): m/z 313.9 ($[M+H]^+$).

H-Ser(TBDMS)-OH

H-Ser(TBDMS)-OH was obtained from Boc-Ser(TBDMS)-OH according to the general procedure for 5 min. ^{1}H NMR (d_8 THF/D_2O 10/1, 300MHz) δ 0.08 (s, 6H, $H_{Si(CH3)2}$), 0.86 (s, 9H, $H_{C(CH3)3}$), 1.65 (t, $^1/_2$ 6H, J = 1.1 Hz, $H_{(CH3)2,\ isobutene}$), 3.64-3.67 (m, $^1/_2$ 2H, $H_{CH2,\ isobutene}$), 3.99-4.01 (m, 2H, H_{CH2CH}), 4.56-4.58 (m, 1H, H_{CHCH2}); LC/MS r_t : 1.15; MS (ESI+): m/z 219.8 ($[M+H]^+$).

Solid-Phase Peptide Synthesis

TFA.H-Arg(Tos)-Asp(OcHx)-Phe-OH

Boc-Phe-Merrifield resin (0.80 mmol/g, 1.00 eq) placed in a syringe shaped vessel was swollen in DCM for 5 min. The resin was then filtered, and a solution of 50% TFA in DCM was added. After shaking at room temperature for 30 min (x2), the resin was filtered and washed with DMF (x3), MeOH (x3), TEA (x3) and DCM (x3). To the resin was then added a mixture of Fmoc-Asp(OcHx)-OH (4.00 eq), HATU (4.00 eq) and DIEA (6.00 eq) in DMF (0.5 M). After shaking at room temperature for 15 min, the resin was filtered and washed with DMF (x3), MeOH (x3) and DCM (x3). The resin was then treated with a solution of 50% piperidine in DMF. After shaking for 1 min (x2), the resin was washed with DMF (x3), MeOH (x3) and DCM (x3). To the resin was then added a mixture of Boc-Arg(Tos)-OH (4.00 eq), HATU (4.00 eq) and DIEA (6.00 eq) in DMF (0.5 M). After shaking at room temperature for 15 min, the resin was filtered and washed with DMF (x3), MeOH (x3) and DCM (x3). The resin was then dried *in vacuo* for 2h. Anhydrous 2-MeTHF (0.06 M) was added to the dried protected peptide resin and to MgI_2 (10 eq), under argon atmosphere. The suspension was heated at 120°C using MW irradiation in a sealed reactor, and stirred at 430 rpm. After ~~heating for~~ 2h, the resin was filtered, and washed with DMF (x3), MeOH (x3) and DCM (x3). The filtrate was treated with a 0.1 M $Na_2S_2O_3$ solution and then concentrated in vacuo. The residue was diluted in H_2O:MeCN 3:1 and purified by preparative HPLC affording the desired peptide (92% overall yield). ^{1}H NMR (DMSO, 400MHz) δ 1.17-1.39 (m, 5H), 1.45-1.47 (m, 3H), 1.63-1.66 (m, 4H), 1.75-1.79 (m, 2H), 2.33 (s, 3H), 2.55-2.58 (m, 1H), 2.73 (dd, 1H, J = 4.9 Hz, J = 16.5 Hz), 2.90 (dd, 1H, J = 8.7 Hz, J = 13.9 Hz), 3.01-3.06 (m, 3H), 4.38-4.44 (m, 1H), 4.62-4.69 (m, 2H), 6.65-

6.79 (b, 1H), 6.94-7.07 (b, 1H), 7.17-7.24 (m, 4H), 7.25-7.29 (m, 3H), 7.65 (d, 2H, *J* = 8.1 Hz), 8.15 (b, 2H), 8.29 (d, 1H, *J* = 6.6 Hz), 8.68 (d, 1H, *J* = 7.6 Hz); ^{13}C NMR (DMSO, 100MHz) δ 20.96, 23.30, 24.92, 28.66, 31.09, 31.13, 36.43, 36.62, 49.55, 51.88, 53.77, 72.57, 125.74, 126.58, 128.30, 129.18, 137.41, 141.26, 141.63, 156.79, 168.48, 169.17, 170.03, 172.73; LC/MS r_t : 1.41; MS (ESI+): m/z 673.3 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{32}H_{44}N_6O_8S + H]^+$: 673.3020, found: 673.3022.

$(TFA)_2$.H-Lys-Phe-Ser(Bn)-NH_2

Fmoc-Rink amide resin (0.48 mmol/g, 1.00 eq) placed in a syringe shaped vessel was swollen in DCM for 5 min. The resin was then filtered and a solution of 50% piperidine in DMF was added. After shaking at room temperature for 1 min (x2), the resin was filtered and washed with DMF (x3), MeOH (x3) and DCM (x3). A mixture of Cbz-Ser(Bn)-OH (4.00 eq), HBTU reagent (4.00 eq) and DIEA (6.00 eq) in DMF (0.5 M) was then added to the resin. After shaking at room temperature for 15 min, the resin was filtered and washed with DMF (x3), MeOH (x3) and DCM (x3). The resin was then dried *in vacuo* for 2h. Anhydrous 2-MeTHF (0.06 M) was added to the dried protected peptide resin and to MgI_2 (10 eq), under argon atmosphere. The suspension was heated at 120°C using MW irradiation, in a sealed reactor, and stirred at 430 rpm for 4h. A $Na_2S_2O_3$ aqueous solution (0.1 M) was then added and the resulting mixture was filtered and washed with DMF (x3), MeOH (x3) and DCM (x3).

To the resin was then added a mixture of Cbz-Phe-OH (4.00 eq), HBTU reagent (4.00 eq) and DIEA (6.00 eq) in DMF (0.5 M). After shaking at room temperature for 15 min, the resin was filtered and washed with DMF (x3), MeOH (x3) and DCM (x3). The resin was then dried *in vacuo* for 2h. Anhydrous 2-MeTHF (0.06 M) was added to the dried protected peptide resin and to MgI_2 (10 eq), under argon atmosphere. The suspension was heated at 120°C using MW irradiation in a sealed reactor, and stirred at 430 rpm for 4h. A $Na_2S_2O_3$ aqueous solution (0.1 M) was then added and the resulting mixture was filtered and washed with DMF (x3), MeOH (x3) and DCM (x3).

To the resin was then added a mixture of Cbz-Lys(Boc)-OH (4.00 eq), HBTU reagent (4.00 eq) and DIEA (6.00 eq) in DMF (0.5 M). After shaking at room temperature for 15 min, the resin was filtered and washed with DMF (x3), MeOH (x3) and DCM (x3). The resin was then dried in vacuo for 2h. Anhydrous 2-MeTHF (0.06 M) was added to the dried protected peptide resin and to MgI_2 (10 eq), under argon atmosphere. The suspension was heated at 120°C using MW irradiation in a sealed reactor, and stirred at 430 rpm for 4h. A $Na_2S_2O_3$ aqueous solution (0.1 M) was then added and the resulting mixture was filtered and washed with DMF (x3), MeOH (x3) and DCM (x3).

The resin was then cleaved with a solution of 90% TFA, 5% TIS, and 5% H_2O. After shaking at room temperature for 30 min (x2), the resin was filtered, and washed with DCM (x3). The filtrate was concentrated *in vacuo* and the peptide was precipitated in cold Et_2O. The solid obtained was diluted in H_2O/MeCN and purified by preparative HPLC affording the desired peptide (90% overall yield). ^{1}H NMR (DMSO, 400 MHz) δ 1.21-1.36 (m, 2H), 1.48-1.55 (m, 3H), 1.61-1.71 (m, 1H), 2.71-2.75 (m, 2H), 2.82 (dd, 1H, *J* = 9.3 Hz, *J* = 14.0 Hz), 3.09 (dd, 1H, *J* = 4.3 Hz, *J* = 14.0 Hz), 3.67 (dd, 1H, *J* = 6.7 Hz, *J* = 10.5 Hz), 3.78 (dd, 1H, *J* = 3.5 Hz, *J* = 10.5 Hz), 3.98 (m, 1H), 4.14-4.20 (m, 1H), 4.52 (s, 2H), 4.60-4.66 (m, 1H), 7.07 (s, 1H), 7.18-7.24 (m, 1H), 7.25-7.31 (m, 5H), 7.32-7.38 (m, 5H), 8.20 (d, 1H, *J* = 8.0 Hz), 8.68 (d, 1H, *J* = 8.1 Hz); ^{13}C NMR (DMSO, 100MHz) δ 22.33, 26.79, 31.58, 37.46, 38.75, 52.40, 54.37, 68.66, 72.55, 126.56, 127.72, 128.26, 128.37, 129.32, 137.42, 137.63,

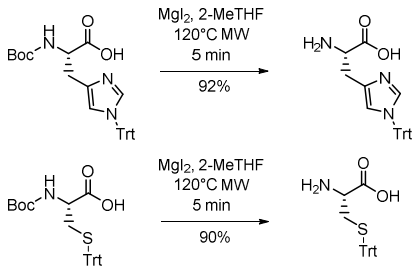
166.43, 170.38, 173.28; LC/MS r_t: 0.86; MS (ESI+): m/z 470.3 ($[M+H]^+$); HRMS (ESI+) m/z: Calcd for $[C_{25}H_{34}N_4O_5 + H]^+$: 470.2767, found: 470.2765.

RESULTS AND DISCUSSION

In this piece of work, we have studied both the removal selectivity and orthogonality of most of the usual amino acid protecting groups used in peptide synthesis. Carbamates including Boc, Fmoc, Cbz, have been developed to protect the amino group of amino acids, Fmoc being actually probably the most commonly used in solid phase peptide synthesis. However, due to the instability of the Fmoc group during conventional ester cleavage in basic conditions, the introduction of an ester group to protect carboxylic entities is underexploited, and yet so convenient. Indeed, a brief overview of the peptide literature evidences a cruel gap in the chemical description of *N*-Fmoc-protected amino acid esters (Fmoc-X_{AA}-OR). The recent development of the MgI_2-assisted methodology can now allow the selective removal of various esters in the presence of the Fmoc protecting group.[4] Thus, methyl, ethyl, *t*-butyl, allyl, and benzyl esters, can be quantitatively removed without affecting the Fmoc group, within reaction times ranging from 10 to 60 minutes.[4] We have also shown that hindered cyclohexyl esters[11,12] were not affected by the MgI_2-assisted methodology. The treatment of Fmoc-Asp(OcHx)-OMe by MgI_2 under MW irradiation for extended times, yielded Fmoc-Asp(OcHx)-OH (Table 1, entries 1-2).

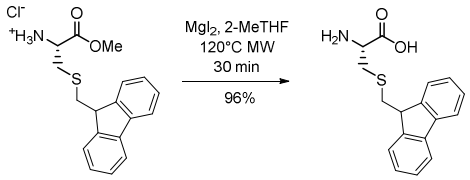
Special attention has been given to explore the scope and limitations of the MgI_2-protocol to specifically remove amino acid protecting groups usually employed in peptide synthesis. The idea was to evaluate the most common *N*- and *C*-PGs used in peptide synthesis, and to evaluate their removal/stability under the MgI_2-MW irradiation experimental conditions. The trityl group is a popular protecting group, which is generally removed in mild acidic conditions, except when it is used to protect the N^{τ}-Im of His or the thiol side-chain of Cys. Applying MgI_2-mediated cleavage experimental conditions, the chemosensitivity of the Trt PG was quite different (N^{α} > O > N-Im > S). We observed that α-amino-trityl protection[4] was more stable than trityl-ethers, N^{τ}-amino side-chain of His was more sensitive to MgI_2 deprotection than *S*-trityl-Cys (Table 1, entries 3-7). Due to the extreme instability of the Boc protecting group under MgI_2 conditions, we succeeded in the selective cleavage of Boc protecting groups, while keeping intact the *S*- and *N*-Trt moiety (Scheme 1 and table 1, entries 4 and 6). To the best of our knowledge, this chemoselectivity is unprecedented, since the selective cleavage of the trityl PG without affecting the Boc moiety was mainly reported, by using TFA in short reaction times.[13–15] We showed that by increasing the reaction time of MW irradiation, Fmoc-Cys(Trt)-OH, Fmoc-His(Trt)-OH and Fmoc-Ser(Trt)-OH, were converted into Fmoc-Cys(SS)-OH, Fmoc-His-OH and Fmoc-Ser-OH respectively, in good yields (Table 1, entries 3, 5 and 7), showing that the *N*-trityl of the imidazole of His, as well as the *O*-Trt of Ser, could be selectively removed in the presence of the *N*-Fmoc PG. While the *S*-Trt could be removed, it was followed by

formation of the disulphide bridge. Then, only Fmoc-Cys(SS)-OH was recovered the sulphydryl group being oxidized into disulphide by residual I_2.



Scheme 1. Selective cleavage of the Boc protecting group in the presence of a Trt moiety

To further explore the thiol functional group protections in Cys and Cys-containing peptides, in the MgI_2-mediated cleavage experimental conditions, we have studied the base-labile *S*-Fm,[16,17] and the *S*-Acm, a PG generally sensitive to iodine oxidation[18] being partially removed in acidic conditions.[1] Both *S*-Fm and *S*-Acm PGs were unaffected under the MgI_2-MW irradiation experimental conditions, allowing to selectively cleave a Boc or a methyl ester group (Scheme 2, Table 1 entries 14-18). However, treatment of HCl.H-Cys(Fm)-OMe by MgI_2 under MW irradiation for 30 minutes, afforded HCl.H-Cys(Fm)-OH (yield > 95%), along with an iodine-containing by-product (iodomethane), as revealed by ^{1}H NMR analysis of the crude reaction, according to the proposed mechanism of Subramanian et al.[19] The quantitative conversions constantly observed by LC/MS analyses, as well as the usual good yields obtained for the expected removal of protecting groups, support the efficiency, the comfort and the simplicity of the MgI_2-mediated cleavage method.



Scheme 2. Selective removal of the methyl ester from H-Cys(Fm)-OMe

A total, but perhaps less impressive chemoselectivity, concerned acyl moieties amine PGs, such as acetyl, formyl, benzoyl, or even phthaloyl. They remained unaffected by the MgI_2-MW protocols (Table 1, entries 8-13). Formamides[20,21] and benzamides[22] are sensitive to acidic environment (usually HCl 6N). MW conditions using MgI_2 allowed the selective removal of the *t*-butyl ester in the presence of a benzoyl group (Table 1, entry 9). The Boc group could also be removed from Boc-Trp(For)-OH, without affecting the N-in formyl moiety (Table 1, entry 13). It is well known that a *t*-butyl ester or a Boc N-protecting group, are more sensitive to acidic conditions than a N-benzoyl[23–26] or a N-formyl group.[27] The same occurred in the MgI_2-MW mediated cleavage. The formyl group is also base-labile,[28–30] and its orthogonality with methyl or ethyl esters is rarely described. MgI_2-mediated cleavage, allowing to

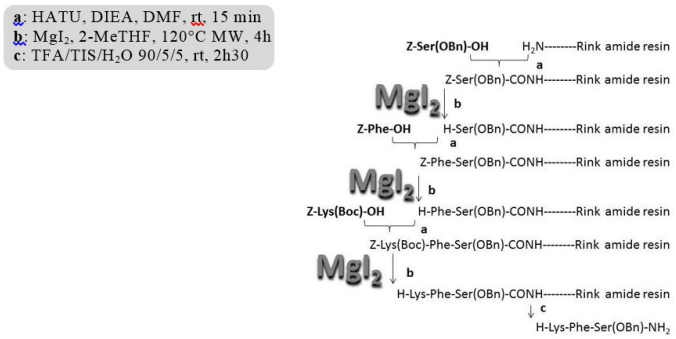
selectively remove methyl esters while keeping the N-formyl group is an interesting alternative (Table 1, entries 11-12).

Silyl ethers constitute a variety of PGs sometimes used in peptide synthesis to mask the hydroxyl group of Ser, Thr, and Tyr. The usual procedure to cleave the *O*-TBDMS ether involves either TBAF or acidic conditions, with an incompatibility of using concomitantly *N*-Boc or *t*-butyl ester PGs.[31,32] In that sense, the MgI_2-MW protocol could be an interesting method to selectively and efficiently cleave the Boc group within a short period of time (5 minutes), while preserving the *O*-TBDMS ether. The Fmoc PG remained repetitively not affected (Table 1, entries 21-22).

Regarding the most popular PGs *N*-Tos,[33,34] *N*-Pmc, or *N*-Pbf, engaged to mask the guanidine group of arginine, and usually removed in acidic conditions, they remained unaffected under MgI_2-MW experimental conditions (Table 1 entries 19-20, 23-24), offering, along with the cyclohexyl group, an orthogonal strategy for the solid phase peptide synthesis on the Merrifield resin (Table 1, entry 25).

We finally explored the challenging Cbz strategy in solid phase peptide synthesis, a strategy difficult to apply, due to the fastidious and non-automatable deprotection protocol involving hydrogenation conditions. Since the Rink amide resin remained unaffected under MgI_2-MW conditions, the model tripeptide H-Lys-Phe-Ser(Bn)-NH_2 was built by coupling Cbz-protected amino acids. The selective removal of Cbz PGs after each coupling step was performed using MgI_2-MW, followed by treatment in usual acidic conditions to release the peptide H-Lys-Phe-Ser(Bn)-$NH_2$from the Rink amide in a good yield (90%) (Scheme 3, and Table 1, entry 26). The strategy developed here allows the use of N-protected Cbz-amino acids in solid phase peptide synthesis, and represents an interesting alternative to the Fmoc strategy. On the other hand, from an eco-friendly point of view, this strategy avoids the use of the piperidine/DMF mixture, conventionally engaged in Fmoc deprotection strategies.

Another interesting application of the MgI_2-MW protocol concern the possibility of using polystyrene resins (Merrifield resins) in SPPS, by the Boc/Fmoc strategy. One of the drawback of using the Merrifield resin concerns the final release of the peptide from the resin, which usually needs strong acid treatments (Liquid HF, TFMSA). We prepared the model tripeptide H-Arg(Tos)-Asp(OcHx)-Phe-OH on the Merrifield resin, by the Boc/Fmoc strategy. The release of the peptide from the Merrifield resin was performed using the MgI_2-MW protocol, and the partially protected peptide H-Arg(Tos)-Asp(OcHx)-Phe-OH was obtained in 92% overall yield (Table 1, entry 25). This synthesis suggested that solid phase synthesis, including peptides, could be performed on the Merrifield support, with final removal of the peptide from the resin under gentle experimental conditions, within 2 hours, while some specific PGs, such as the cyclohexyl ester on Asp, could be preserved for eventual further fragment coupling.



Scheme 3. SPPS of H-Lys-Phe-Ser(Bn)-NH_2 using Cbz-amino acids

CONCLUSION

In this piece of work, we report an efficient, convenient, and automatable protocol for removal of PGs, which highlights easy to implement novel orthogonalities for known protecting groups used in peptide chemistry. In particular, the use of convenient N-Cbz protected amino acids can be considered, as well as the use of the Merrifield resin for the solid phase peptide synthesis. In addition, this simple protocol, using the harmless MgI_2 in 2-MeTHF under MW irradiation, allows not only alternative strategies in peptide synthesis, but also a more eco-friendly way to build peptides.

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Table 1. Cleavage of PGs with MgI_2 under MW irradiation in anhydrous 2-MeTHF at 120°C.

Entry	Substrate	Time (min)	Yield %[a]	Conversion (%)	Product
1	Fmoc-Asp(OcHx)-OMe	60	97	100	Fmoc-Asp(OcHx)-OH
2	Fmoc-Asp(OcHx)-OMe	300	95	100	Fmoc-Asp(OcHx)-OH
3	Fmoc-Cys(Trt)-OH	480	91	100	Fmoc-Cys(SS)-OH
4	Boc-Cys(Trt)-OH	5	90	100	H-Cys(Trt)-OH
5	Fmoc-His(Trt)-OH	150	93	100	Fmoc-His-OH
6	Boc-His(Trt)-OH	5	92	100	H-His(Trt)-OH
7	Fmoc-Ser(Trt)-OH	120	95	100	Fmoc-Ser-OH
8	Bz-Phe-OMe	30	95	100	Bz-Phe-OH
9	Bz-Phe-O*t*Bu	30	98	100	Bz-Phe-OH
10	Pht-Lys-OH	300	-	<10	H-Lys-OH
11	For-Phe-OMe	30	97	100	For-Phe-OH
12	For-Phe-OMe	300	96	100	For-Phe-OH
13	Boc-Trp(For)-OH	5	>95[b]	100	H-Trp(For)-OH
14	Boc-Cys(Fm)-OH	5	96	100	H-Cys(Fm)-OH
15	Boc-Cys(Fm)-OH	300	95	100	H-Cys(Fm)-OH
16	HCl.H-Cys(Fm)-OMe	30	> 95[b]	100	H-Cys(Fm)-OH
17	Fmoc-Cys(Acm)-OMe	30	95	100	Fmoc-Cys(Acm)-OH
18	Fmoc-Cys(Acm)-OMe	300	94	100	Fmoc-Cys(Acm)-OH
19	Fmoc-Arg(Tos)-OH	300	-	n.r[e]	Fmoc-Arg-OH
20	Tos-Arg-OH	300	-	n.r[e]	H-Arg-OH
21	Fmoc-Ser(TBDMS)-OH	300	-	< 10	Fmoc-Ser-OH
22	Boc-Ser(TBDMS)-OH	5	> 95[b]	100	H-Ser(TBDMS)-OH
23	Fmoc-Arg(Pmc)-OH	300	-	< 10	Fmoc-Arg-OH
24	Fmoc-Arg(Pbf)-OH	300	-	< 10	Fmoc-Arg-OH
25	Boc-Arg(Tos)-Asp(OcHx)-Phe-Merrifield resin	120[c]	92%[d]	-	H-Arg(Tos)-Asp(OcHx)-Phe-OH
26	Cbz-Lys(Boc)-Phe-Ser(Bn)-Rink amide resin	240[c]	90[d]	-	H-Lys-Phe-Ser(Bn)-NH_2

[a]Yields of isolated pure product; [b]Yields evaluated by ^{1}HNMR analysis; [c]Reaction time for MgI_2 deprotection steps; [d]Overall yield for isolated product synthesized from Boc-Phe-Merrifield resin (entry 25) or from Fmoc-Rink-Amide resin (entry 26); [e]No reaction.

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Table 1. Cleavage of PGs with MgI_2 under MW irradiation in anhydrous 2-MeTHF at 120°C.

Entry	Substrate	Time (min)	Yield %[a]	Conversion (%)	Product
1	Fmoc-Asp(OcHx)-OMe	60	97	100	Fmoc-Asp(OcHx)-OH
2	Fmoc-Asp(OcHx)-OMe	300	95	100	Fmoc-Asp(OcHx)-OH
3	Fmoc-Cys(Trt)-OH	480	91	100	Fmoc-Cys(SS)-OH
4	Boc-Cys(Trt)-OH	5	90	100	H-Cys(Trt)-OH
5	Fmoc-His(Trt)-OH	150	93	100	Fmoc-His-OH
6	Boc-His(Trt)-OH	5	92	100	H-His(Trt)-OH
7	Fmoc-Ser(Trt)-OH	120	95	100	Fmoc-Ser-OH
8	Bz-Phe-OMe	30	95	100	Bz-Phe-OH
9	Bz-Phe-O*t*Bu	30	98	100	Bz-Phe-OH
10	Pht-Lys-OH	300	-	<10	H-Lys-OH
11	For-Phe-OMe	30	97	100	For-Phe-OH
12	For-Phe-OMe	300	96	100	For-Phe-OH
13	Boc-Trp(For)-OH	5	>95[b]	100	H-Trp(For)-OH
14	Boc-Cys(Fm)-OH	5	96	100	H-Cys(Fm)-OH
15	Boc-Cys(Fm)-OH	300	95	100	H-Cys(Fm)-OH
16	HCl.H-Cys(Fm)-OMe	30	> 95[b]	100	H-Cys(Fm)-OH
17	Fmoc-Cys(Acm)-OMe	30	95	100	Fmoc-Cys(Acm)-OH
18	Fmoc-Cys(Acm)-OMe	300	94	100	Fmoc-Cys(Acm)-OH
19	Fmoc-Arg(Tos)-OH	300	-	n.r[e]	Fmoc-Arg-OH
20	Tos-Arg-OH	300	-	n.r[e]	H-Arg-OH
21	Fmoc-Ser(TBDMS)-OH	300	-	< 10	Fmoc-Ser-OH
22	Boc-Ser(TBDMS)-OH	5	> 95[b]	100	H-Ser(TBDMS)-OH
23	Fmoc-Arg(Pmc)-OH	300	-	< 10	Fmoc-Arg-OH
24	Fmoc-Arg(Pbf)-OH	300	-	< 10	Fmoc-Arg-OH
25	Boc-Arg(Tos)-Asp(OcHx)-Phe-Merrifield resin	120[c]	92%[d]	-	H-Arg(Tos)-Asp(OcHx)-Phe-OH
26	Cbz-Lys(Boc)-Phe-Ser(Bn)-Rink amide resin	240[c]	90[d]	-	H-Lys-Phe-Ser(Bn)-NH_2

[a]Yields of isolated pure product; [b]Yields evaluated by ^{1}HNMR analysis; [c]Reaction time for MgI_2 deprotection steps; [d]Overall yield for isolated product synthesized from Boc-Phe-Merrifield resin (entry 25) or from Fmoc-Rink-Amide resin (entry 26); [e]No reaction.