

Facile and Rapid Access to Linear and Truncated Microcystin Analogues for the Implementation of Immunoassays

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Supporting Information

ESI mass spectrum of **19** recorded in the positive mode.

ESI mass spectrum of free aminoxy tripod recorded in the positive mode.

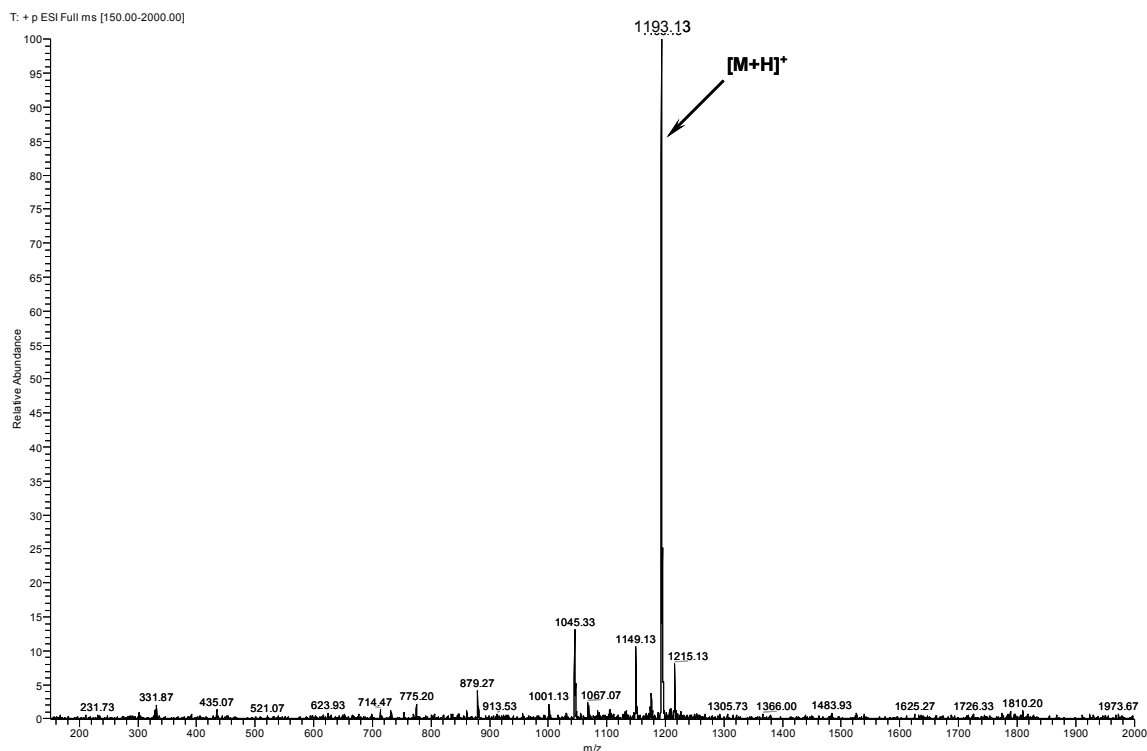
ESI mass spectrum of free aminoxy tripod recorded in the negative mode.

ESI mass spectrum of free sulfhydryl tripod recorded in the positive mode.

ESI mass spectrum of free sulfhydryl tripod recorded in the negative mode.

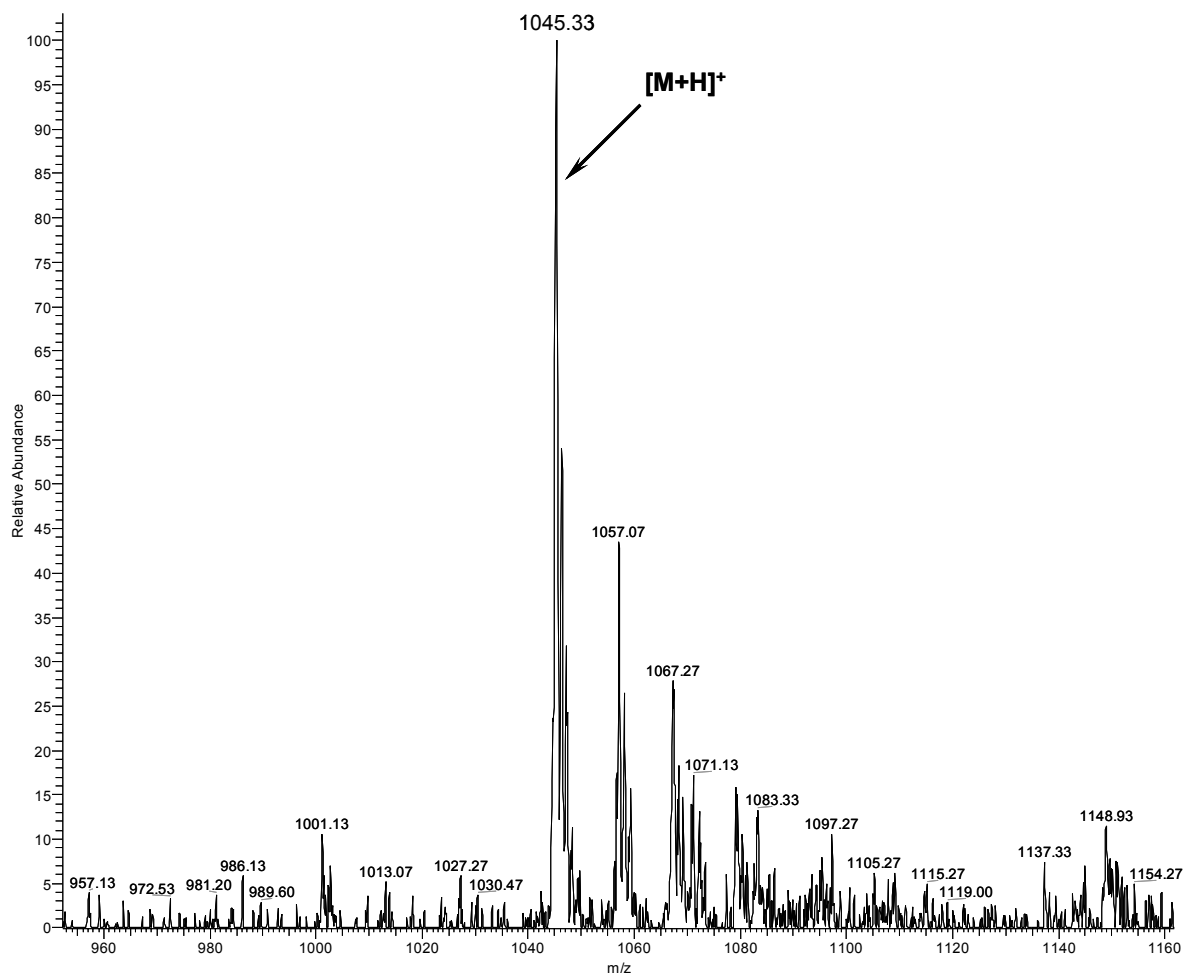
ESI mass spectrum of "microcystin tripod" **20** recorded in the positive mode.

ESI mass spectrum of **19** recorded in the positive mode.^a



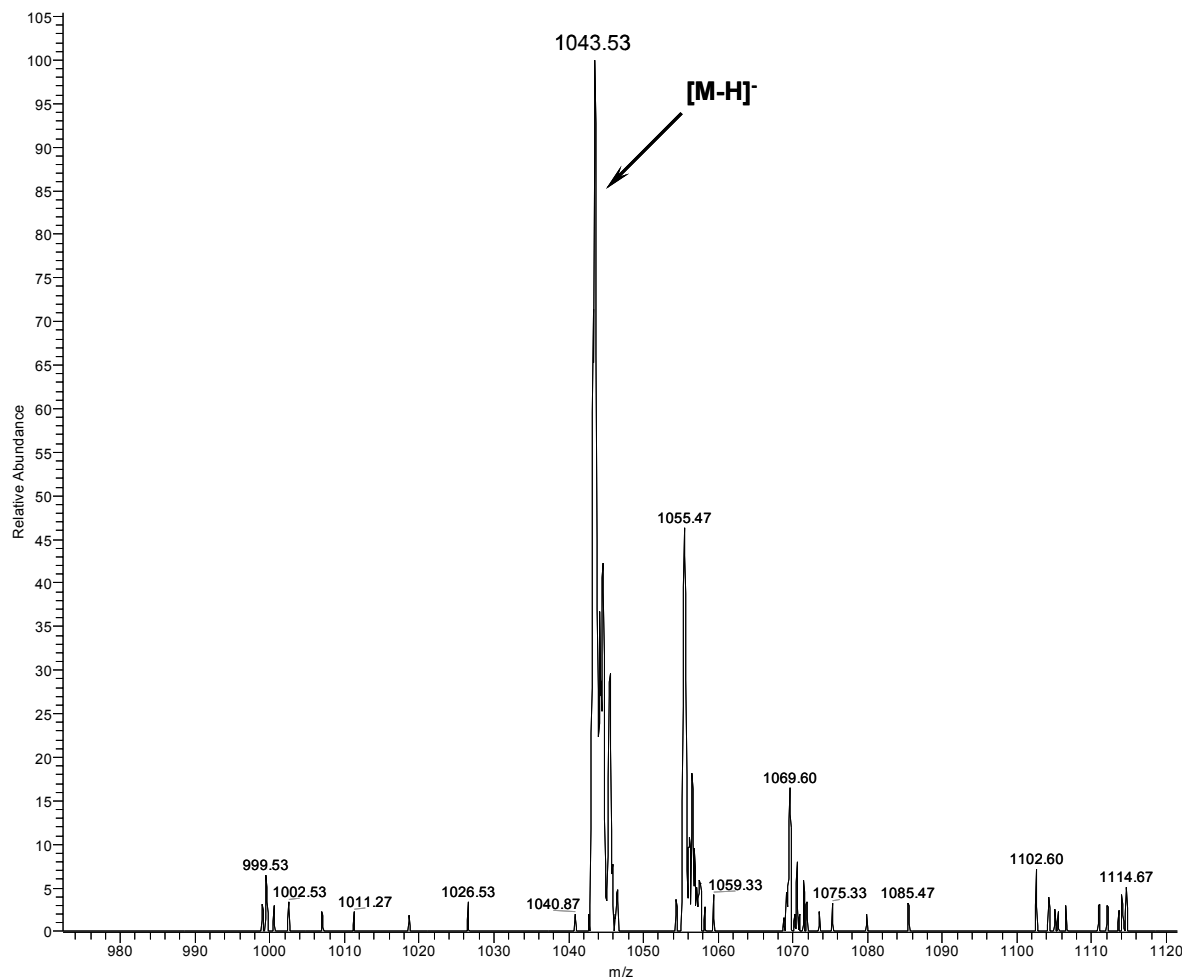
Under our ionisation conditions, the expected $[M + H]^+$ at $m/z = 1159.50$ was not observed. We suspected that oxidation of Adda side-chain occurs during the ESI analysis, especially hydroxylation of (C4-C5) or (C6-C7) double bond, probably mediated by reactive oxygen species (ROS) generated under discharge conditions (see Boys *et al.* (*Anal. Chem.*, 2009, **81**, 4027) for a comprehensive study about oxidative modifications during electrospray ionisation). Furthermore, hydration of phthalimide moiety also occurred (ΔM of +18 Da).

ESI mass spectrum of free aminoxy tripod recorded in the positive mode.^a



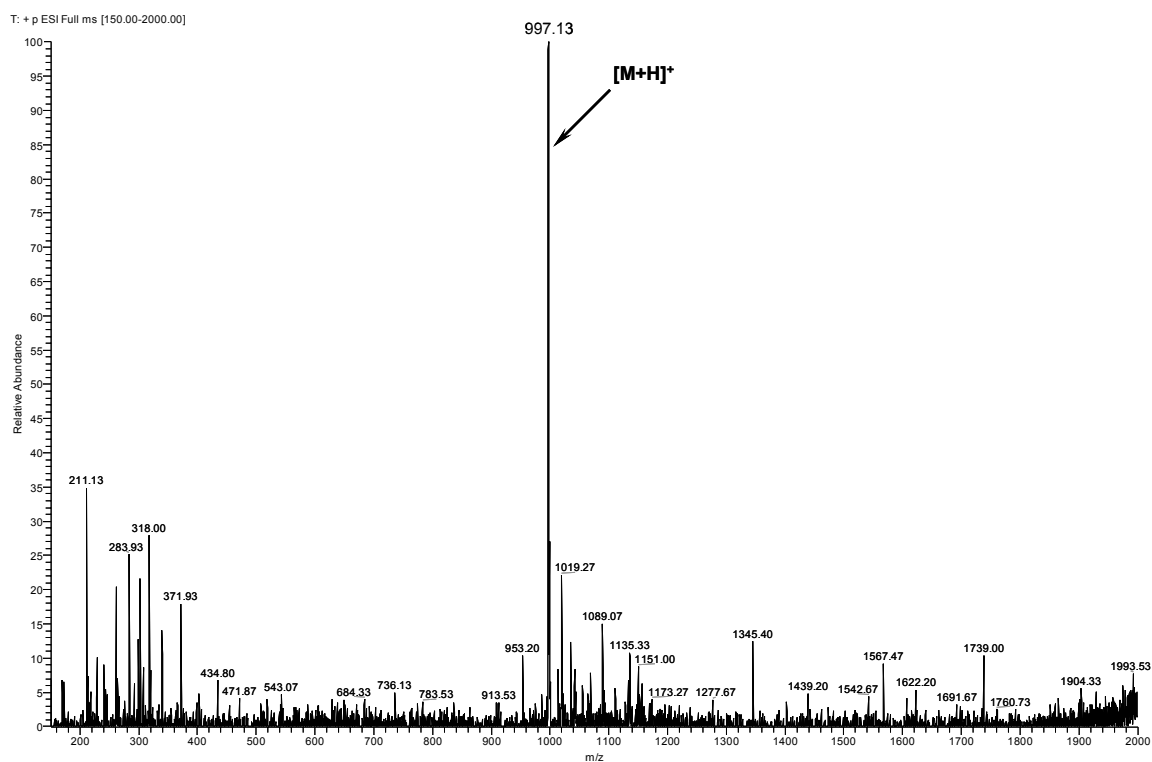
Under our ionisation conditions, the expected $[M + H]^+$ at $m/z = 1029.50$ was not observed. We suspected that oxidation of Adda side-chain occurs during the ESI analysis, especially hydroxylation of (C4-C5) or (C6-C7) double bond, probably mediated by reactive oxygen species (ROS) generated under discharge conditions (see Boys *et al.* (*Anal. Chem.*, 2009, **81**, 4027) for a comprehensive study about oxidative modifications during electrospray ionisation).

ESI mass spectrum of free aminoxy tripod recorded in the negative mode.^a



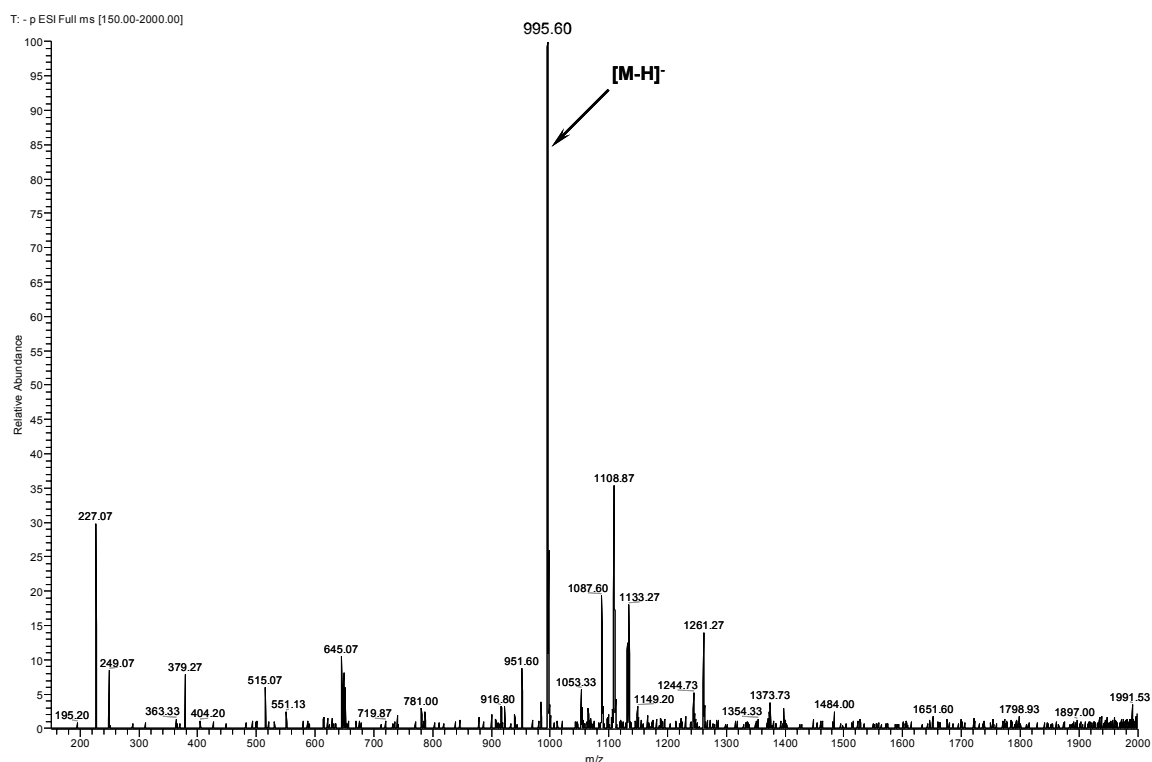
Under our ionisation conditions, the expected [M - H]⁻ at $m/z = 1027.48$ was not observed. We suspected that oxidation of Adda side-chain occurs during the ESI analysis, especially hydroxylation of (C4-C5) or (C6-C7) double bond, probably mediated by reactive oxygen species (ROS) generated under discharge conditions (see Boys *et al.* (*Anal. Chem.*, 2009, **81**, 4027) for a comprehensive study about oxidative modifications during electrospray ionisation).

ESI mass spectrum of free sulfhydryl tripod recorded in the positive mode.^a



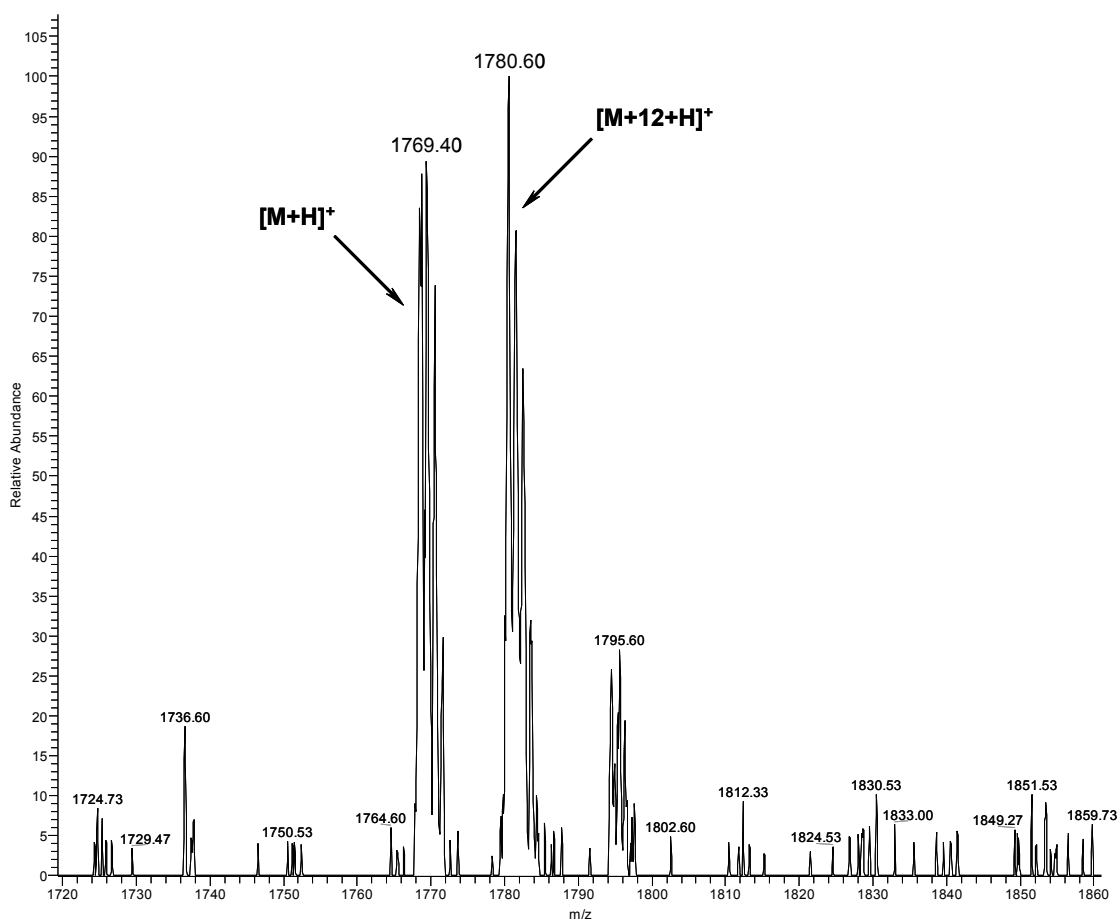
Under our ionisation conditions, the expected $[M + H]^+$ at $m/z = 969.49$ was not observed. We suspected that oxidation of Adda side-chain occurs during the ESI analysis, especially hydroxylation of (C4-C5) or (C6-C7) double bond, probably mediated by reactive oxygen species (ROS) generated under discharge conditions (see Boys *et al.* (*Anal. Chem.*, 2009, **81**, 4027) for a comprehensive study about oxidative modifications during electrospray ionisation). Furthermore, formation of an oxime (ΔM of +12 Da) between the aminoxy group and formaldehyde (generated from methanol under discharge conditions) was also observed. Such ligation reaction was also observed with the *S*Et derivative (see the corresponding mass spectrum: peak with $m/z = 1057.07$, *vide supra*).

ESI mass spectrum of free sulfhydryl tripod recorded in the negative mode.^a



Under our ionisation conditions, the expected $[M - H]^-$ at $m/z = 967.48$ was not observed. We suspected that oxidation of Adda side-chain occurs during the ESI analysis, especially hydroxylation of (C4-C5) or (C6-C7) double bond, probably mediated by reactive oxygen species (ROS) generated under discharge conditions (see Boys *et al.* (*Anal. Chem.*, 2009, 81, 4027) for a comprehensive study about oxidative modifications during electrospray ionisation). Furthermore, formation of an oxime (Δm of +12 Da) between the aminoxy group and formaldehyde (generated from methanol under discharge conditions) was also observed. Such ligation reaction was also observed with the *SEt* derivative (see the corresponding mass spectra: peak with $m/z = 1055.47$, *vide supra*).

ESI mass spectrum of "microcystin tripod" **20** recorded in the positive mode.^a



Under our ionisation conditions, the expected $[M + H]^+$ at $m/z = 1737.49$ was not observed. We suspected that oxidation of Adda side-chain occurs during the ESI analysis, especially hydroxylation of (C4-C5) or (C6-C7) double bond, probably mediated by reactive oxygen species (ROS) generated under discharge conditions (see Boys *et al.* (*Anal. Chem.*, 2009, **81**, 4027) for a comprehensive study about oxidative modifications during electrospray ionisation). Furthermore, hydration of maleimide moiety also occurred (ΔM of +18 Da). A second major peak at $m/z = 1780.60$ corresponding to the formaldehyde-oxime derivative was also observed (ΔM of +12 Da).