Supporting information

Post-SELEX modification of quinine aptamers through neoacetalization

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General experimental methods

Reagents and solvents were used as received from commercial suppliers. Oligonucleotide synthesis was performed with ÄKTA oligopilot plus 10 DNA/RNA synthesizer and mass spectra were recorded by Waters ACQUITY RDa mass spectrometer. NMR spectra (^{1}H) were recorded on Bruker Avance 500 MHz NMR spectrometer, and chemical shifts (δ) are reported as parts per million (ppm). The residual proton signal of the deuterated solvent was used as an internal standard.

Aptamer synthesis

Three modified aptamer scaffolds (**MN4-T19**, **MN4-C20** and **MN4-A21**) were synthesized with ÄKTA oligopilot plus 10 DNA/RNA synthesizer in 1.0 µmol scale on Ac-dC-CPG using standard DNA phosphoramidite building blocks and previously synthesized 4-(benzoyloxy)benzylidene protected (2*R*,3*S*)-4-(methoxyamino)butane-1,2,3-triol phosphoramidite building block (MOANA). In each aptamer, MOANA replaced different base (T19, C20 or A21) in the aptamer sequence. After synthesis, aptamer scaffolds were incubated in 25 % aqueous NH_3 for 4 h at 55 °C to cleave the solid support and the protecting groups.

MN4-T19, **MN4-C20** and **MN4-A21** were purified by RP-HPLC. Every modified aptamer scaffold was co-eluted with the corresponding scaffold without the MOANA. To facilitate the separation, **MN4-T19**, **MN4-C20** and **MN4-A21** were derivatized with a hydrophobic cyclohexanecarboxaldehyde. The amount of modified aptamer scaffolds was estimated to be 1 µmol. A 0.1 M solution of cyclohexanecarboxaldehyde in DMSO (100 µl, 10 µmol) was added to 1 ml of each aptamer solution with 25 µl of 2 M TEAA buffer (pH 5.5). The reaction mixtures were incubated at room temperature. After three days, UHPLC-MS analysis showed the completion of all three reactions. The reaction mixtures were neutralized with triethylamine, and the crude products were purified by RP-HPLC using a bioZen oligo LC column (2.6 µm, 150 x 4.6 mm) eluting with a linear gradient of 50 mM aqueous TEAA in ACN (pH 7.0) (5–25 % over 20 min) with a flow rate of 0.6 ml min^{-1}. The detection wavelength was 260 nm.

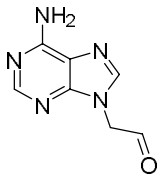
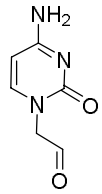
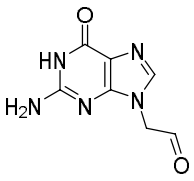
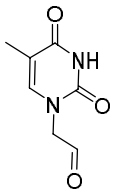
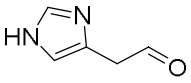
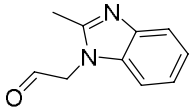
Derivatization of the aptamers with aldehydes

Each aldehyde was dissolved in DMSO to prepare 5 mM stock-solutions. These solutions (8 µl, 0.04 µmol) were combined to prepare different aldehyde mixtures (groups 1, 2, 3.1, 3.2 and 3.3, Table S1) and dissolved into 20 ml of 100 mM cacodylate buffer (pH 5.5). **MN4-T19** (1.89 µl, 52.75 µM) and 2 mM quinine sulfate dihydrate (10 µl, 20 nmol), both dissolved in Milli-Q water, were mixed with each 2 µM aldehyde mixture (100 µl) separately. Another set of reactions were prepared in the same way without quinine sulfate dihydrate. Reaction mixtures were kept in the dark at room temperature, allowing them to reach equilibrium. The progress of the DCC reactions was monitored at regular intervals by UHPLC-MS using an ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm) eluting with a linear gradient of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM) (5–25 % over 4 min) with a flow rate of 0.4 ml min^{-1}. The detection wavelength was 254 nm and the temperature of the column 60 °C. After two weeks, the relative intensities of the starting material and product peaks no longer changed. The same process was

repeated for both **MN4-C20** (1.57 µl, 63.5 µM) and **MN4-A21** (3.46 µl, 28.85 µM) in the presence and absence of quinine. The relative intensities of naked aptamers and their DCC products are listed in Table S2.

Table S1. Aldehydes and their abbreviations used in the derivatization of aptamers.

Group 1

2-(adenin-9-yl) acetaldehyde (**a**) 	2-(cytosin-1-yl) acetaldehyde (**b**) 	2-(guanin-9-yl) acetaldehyde (**c**) 
2-(thymin-1-yl) acetaldehyde (**d**) 	2-(imidazol-4-yl) acetaldehyde (**e**) 	2-(2-methylbenzimidazol-1-yl) acetaldehyde (**f**) 

Group 2

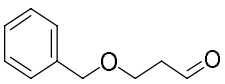
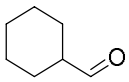
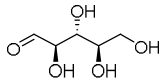
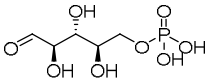
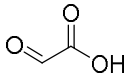
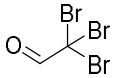
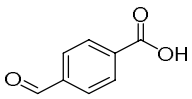
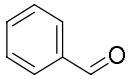
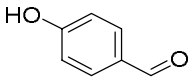
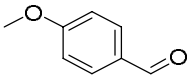
3-Benzyloxypropionaldehyde (**g**) 	Cyclohexanecarboxaldehyde (**h**) 	D-ribose (**i**) 
D-ribose-5-phosphate (**j**) 	Glyoxylic acid (**k**) 	Tribromoacetaldehyde (**l**) 

Table S2 (continued). Aldehydes and their abbreviations used in the derivatization of aptamers.

Group 3.1

4-Carboxybenzaldehyde (**m**) 	Benzaldehyde (**n**) 

Group 3.2

4-Hydroxybenzaldehyde (**o**) 	4-Methoxybenzaldehyde (**p**) 

Group 3.3

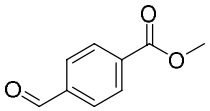
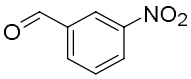
Methyl 4-formylbenzoate (**q**) 	3-Nitrobenzaldehyde (**r**) 

Table S2. Relative concentrations (%) of naked aptamers and their DCC products.

Group 1		Naked aptamer	Formaldehyde	Acetaldehyde	a	b	c	d	e	f	Quinine adduct
	MN4-T19	12.0	11.7	0.0	51.8	0.0	18.5	0.0	6.1	0.0	0.0
	MN4-T19, Quinine	13.5	11.9	0.0	49.1	0.0	12.7	0.0	5.0	0.0	7.8
	MN4-C20	0.0	6.1	0.0	72.7	0.0	15.4	0.0	5.9	0.0	0.0
	MN4-C20, Quinine	13.2	18.1	0.0	39.1	0.0	11.7	0.0	8.0	0.0	10.1
	MN4-A21	27.0	33.5	0.0	22.8	0.0	4.0	0.0	12.6	0.0	0.0
	MN4-A21, Quinine	33.7	49.6	0.0	8.4	0.0	2.9	0.0	5.4	0.0	0.0
Group 2		**Naked aptamer**	**Formaldehyde**	**Acetaldehyde**	**g**	**h**	**i**	**j**	**k**	**l**	**Quinine adduct**
	MN4-T19	36.7	16.5	9.9	36.9	0.0	0.0	0.0	0.0	0.0	0.0
	MN4-T19, Quinine	42.8	28.0	10.5	11.8	0.0	0.0	0.0	0.0	0.0	6.9
	MN4-C20	11.0	6.3	0.0	82.6	0.0	0.0	0.0	0.0	0.0	0.0
	MN4-C20, Quinine	38.1	26.7	8.5	15.9	0.0	0.0	0.0	0.0	0.0	10.8
	MN4-A21	54.7	23.2	13.5	8.5	0.0	0.0	0.0	0.0	0.0	0.0
	MN4-A21, Quinine	59.1	23.2	10.5	0.0	0.0	0.0	0.0	0.0	0.0	7.2

Group 3.1		Naked aptamer	Formaldehyde	Acetaldehyde	m	n	Quinine adduct	N-O cleavage product
	MN4-T19	73.8	13.6	12.7	0.0	0.0	0.0	0.0
	MN4-T19, Quinine	12.7	0.0	0.0	0.0	0.0	3.6	83.7
	MN4-C20	56.1	16.0	9.0	8.8	10.1	0.0	0.0
	MN4-C20, Quinine	54.0	20.5	8.1	0.0	0.0	12.1	5.3
	MN4-A21	75.9	15.8	8.4	0.0	0.0	0.0	0.0
	MN4-A21, Quinine	66.9	19.4	9.0	0.0	0.0	0.0	4.7
Group 3.2		**Naked aptamer**	**Formaldehyde**	**Acetaldehyde**	**o**	**p**	**Quinine adduct**	**N-O cleavage product**
	MN4-T19	61.5	20.0	13.5	3.1	0.0	0.0	1.9
	MN4-T19, Quinine	19.6	0.0	0.0	0.0	0.0	0.0	80.4
	MN4-C20	36.0	64.0	0.0	0.0	0.0	0.0	0.0
	MN4-C20, Quinine	44.0	28.6	10.7	0.0	0.0	9.9	6.8
	MN4-A21	63.3	17.8	14.8	4.0	0.0	0.0	0.0
	MN4-A21, Quinine	47.9	27.0	11.2	2.7	0.0	5.4	5.8
Group 3.3		**Naked aptamer**	**Formaldehyde**	**Acetaldehyde**	**q**	**r**	**Quinine adduct**	**N-O cleavage product**
	MN4-T19	42.8	0.0	0.0	16.4	40.8	0.0	0.0
	MN4-T19, Quinine	19.5	0.0	0.0	6.6	0.0	2.5	71.4
	MN4-C20	24.1	0.0	0.0	33.2	42.6	0.0	0.0
	MN4-C20, Quinine	23.8	16.1	0.0	27.6	13.7	10.9	7.8
	MN4-A21	60.8	16.7	14.7	3.5	4.4	0.0	0.0
	MN4-A21, Quinine	45.6	20.1	17.1	4.1	0.0	7.5	5.6

Isothermal titration calorimetry (ITC)

After identifying the dynamic combinatorial library formed by **MN4-C20**, methyl 4-formylbenzoate and 3-nitrobenzaldehyde as the most responsive to the presence of quinine, methyl 4-formylbenzoate (**MN4-C20q**) and 3-nitrobenzaldehyde (**MN4-C20r**) derivatized aptamer scaffolds were synthesized on a larger scale for isothermal titration calorimetry as described above.

However, due to multiple rounds of lyophilization after purification, methyl-4-formylbenzoate and 3-nitrobenzaldehyde were released from the aptamer scaffolds to some extent. Hence, derivatization of **MN4-C20** with these two aldehydes was repeated on the same samples. **MN4-C20q** in Milli-Q water (500 µl, 1.04 µmol) and 50 mM methyl 4-formylbenzoate in DMSO (210 µl, 10.5 µmol) were dissolved in 200 µl of 100 mM cacodylate buffer (pH 5.5). Similarly, **MN4-C20r** in Milli-Q water (500 µl, 1.13 µmol) and 50 mM 3-nitrobenzaldehyde in DMSO (225 µl, 11.3 µmol) were dissolved in 200 µl of 100 mM cacodylate buffer (pH 5.5). After the completion of the reactions was confirmed by UHPLC-MS, they were neutralized with 10 µl of triethylamine and extracted with DCM (2 ml) to remove the excess of aldehydes. Aptamers were left in neutralized reaction solutions to prevent the release of aldehydes.

For ITC studies, 0.906 mM **MN4-C20q** in Milli-Q water:DMSO:TEA:100 mM cacodylate buffer (pH 5.5) (54:23:1:22, *v*:*v*:*v*:*v*) (80 µl) was diluted with 20 mM Tris•HCl, 140 mM NaCl, 5 mM KCl, (pH 7.4) buffer to make a 207 µM solution. This sample was titrated with 2.6 mM quinine in 20 mM Tris•HCl, 140 mM NaCl, 5 mM KCl, (pH 7.4) buffer with the same percentage of Milli-Q water, DMSO, TEA and 100 mM cacodylate buffer (pH 5.5) as in titrate. Similarly, 1.01 mM **MN4-C20r** in Milli-Q water:DMSO:TEA:100 mM cacodylate buffer (pH 5.5) (53:24:1:21, *v*:*v*:*v*:*v*) (71 µl) was diluted with 20 mM Tris•HCl, 140 mM NaCl, 5 mM KCl, (pH 7.4) buffer to make a 204 µM solution. This sample was titrated with 2.6 mM quinine in 20 mM Tris•HCl, 140 mM NaCl, 5 mM KCl, (pH 7.4) buffer with the same percentage of Milli-Q water, DMSO, TEA and 100 mM cacodylate buffer (pH 5.5) as in the titrate.

ITC experiments were performed by MicroCal iTC200 (Malvern Panalytical Ltd.) instrument. The titrations were conducted at 25 °C, and every experiment consisted of 32 consecutive injections. The titrant was injected every 300 s, the first injection being 0.5 µl and the others 2.4 µl. In every experiment, the reference power was 11 µCal/s, initial delay 60 s and stirring speed 1000 rpm. Control measurements were performed by titrating quinine into 20 mM Tris•HCl, 140 mM NaCl, 5 mM KCl, (pH 7.4) buffer. In control measurements, the same percentage of Milli-Q water, DMSO, TEA and 100 mM cacodylate buffer (pH 5.5) were added both to the titrant and the titrate as

used in the main experiments. The heat of dilution of the titrant was subtracted from raw data, and the first injection (0.5 µl) was removed from each dataset. Data were fitted to a one-site binding model using Origin 7.0 software.

The binding of naked **MN4-C20** to quinine was also investigated by ITC. The **MN4-C20** (150 µM) was titrated with 3 mM quinine in 20 mM Tris•HCl, 140 mM NaCl, 5 mM KCl, (pH 7.4) buffer at 20 °C, and experiment consisted of 34 consecutive injections. The titrant was injected every 300 seconds, with the first injection being 1 µl, the next nine injections 4 µl each, and the remaining injections 6.5 µl each. The reference power, initial delay and stirring speed were same as described above. The control measurement was performed by titrating quinine into the buffer. However, only weak binding was observed, and the thermodynamic parameters for **MN4-C20** could not be determined.

UV thermal melting studies

MN4-C20q (0.91 µl, 0.906 mM) and **MN4-C20r** (0.80 µl,1.01 mM) were diluted separately with 1 ml of 20 mM Tris•HCl, 140 mM NaCl, 5 mM KCl, (pH 7.4) buffer. Melting temperatures of the aptamers were determined by a PerkinElmer Lambda 35 UV/Vis spectrophotometer with a Peltier temperature control unit. The UV melting curves were obtained by three heating and cooling cycles between 10 °C and 90 °C, at the rate of 0.5 °C/min. Absorbance was recorded at 0.5 °C intervals at 260 nm. Biphasic melting curves were obtained for both aptamers, with melting temperatures for **MN4-C20q** 17.4 ± 1.0 °C and 54.4 ± 0.2 °C, and for **MN4-C20r** 14.1 ± 0.6 °C and 55.2 ± 0.1 °C.

Polyacrylamide gel electrophoresis (PAGE)

MN4-C20q and **MN4-C20r** were analyzed with varying quinine concentrations (0 µM, 4 µM, 200 µM, 500 µM and 1 mM) by PAGE. Samples were prepared by mixing 5 µl of 0.6 µM **MN4-C20q** or **MN4-C20r** in 20 mM Tris•HCl, 140 mM NaCl, 5 mM KCl, (pH 7.4) buffer with 3 µl acrylamide (TBE) sample buffer. A commercial 10 % TBE gel (Thermo Fisher Scientific) was loaded into an electrophoresis chamber, and it was filled with 90 mM tris, 90 mM borate, and 2 mM EDTA (pH 8.3) buffer. The electrophoresis was performed with 200 V (45 mA) for 15 min, after which the gel was stained by SYBR gold nucleic acid gel stain (Thermo Fisher Scientific).

N-O bond cleavage

The cleavage of the N-O bond was investigated in the presence of quinine but without the aldehydes. **MN4-T19** (1.89 µl, 52.75 µM) and 2 mM quinine sulfate dihydrate (10 µl, 20 nmol) in Milli-Q water

were dissolved in 100 µl of 100 mM cacodylate buffer (pH 5.5). Reaction mixture was kept in the dark at room temperature and the progress of the N-O cleavage was monitored by UHPLC-MS using an ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm) eluting with a linear gradient of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM) (5–25 % over 4 min) with a flow rate of 0.4 ml min^{-1}. The detection wavelength was 254 nm and the temperature of the column 60 °C.

After the cleavage of N-O bond was detected, the reaction mixture containing decomposed **MN4-T19** in the presence quinine, was neutralized with 200 mM cacodylate buffer (pH 7.4) and 1 µl of nuclease P_1 was added to the mixture. The reaction mixture was kept at room temperature. After one day, monomeric nucleotides along with a trimer consisting of phosphodiester-linked (2*R*,3*S*)-4-aminobutane-1,2,3-triol, thymidine and deoxycytidine were found by UHPLC-MS analysis (XBridge C18 column (5 µm, 4.6 x 30 mm); linear gradient of 0.1 % formic acid in MeOH (5–100 % over 2.2 min); flow rate of 0.8 ml min^{-1}; λ = 254 nm).

The methoxy group cleavage was investigated also by NMR. (2*R*,3*S*)-4-(methoxyamino)butane-1,2,3-triol (12 mg, 79 µmol) and quinine sulfate dihydrate (93 mg, 0.48 mmol) were incubated in deuterated acetate buffer (pH 5.5) at the room temperature. For the reference, (2*R*,3*S*)-4-(methoxyamino)butane-1,2,3-triol was also incubated in deuterated acetate buffer (pH 5.5) without the quinine. The progress of the reactions was monitored by NMR at regular intervals (Figures S80 and S81). Even after two weeks, no change in NMR spectrum was observed.

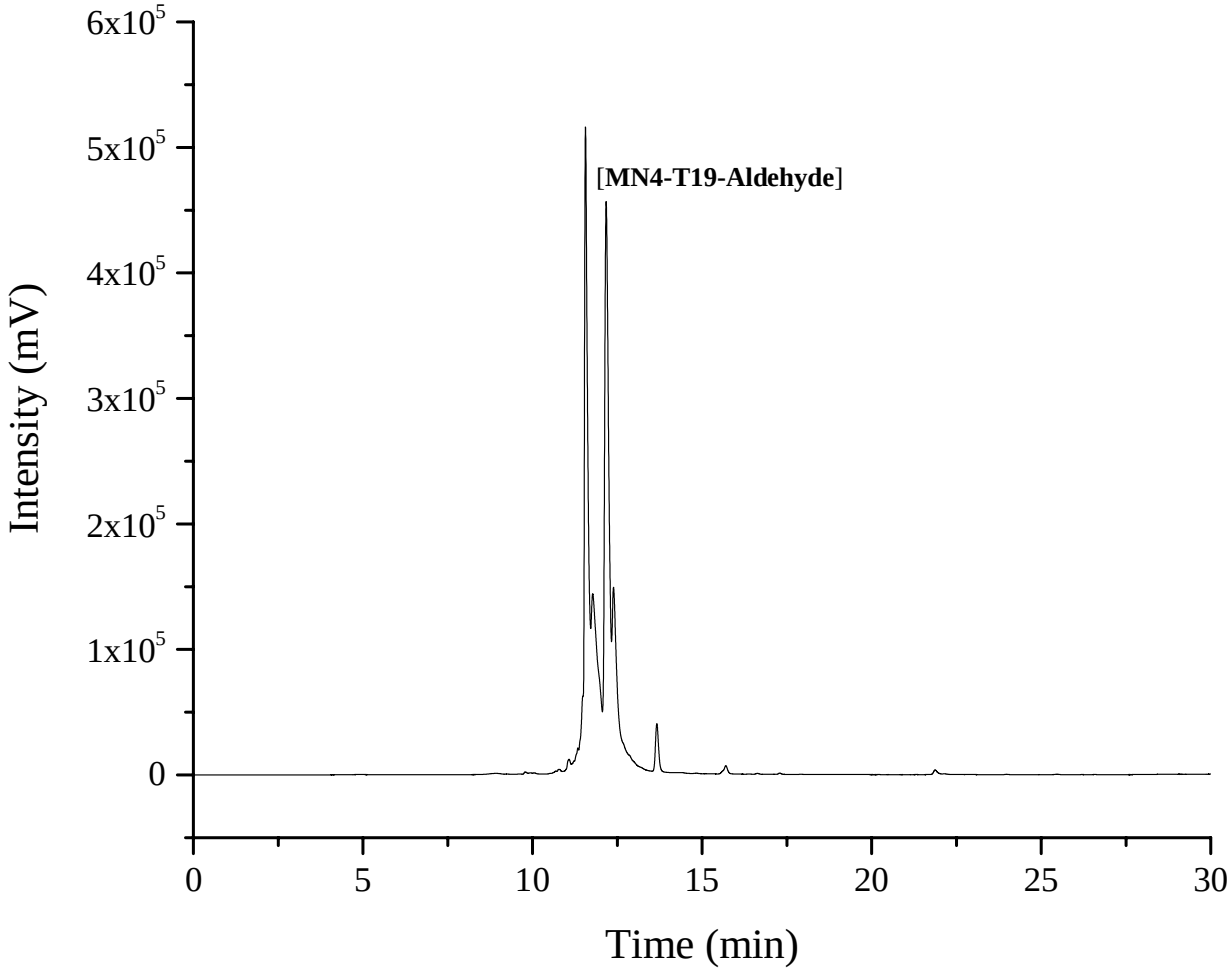


Figure S1. RP-HPLC traces of crude **MN4-T19** derivatized with cyclohexanecarboxaldehyde; bioZen oligo LC column (2.6 µm, 150 x 4.6 mm); flow rate = 0.6 ml min^{-}1; linear gradient (5-25 % over 20 min) of 50 mM aqueous TEAA in ACN (pH 7.0); λ = 260 nm.

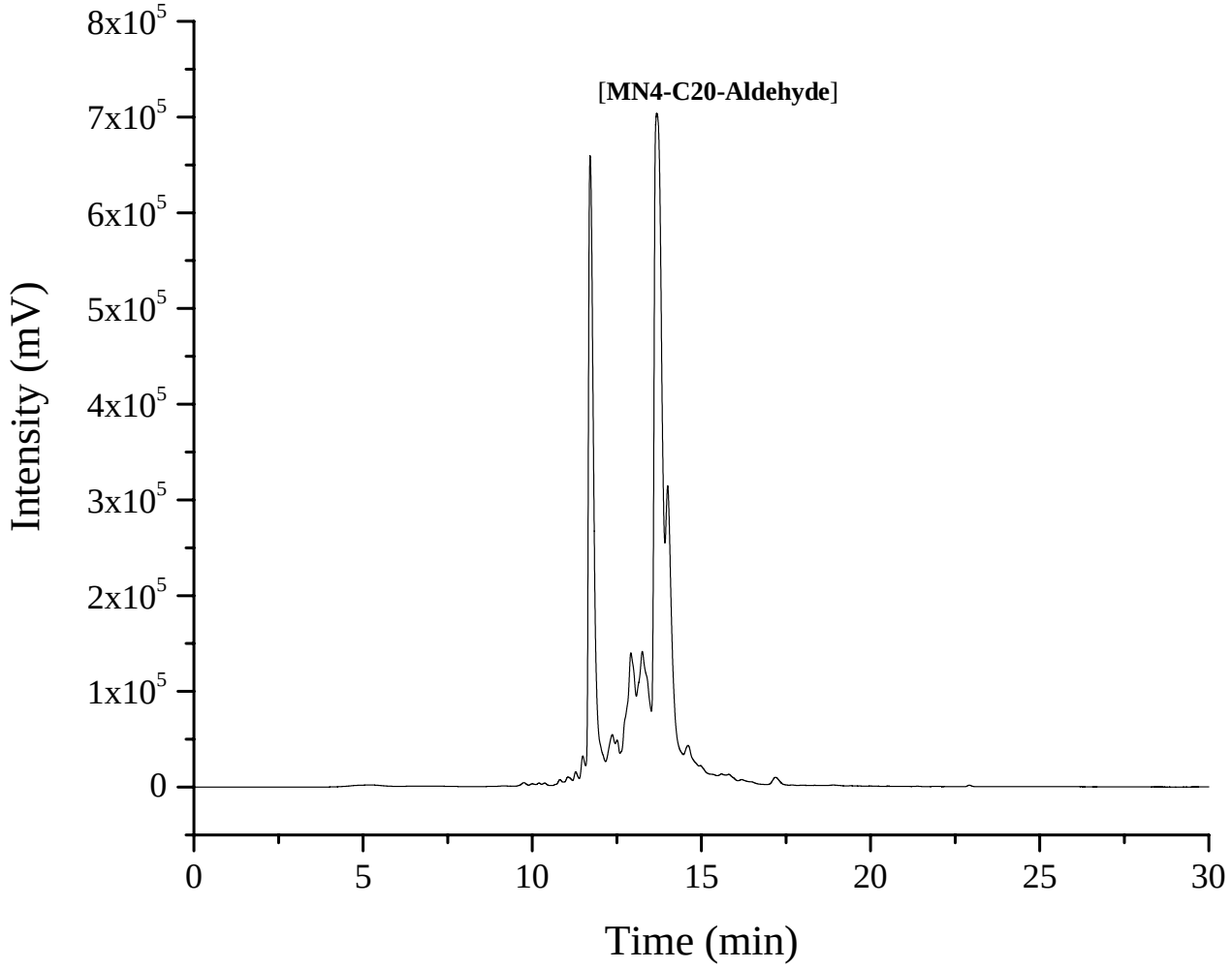


Figure S2. RP-HPLC traces of crude **MN4-C20** derivatized with cyclohexanecarboxaldehyde; bioZen oligo LC column (2.6 µm, 150 x 4.6 mm); flow rate = 0.6 ml min^{-}1; linear gradient (5-25 % over 20 min) of 50 mM aqueous TEAA in ACN (pH 7.0); λ = 260 nm.

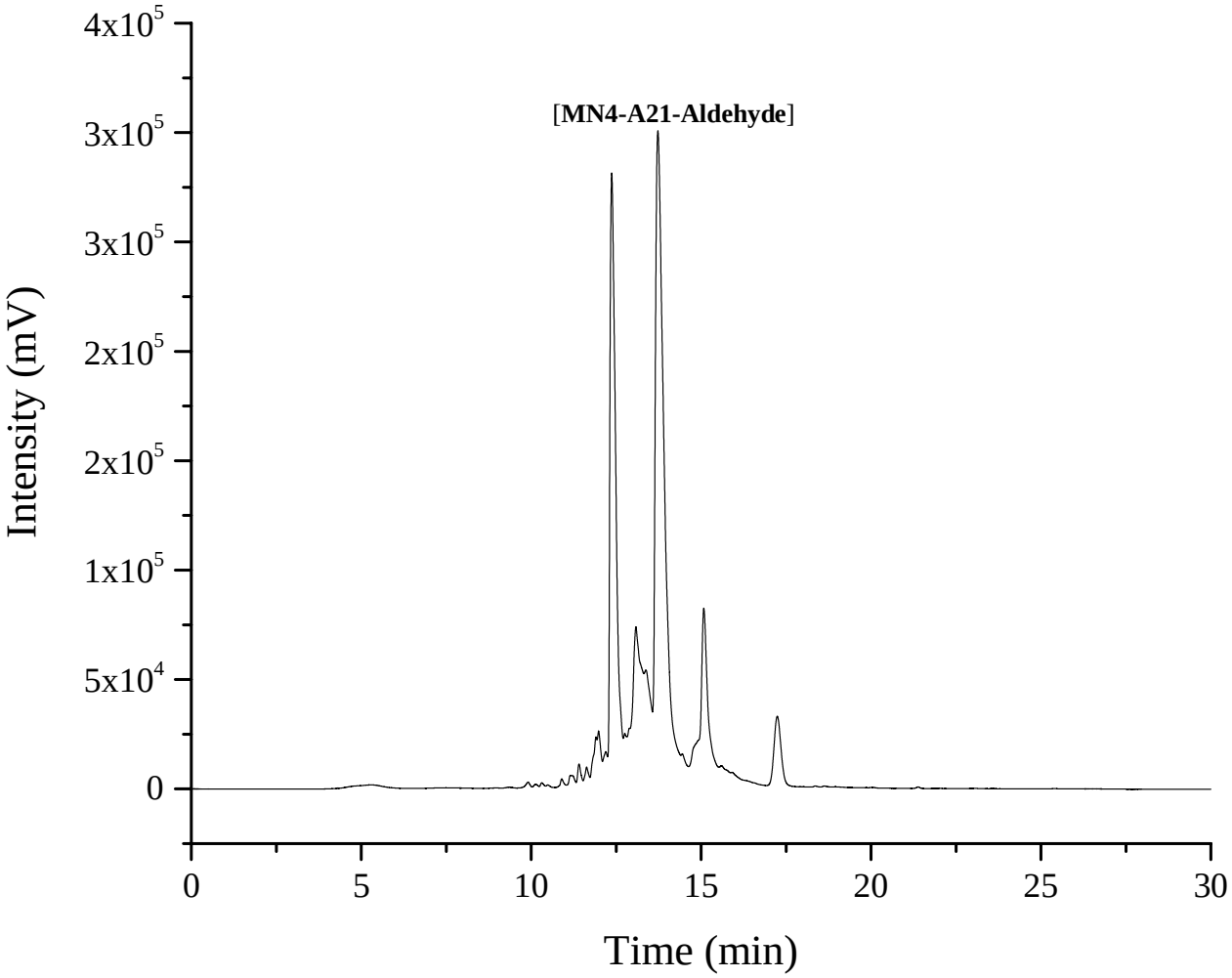


Figure S3. RP-HPLC traces of crude **MN4-A21** derivatized with cyclohexanecarboxaldehyde; bioZen oligo LC column (2.6 µm, 150 x 4.6 mm); flow rate = 0.6 ml min^{-}1; linear gradient (5-25 % over 20 min) of 50 mM aqueous TEAA in ACN (pH 7.0); λ = 260 nm.

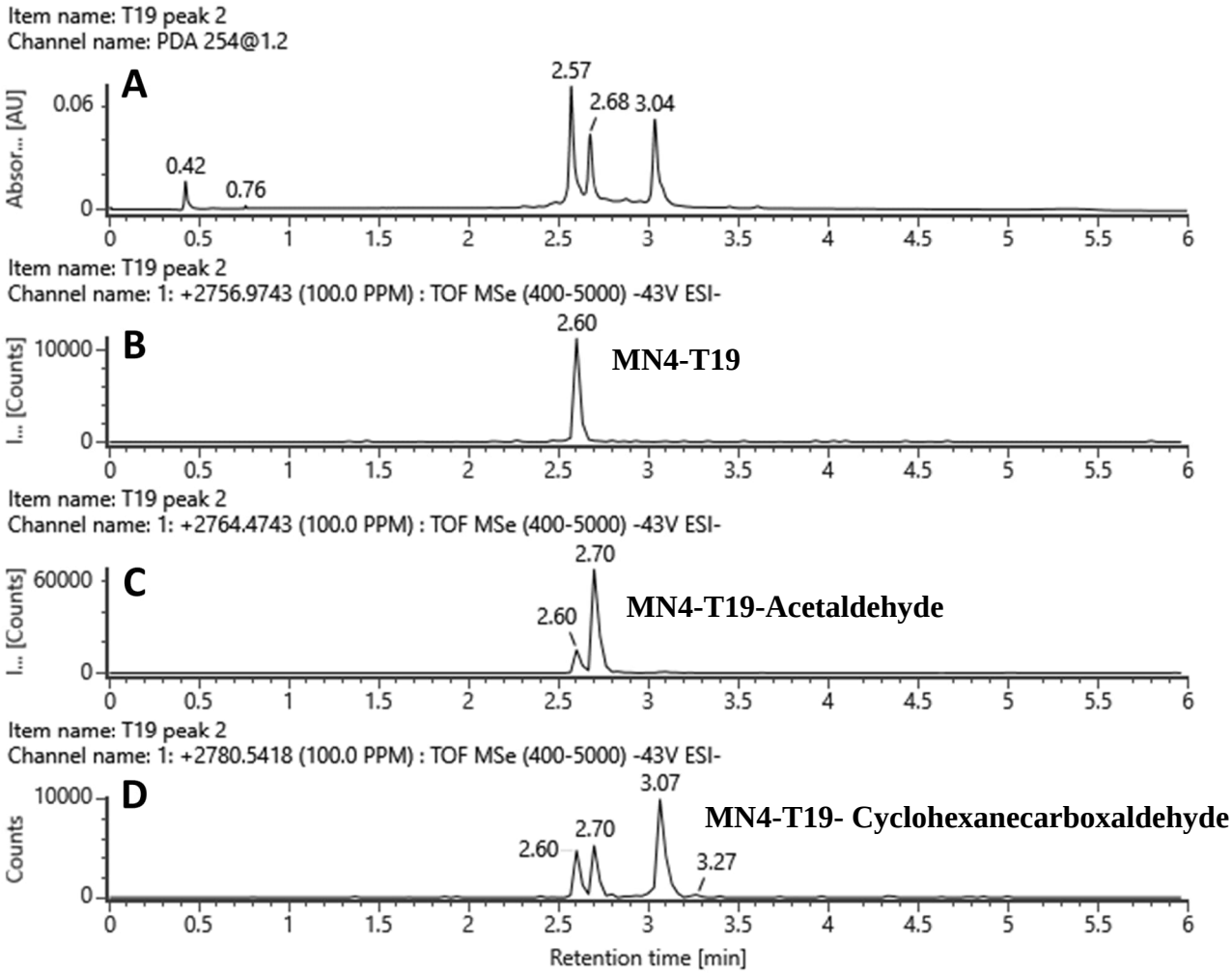


Figure S4. UV (A) and extracted ion (B—D) UPLC traces of purified **MN4-T19**; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5— 25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

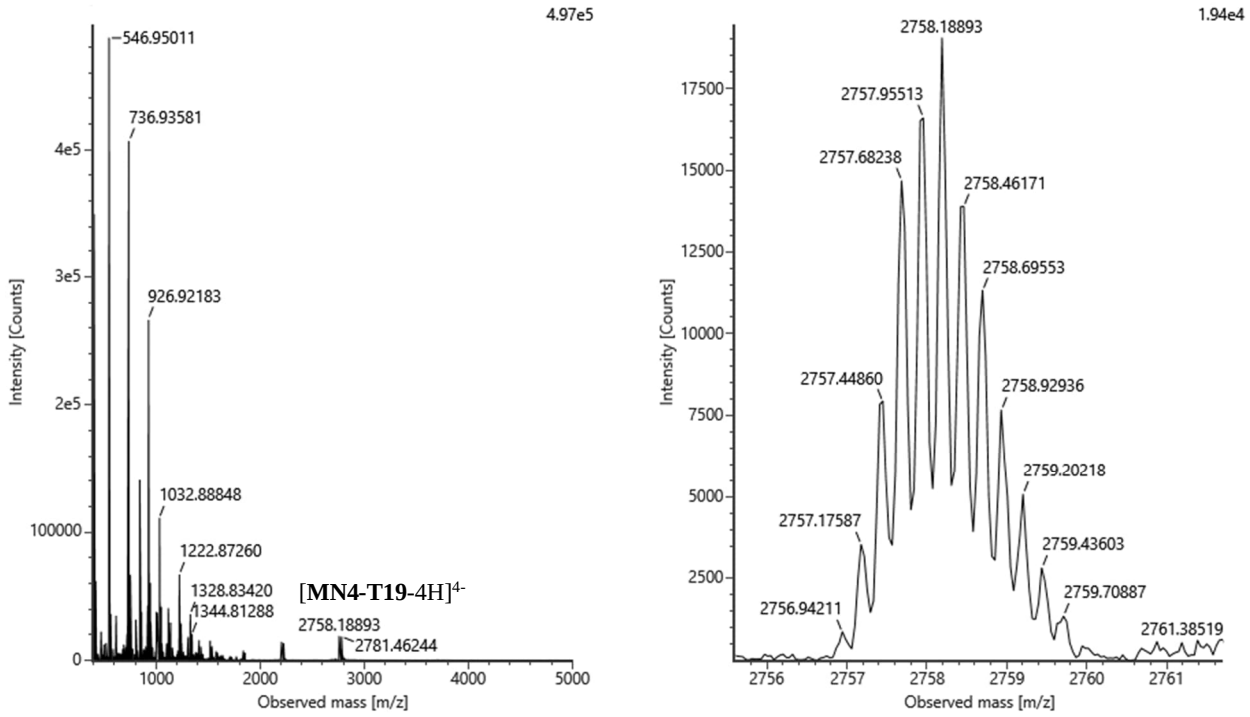


Figure S5. Mass spectrum of purified **MN4-T19**.

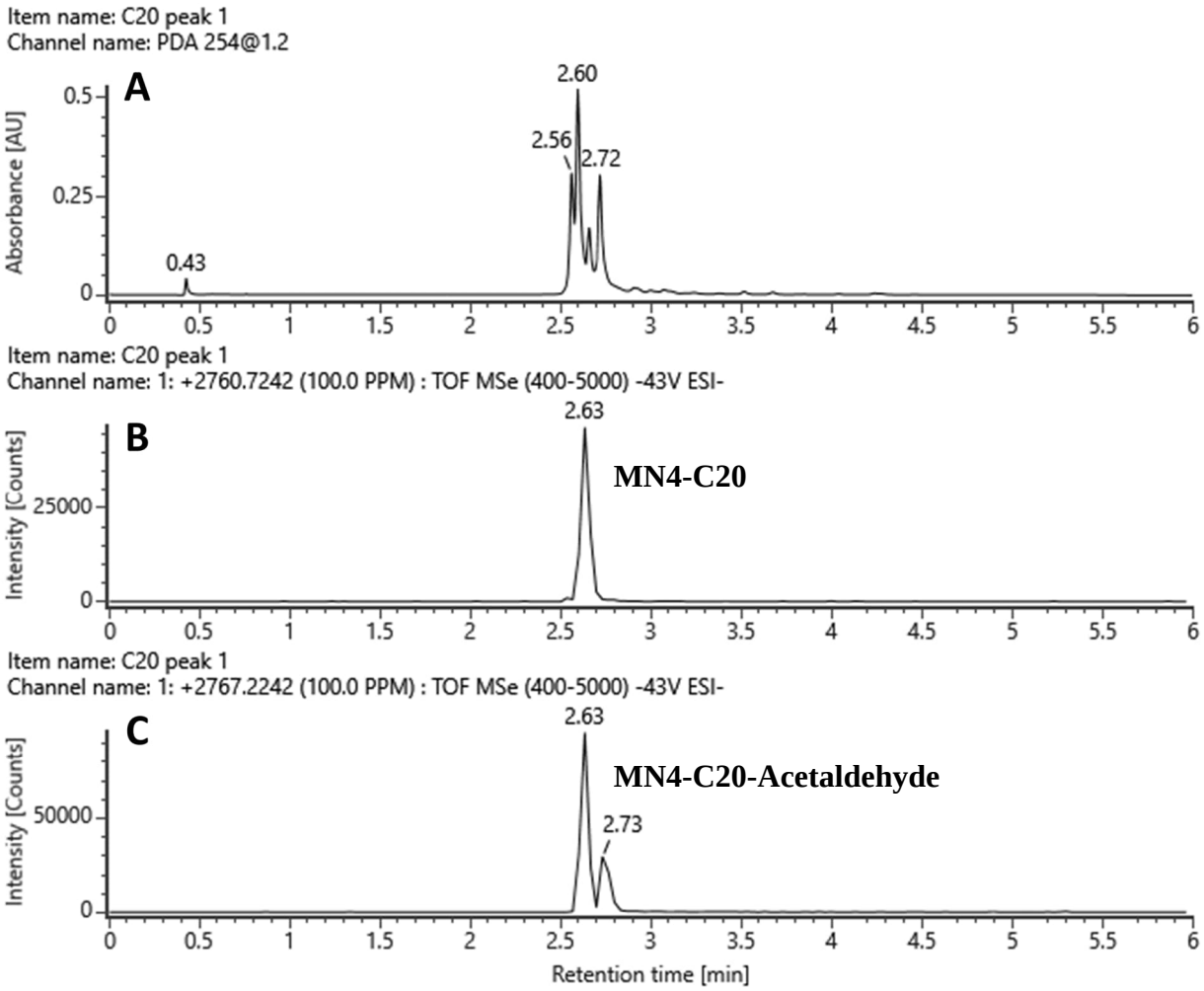


Figure S6. UV (A) and extracted ion (B and C) UPLC traces of purified **MN4-C20**; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5— 25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

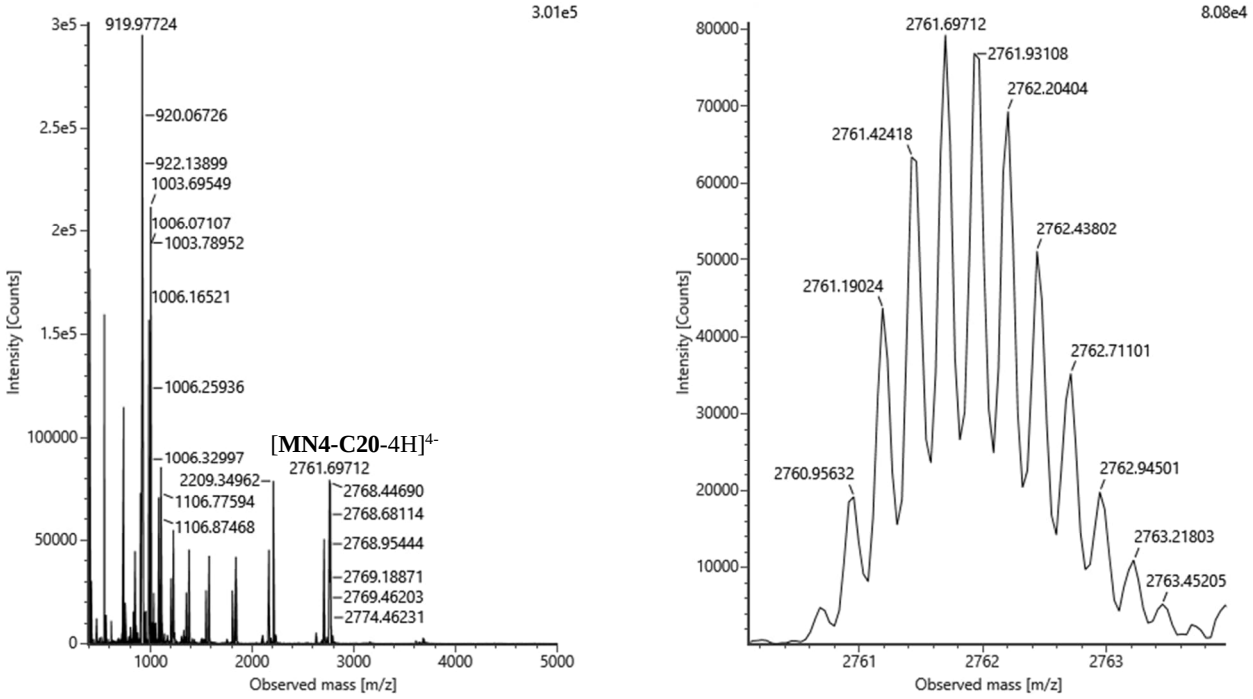


Figure S7. Mass spectrum of purified **MN4-C20**.

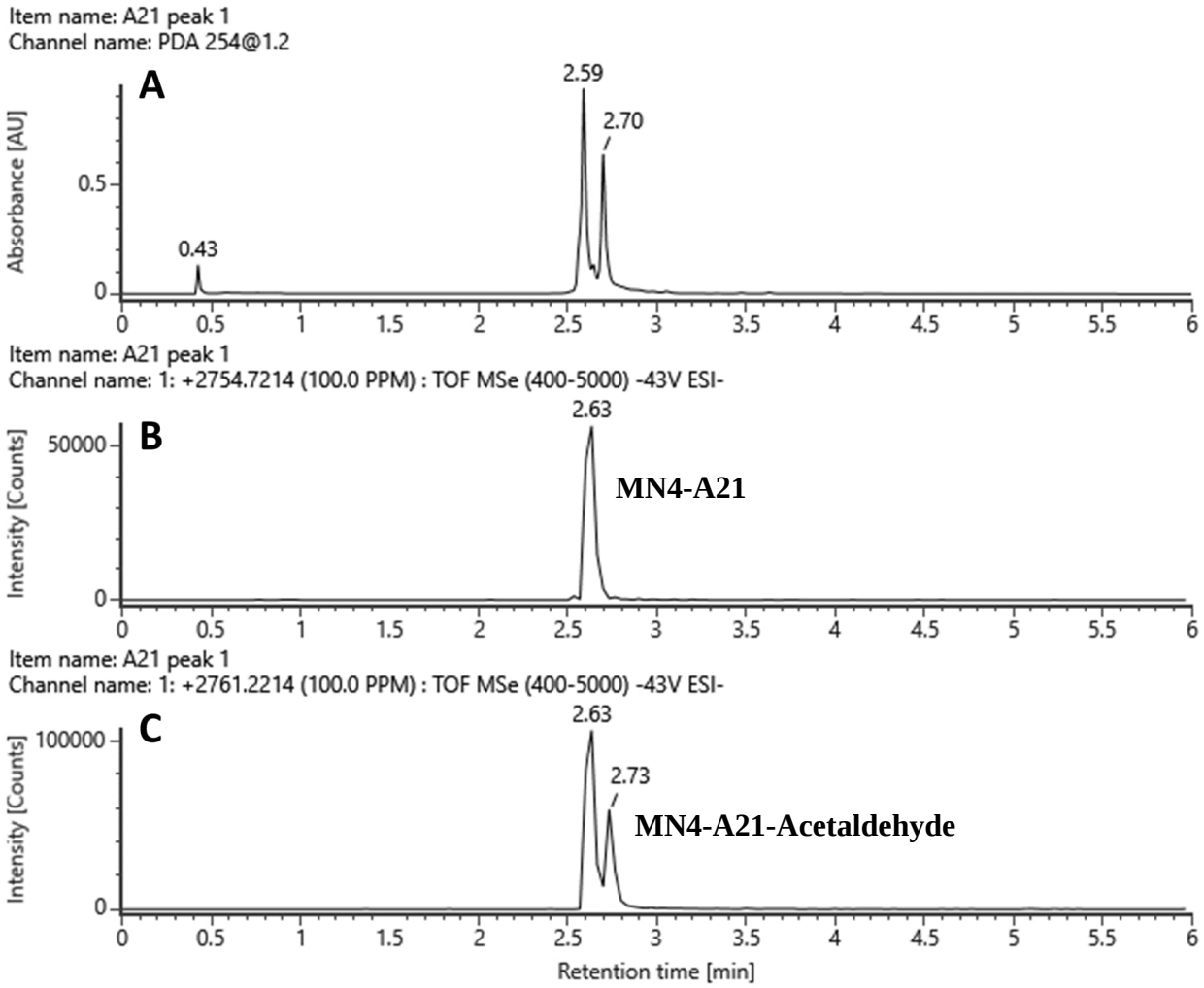


Figure S8. UV (A) and extracted ion (B and C) UPLC traces of purified **MN4-A21**; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5— 25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

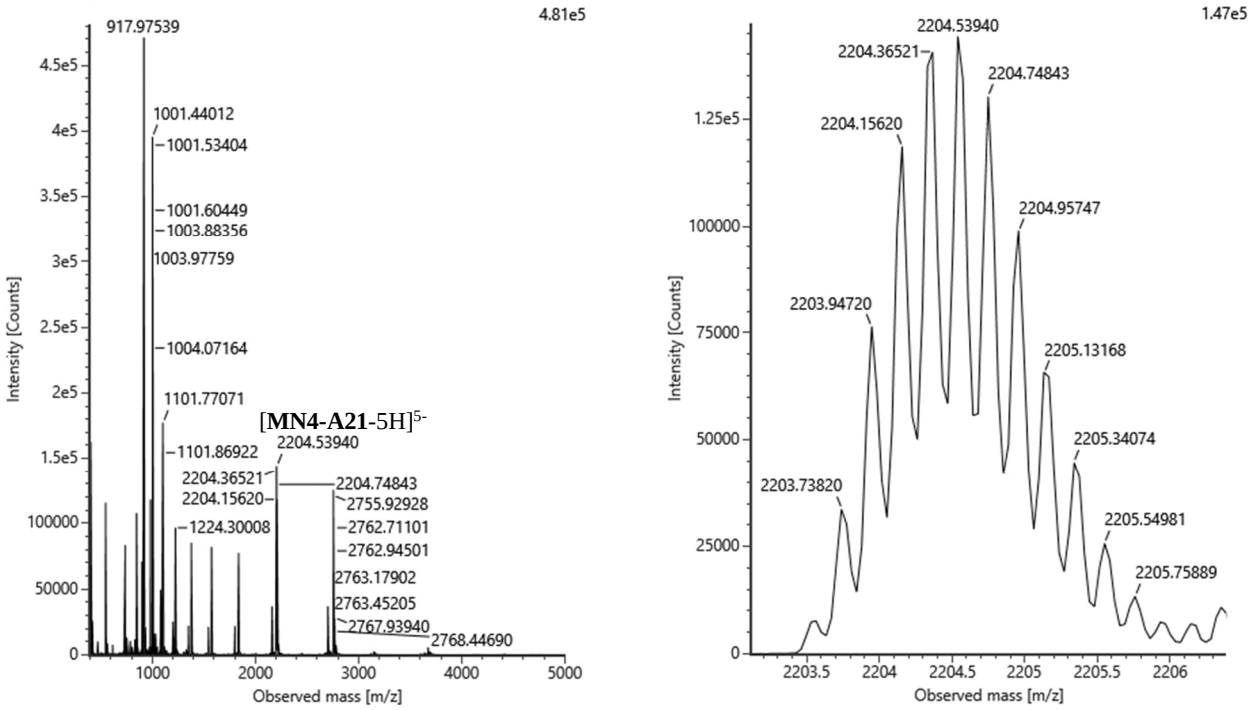


Figure S9. Mass spectrum of purified **MN4-A21**.

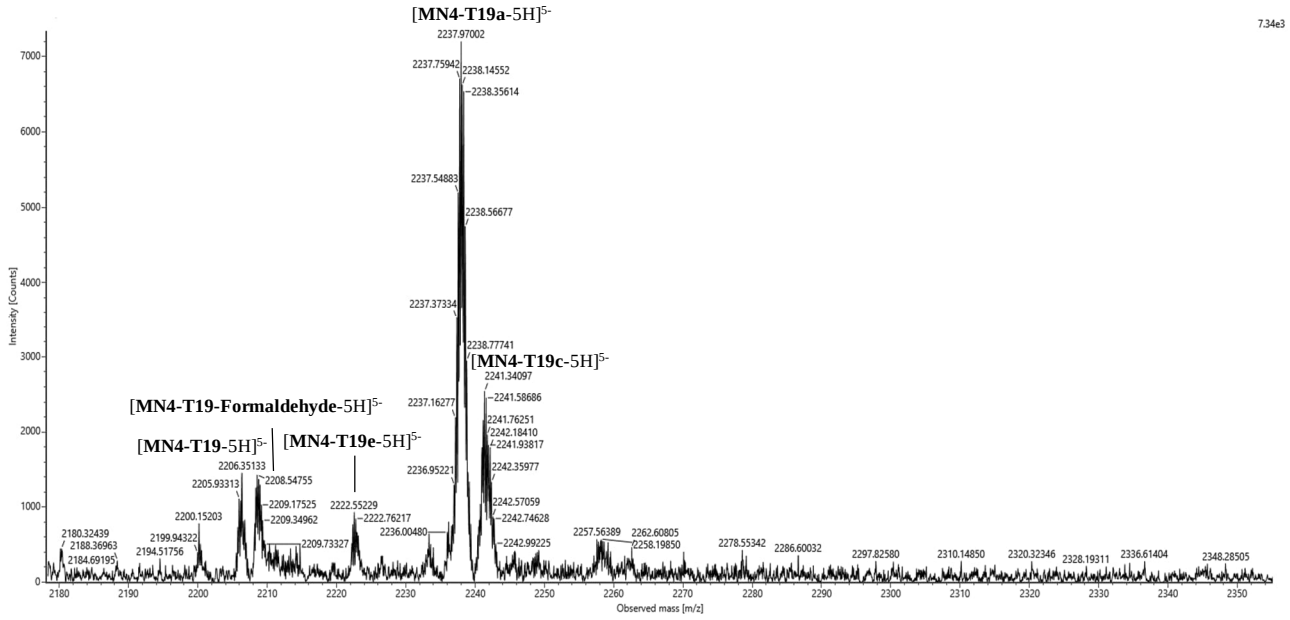


Figure S10. Mass spectrum of **MN4-T19** with aldehyde mixture 1 in the absence of quinine.

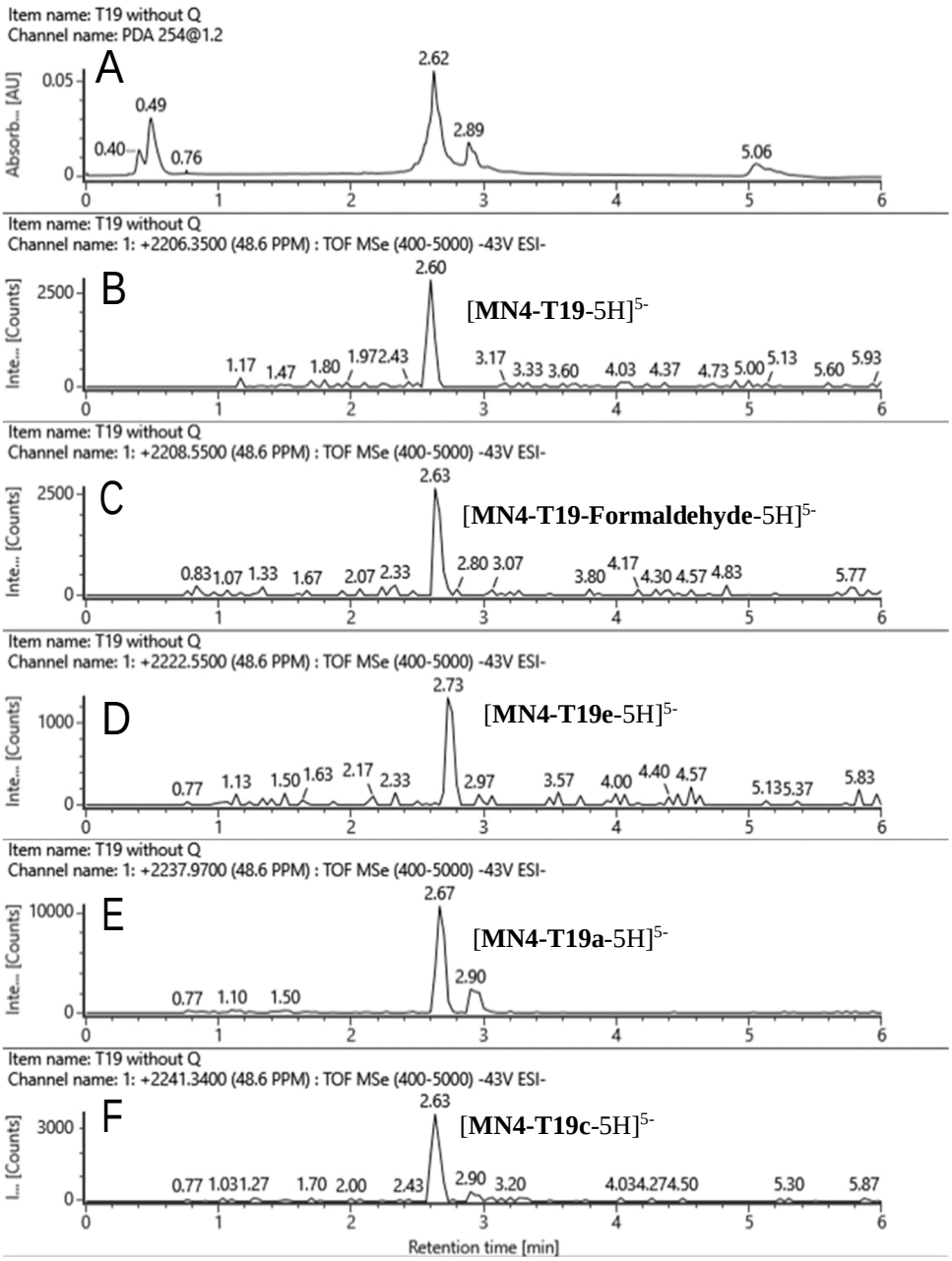


Figure S11. UV (A) and extracted ion (B—F) UPLC traces of **MN4-T19** with aldehyde mixture 1 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

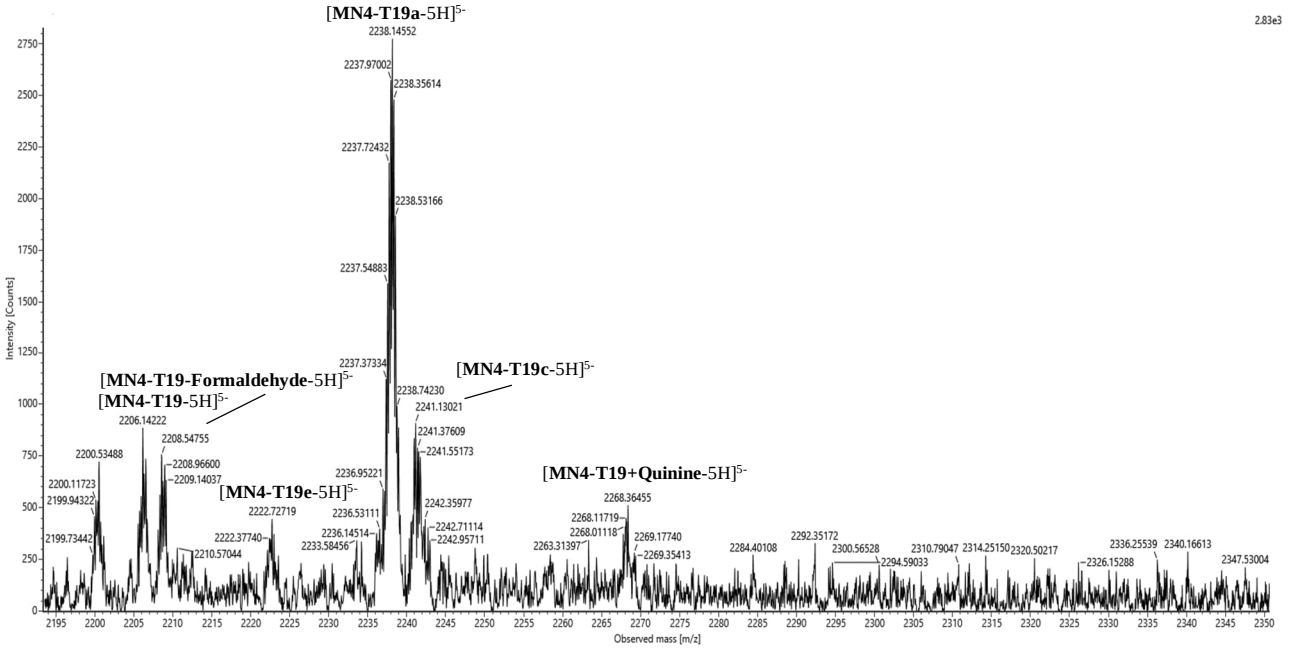


Figure S12. Mass spectrum of **MN4-T19** with aldehyde mixture 1 in the presence of quinine.

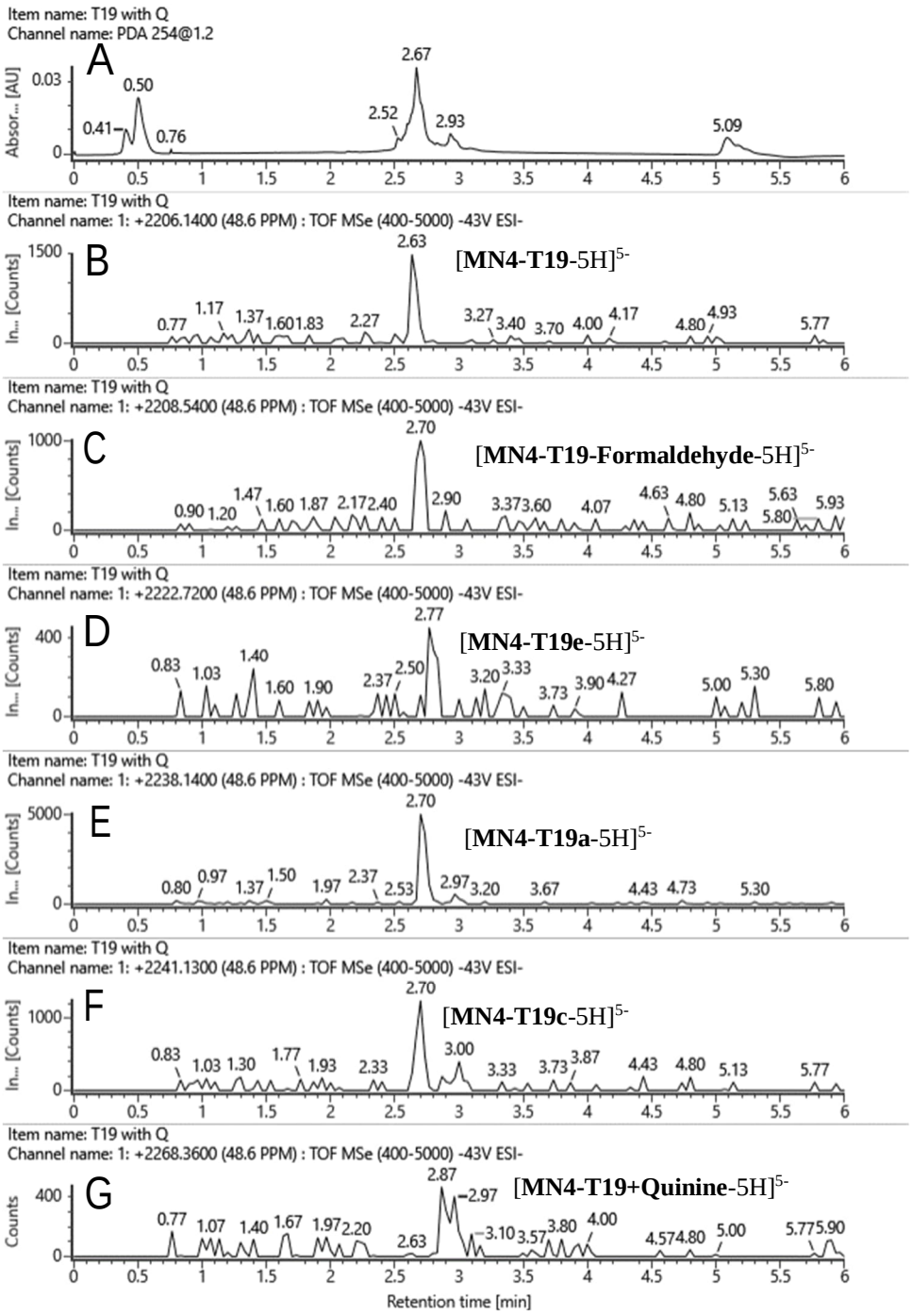


Figure S13. UV (A) and extracted ion (B—G) UPLC traces of **MN4-T19** with aldehyde mixture 1 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

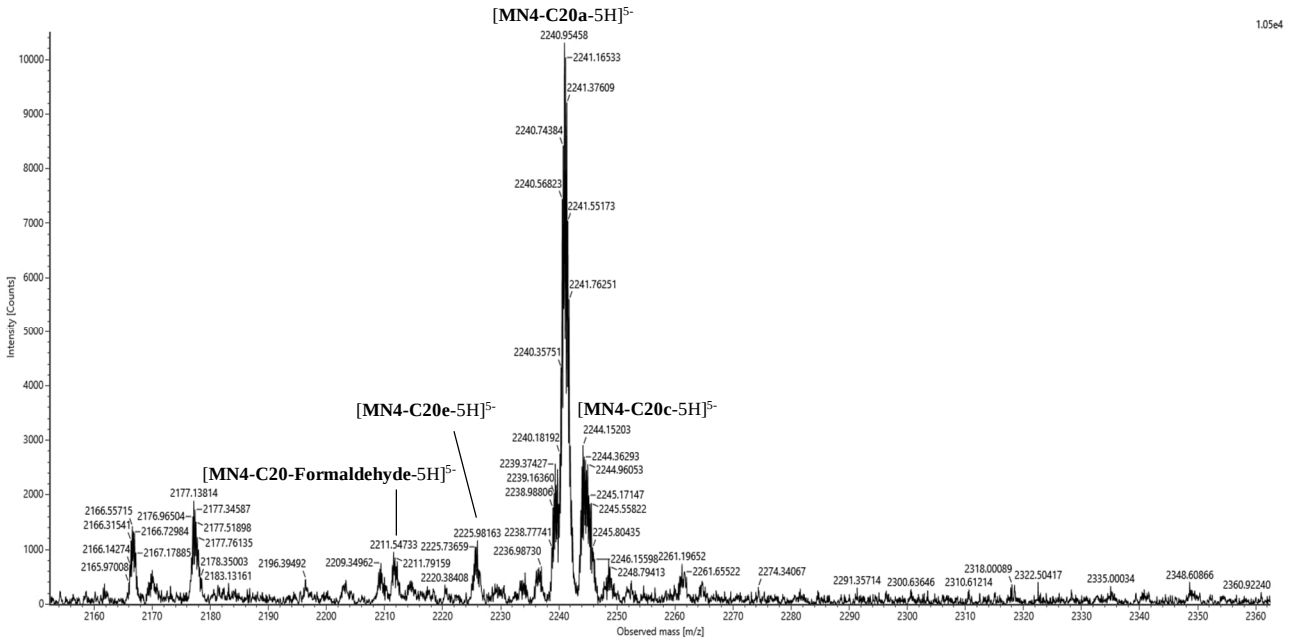


Figure S14. Mass spectrum of **MN4-C20** with aldehyde mixture 1 in the absence of quinine.

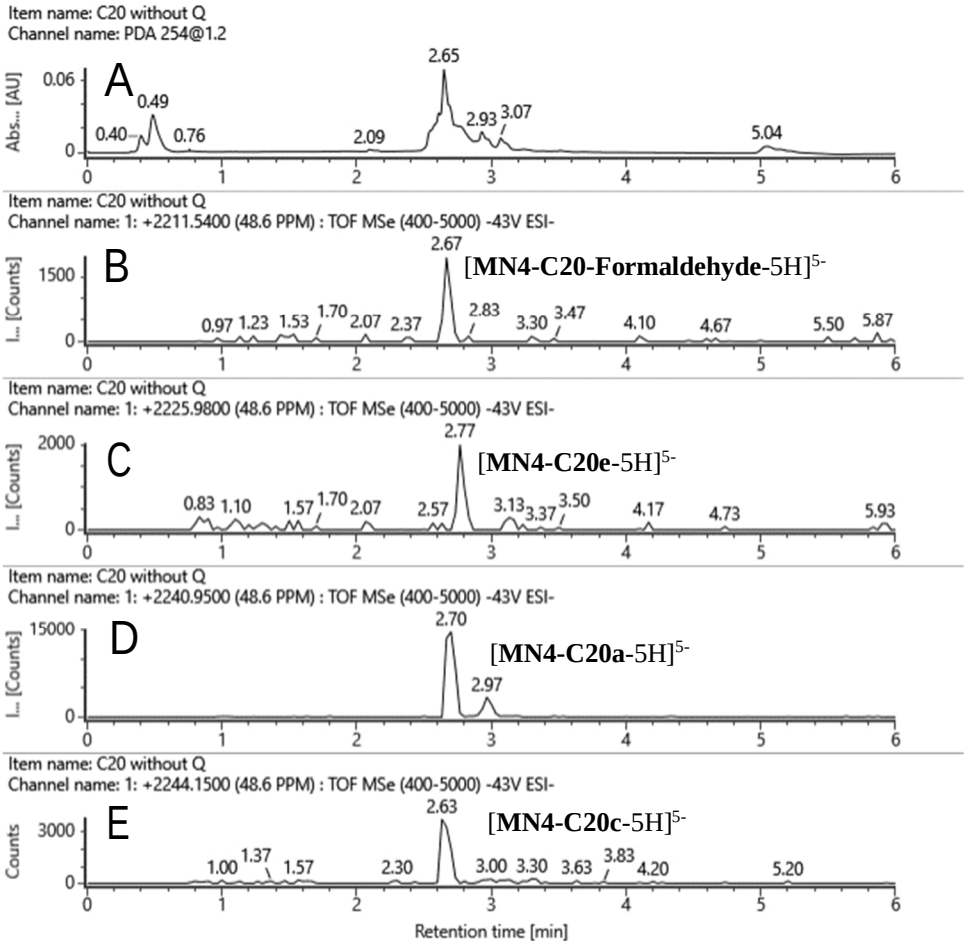


Figure S15. UV (A) and extracted ion (B—E) UPLC traces of **MN4-C20** with aldehyde mixture 1 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4

ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

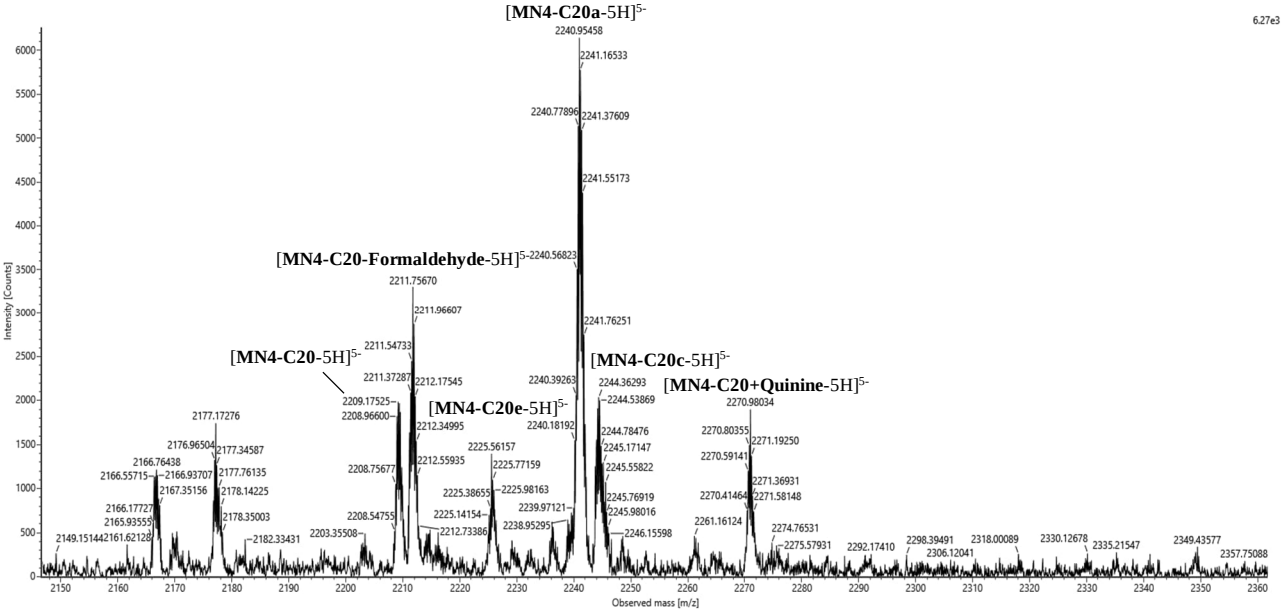


Figure S16. Mass spectrum of **MN4-C20** with aldehyde mixture 1 in the presence of quinine.

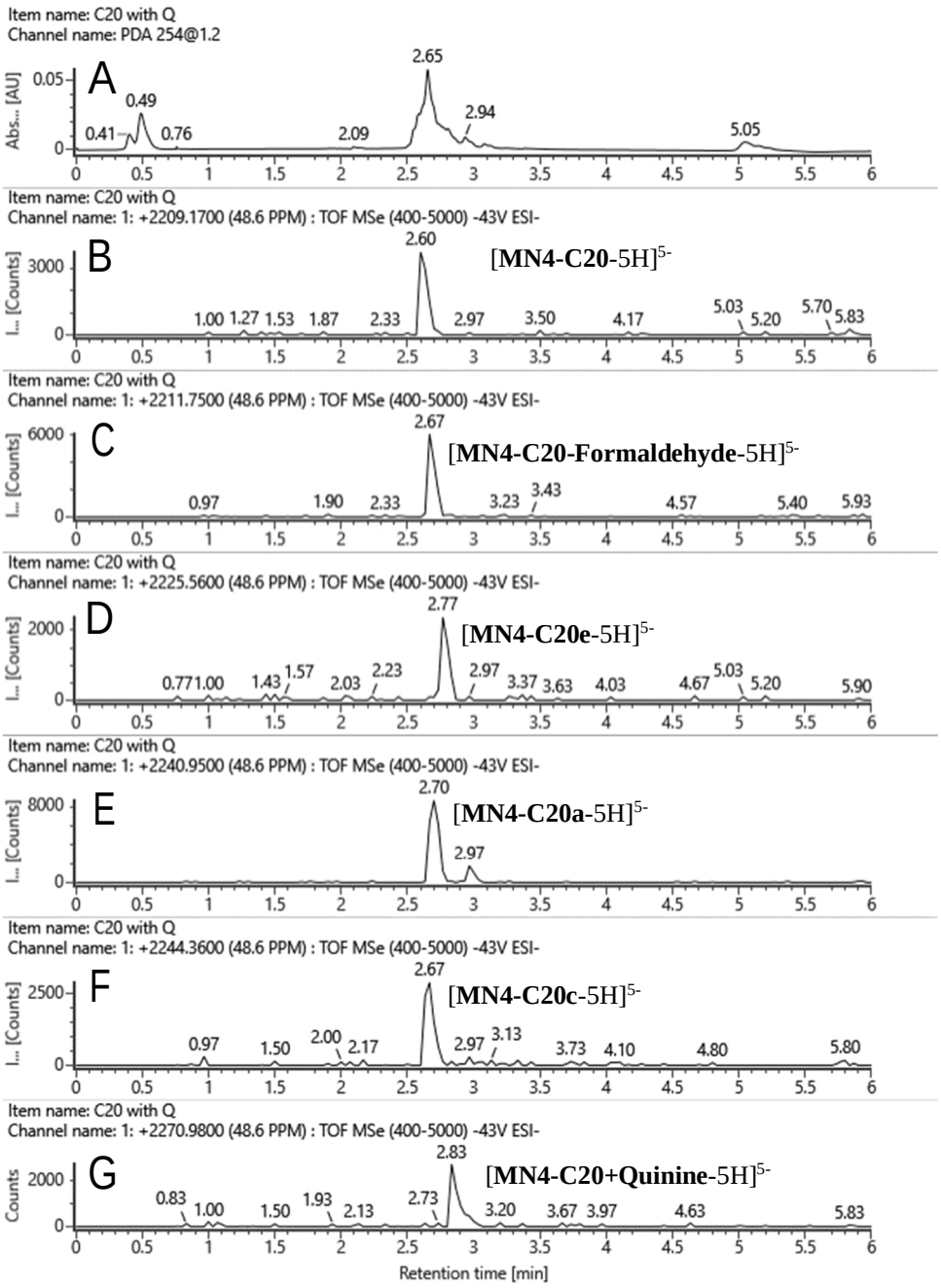


Figure S17. UV (A) and extracted ion (B—G) UPLC traces of **MN4-C20** with aldehyde mixture 1 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

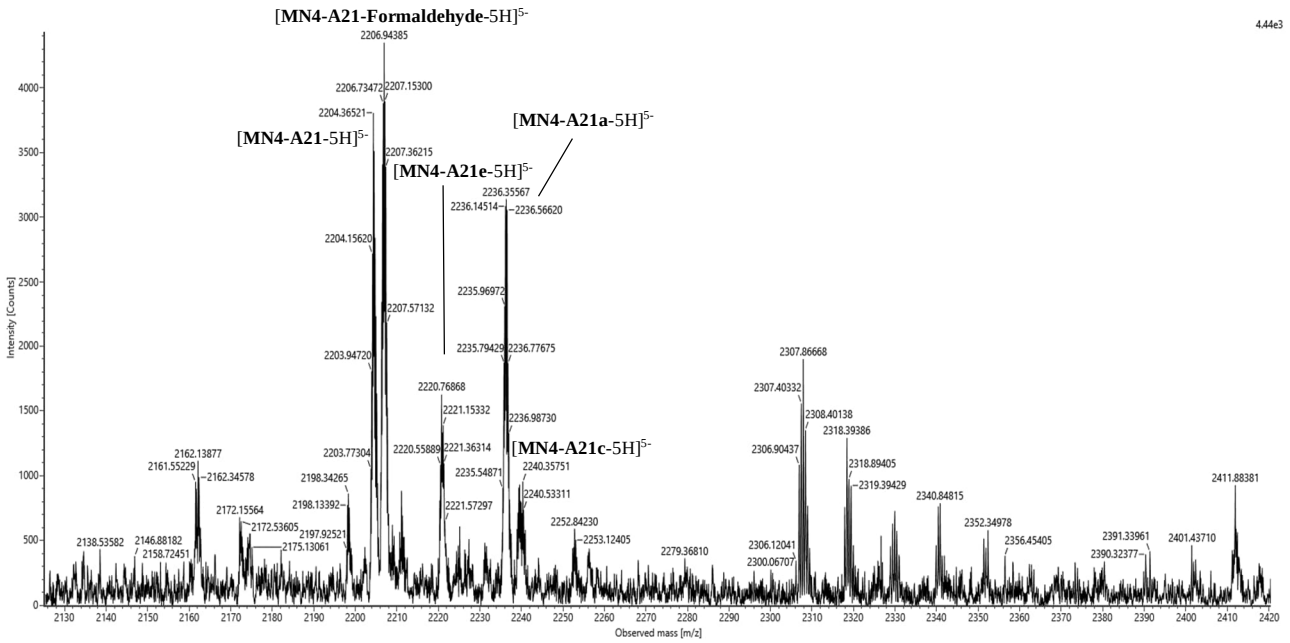


Figure S18. Mass spectrum of **MN4-A21** with aldehyde mixture 1 in the absence of quinine.

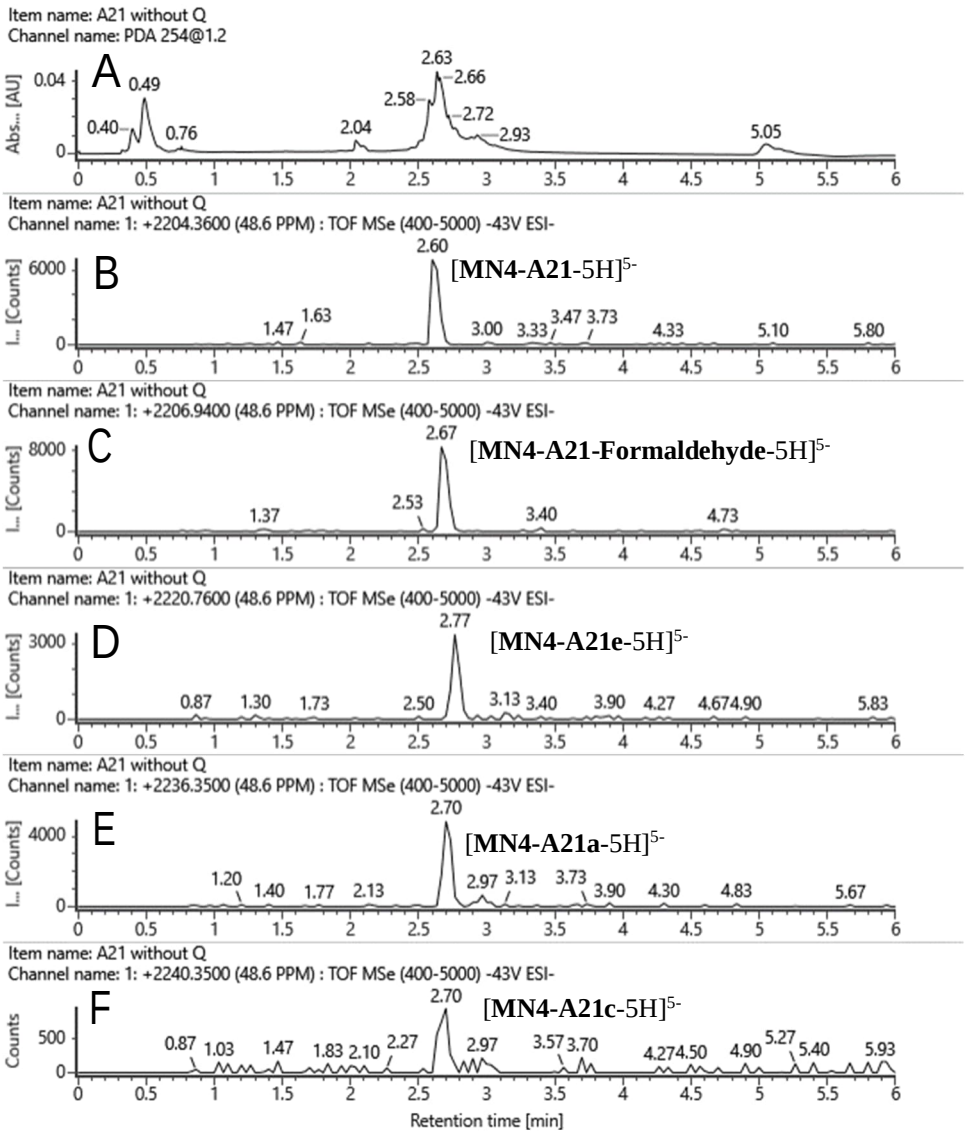


Figure S19. UV (A) and extracted ion (B—F) UPLC traces of **MN4-A21** with aldehyde mixture 1 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

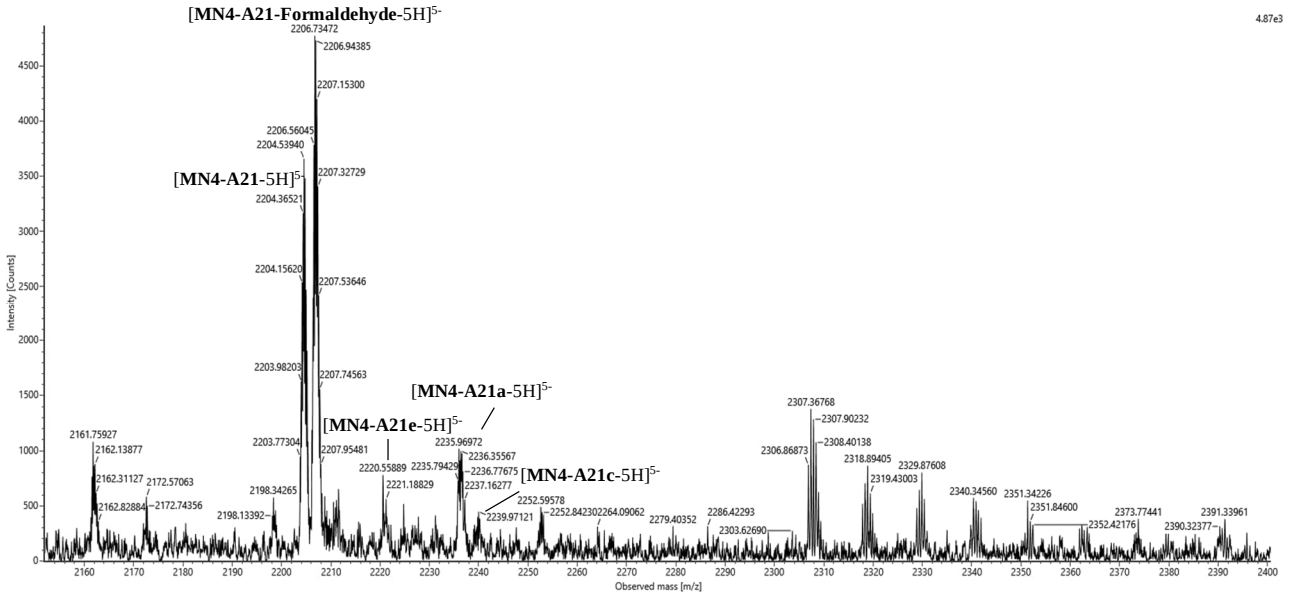


Figure S20. Mass spectrum of **MN4-A21** with aldehyde mixture 1 in the presence of quinine.

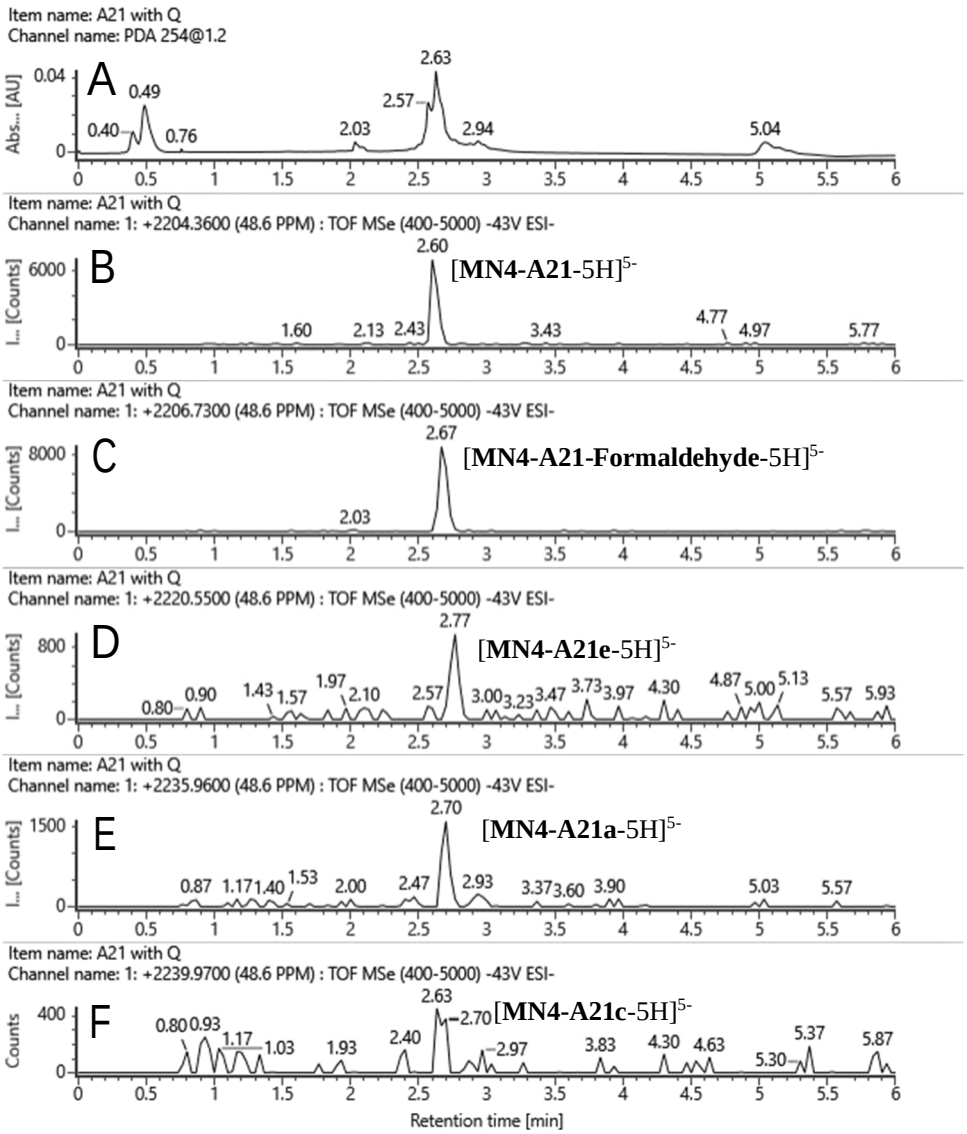


Figure S21. UV (A) and extracted ion (B—F) UPLC traces of **MN4-A21** with aldehyde mixture 1 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

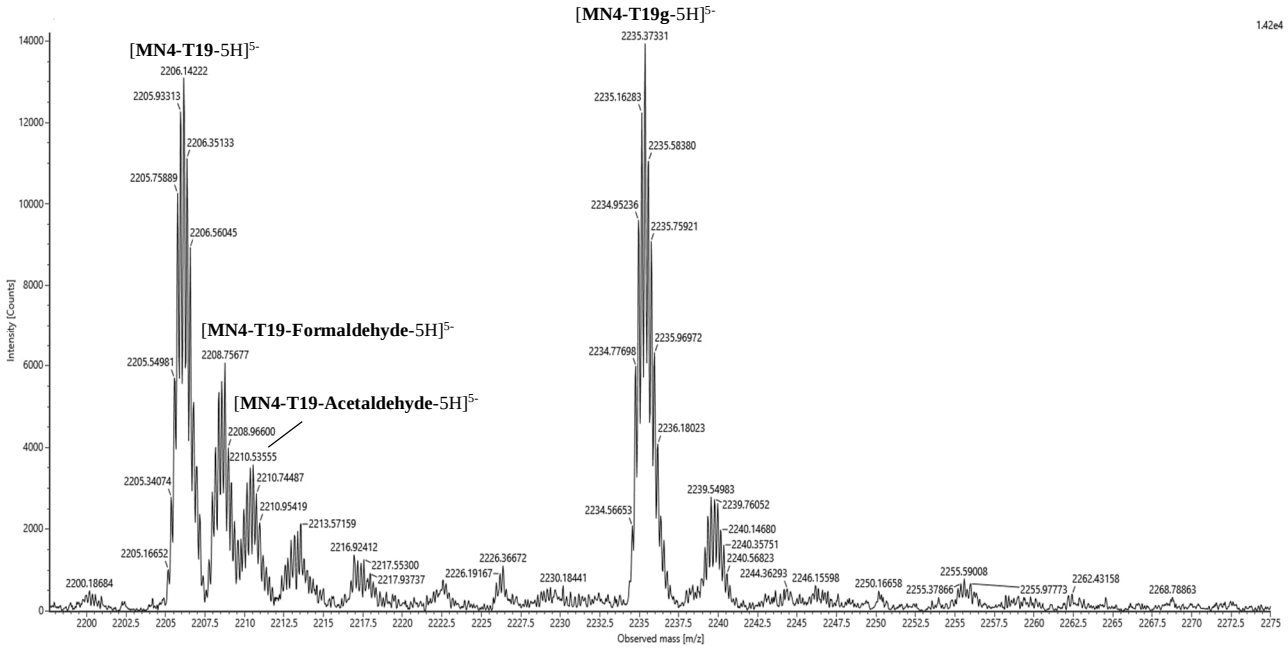


Figure S22. Mass spectrum of **MN4-T19** with aldehyde mixture 2 in the absence of quinine.

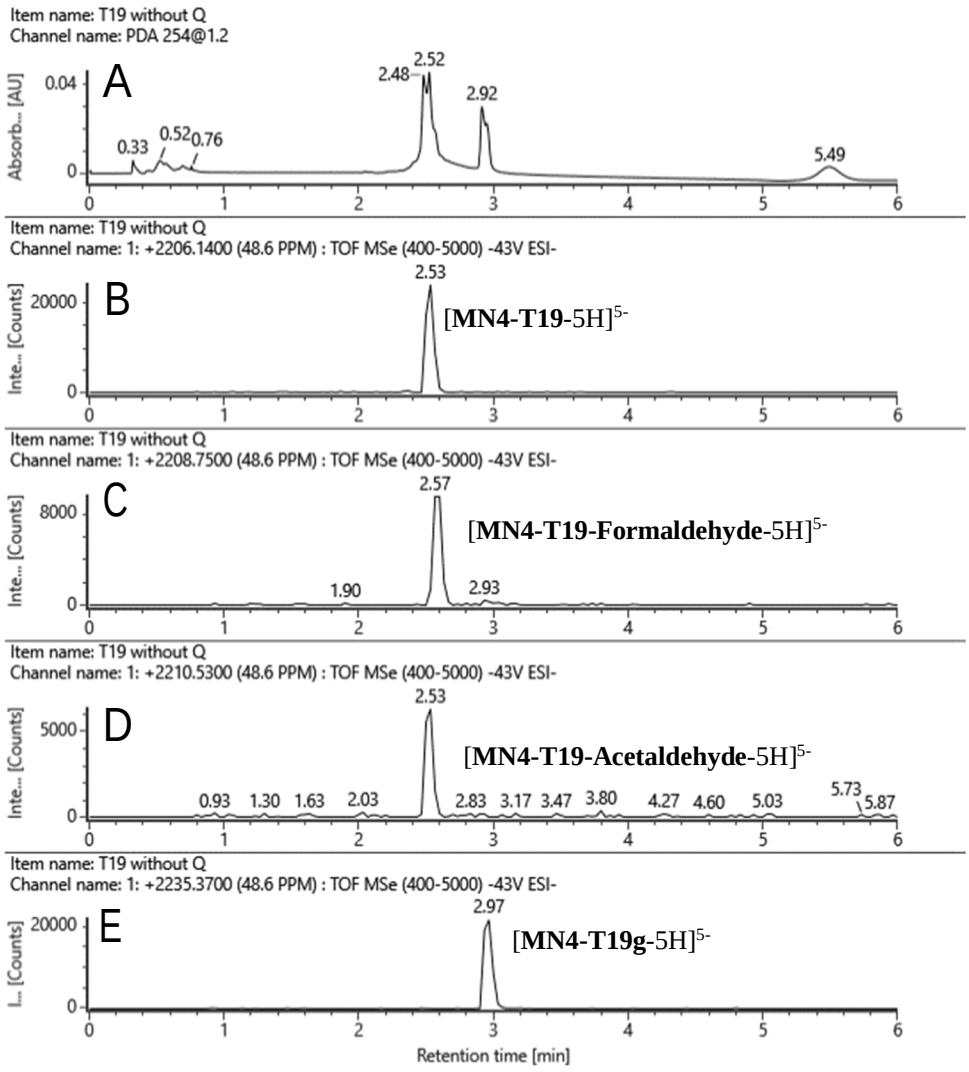


Figure S23. UV (A) and extracted ion (B—E) UPLC traces of **MN4-T19** with aldehyde mixture 2 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

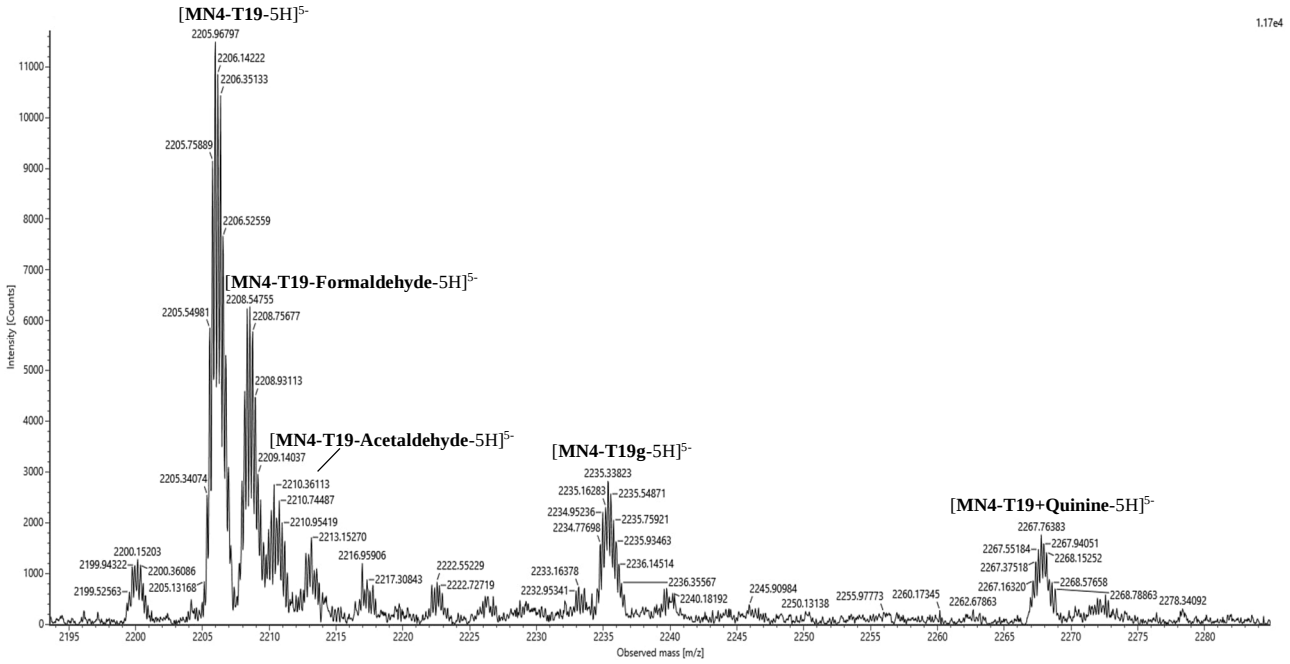


Figure S24. Mass spectrum of **MN4-T19** with aldehyde mixture 2 in the presence of quinine.

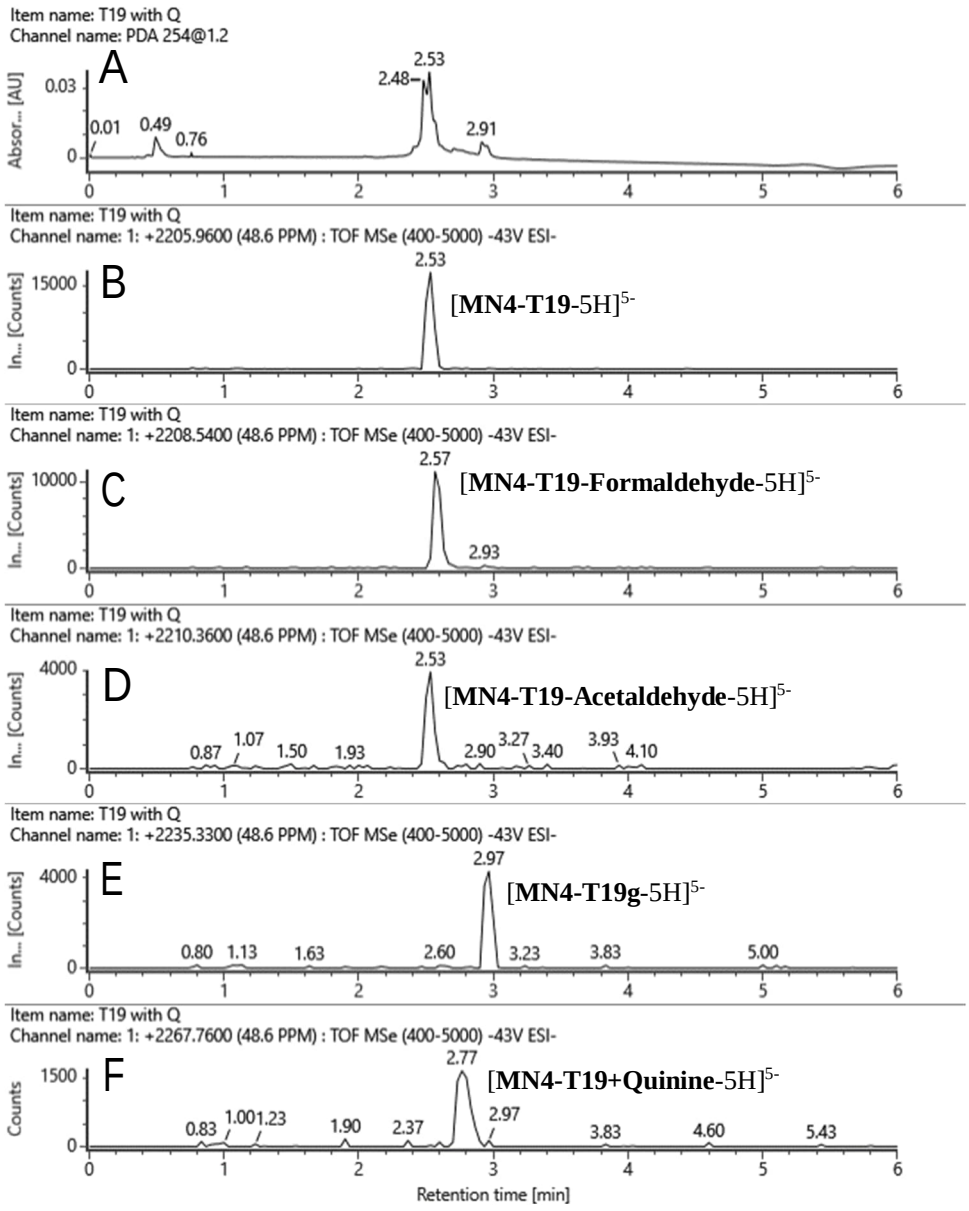


Figure S25. UV (A) and extracted ion (B—F) UPLC traces of **MN4-T19** with aldehyde mixture 2 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

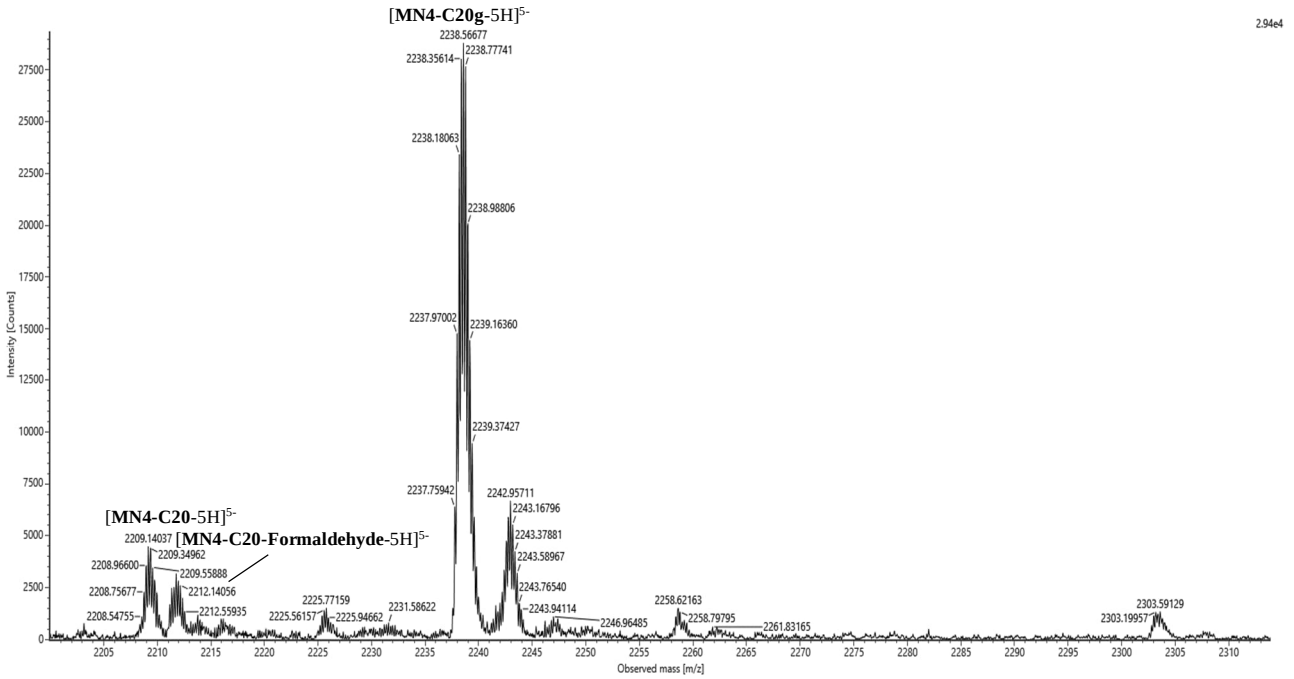


Figure S26. Mass spectrum of **MN4-C20** with aldehyde mixture 2 in the absence of quinine.

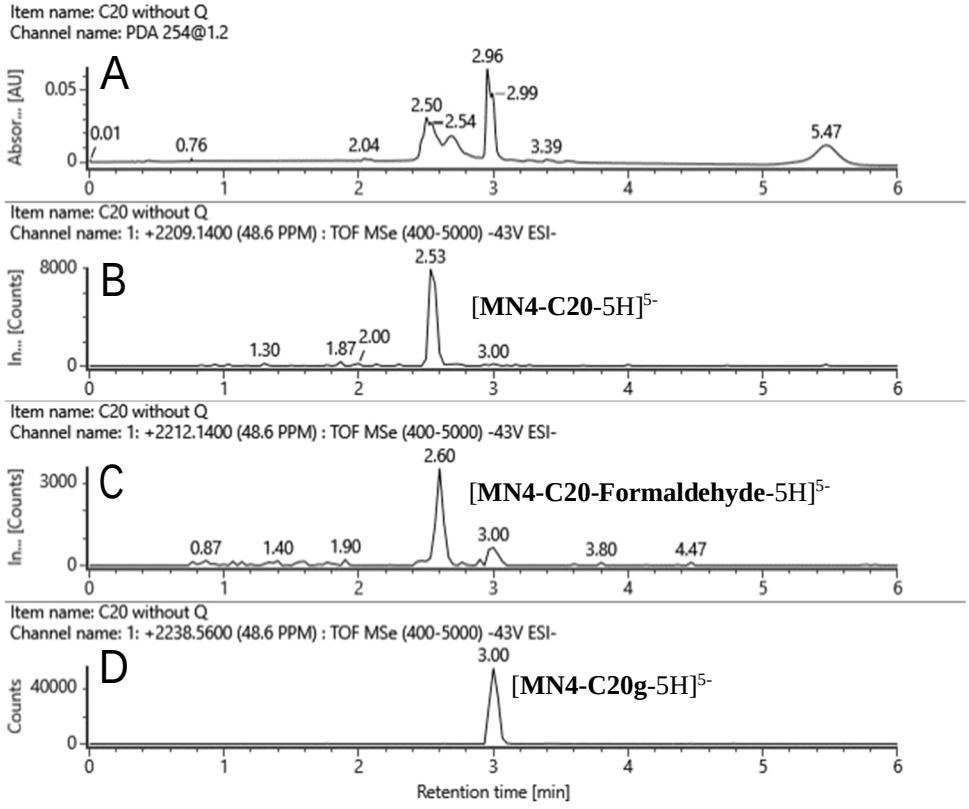


Figure S27. UV (A) and extracted ion (B—D) UPLC traces of **MN4-C20** with aldehyde mixture 2 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

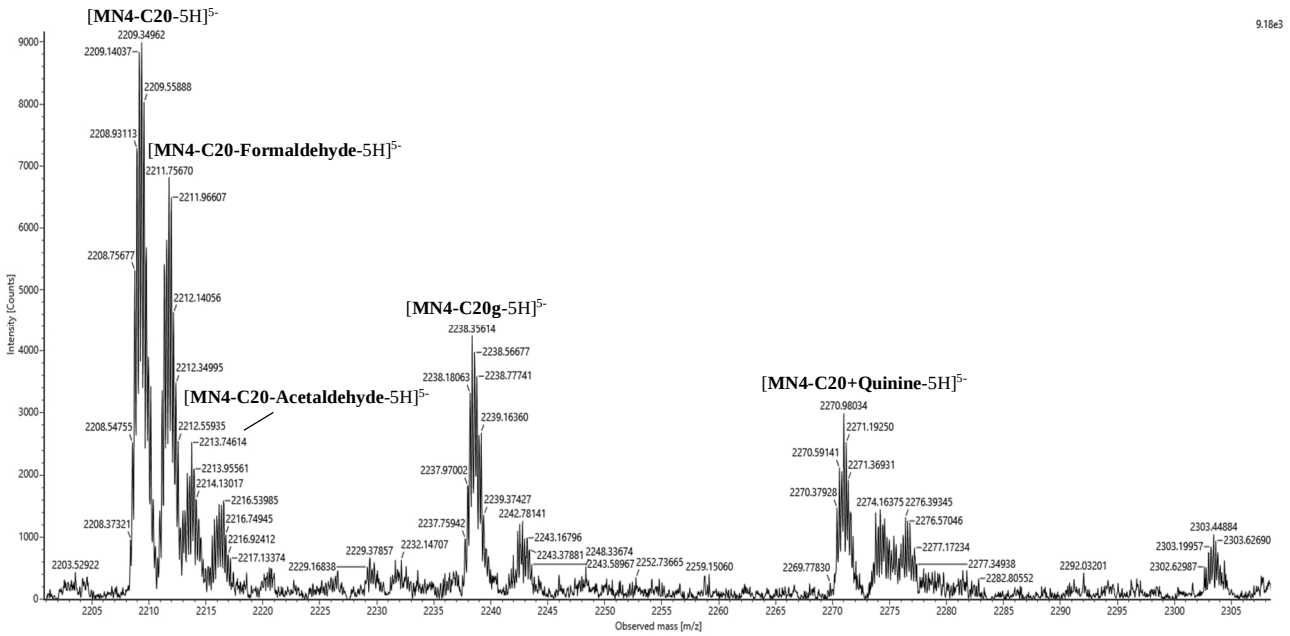


Figure S28. Mass spectrum of **MN4-C20** with aldehyde mixture 2 in the presence of quinine.

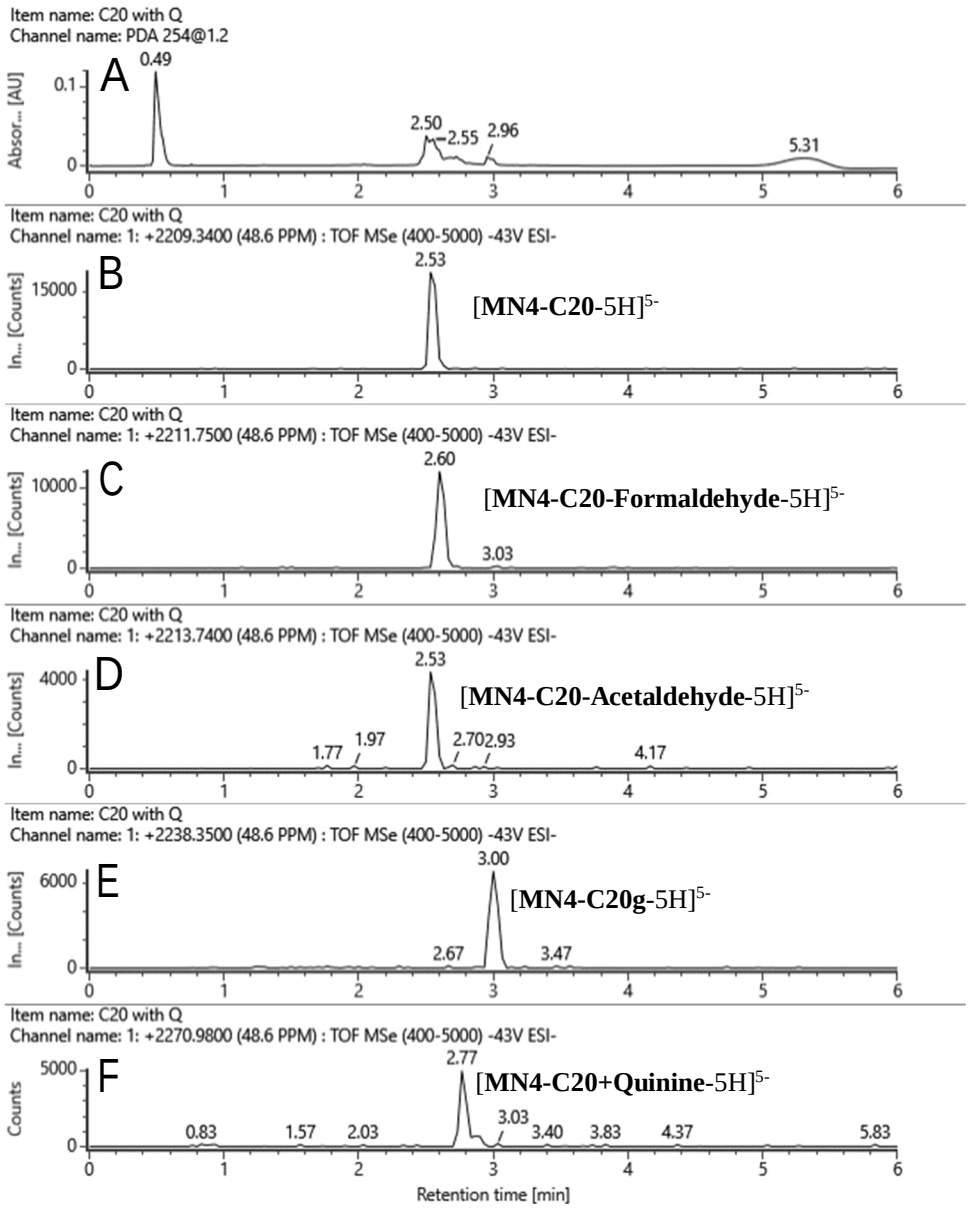


Figure S29. UV (A) and extracted ion (B—F) UPLC traces of **MN4-C20** with aldehyde mixture 2 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

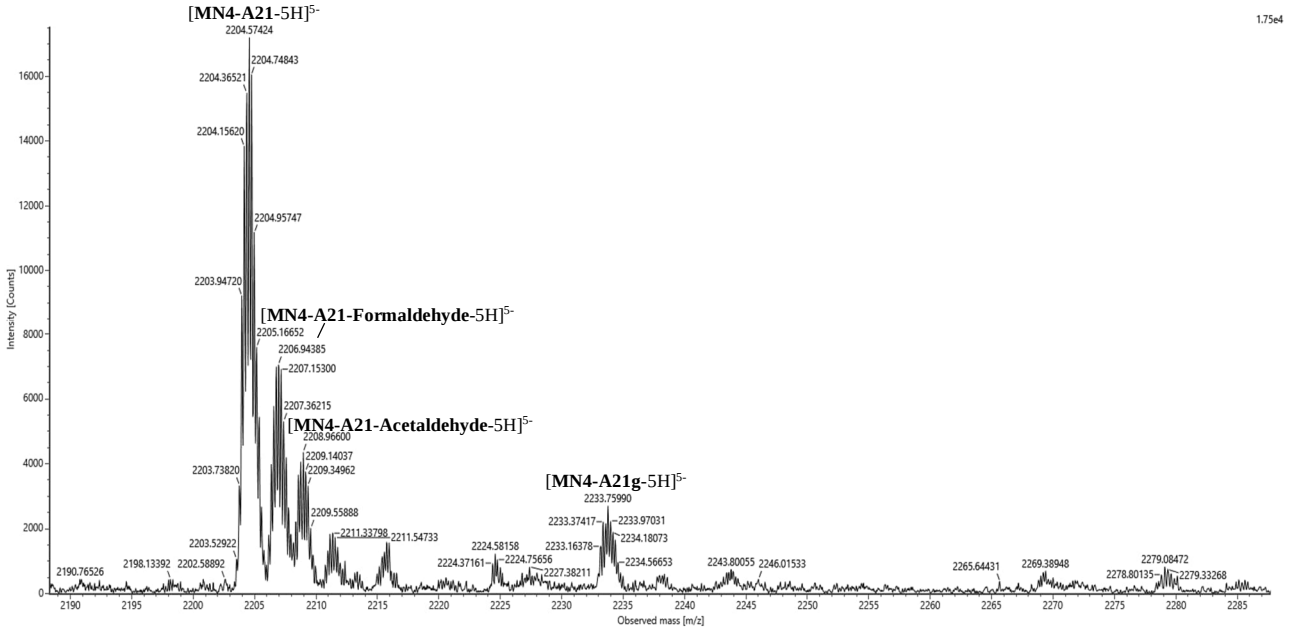


Figure S30. Mass spectrum of **MN4-A21** with aldehyde mixture 2 in the absence of quinine.

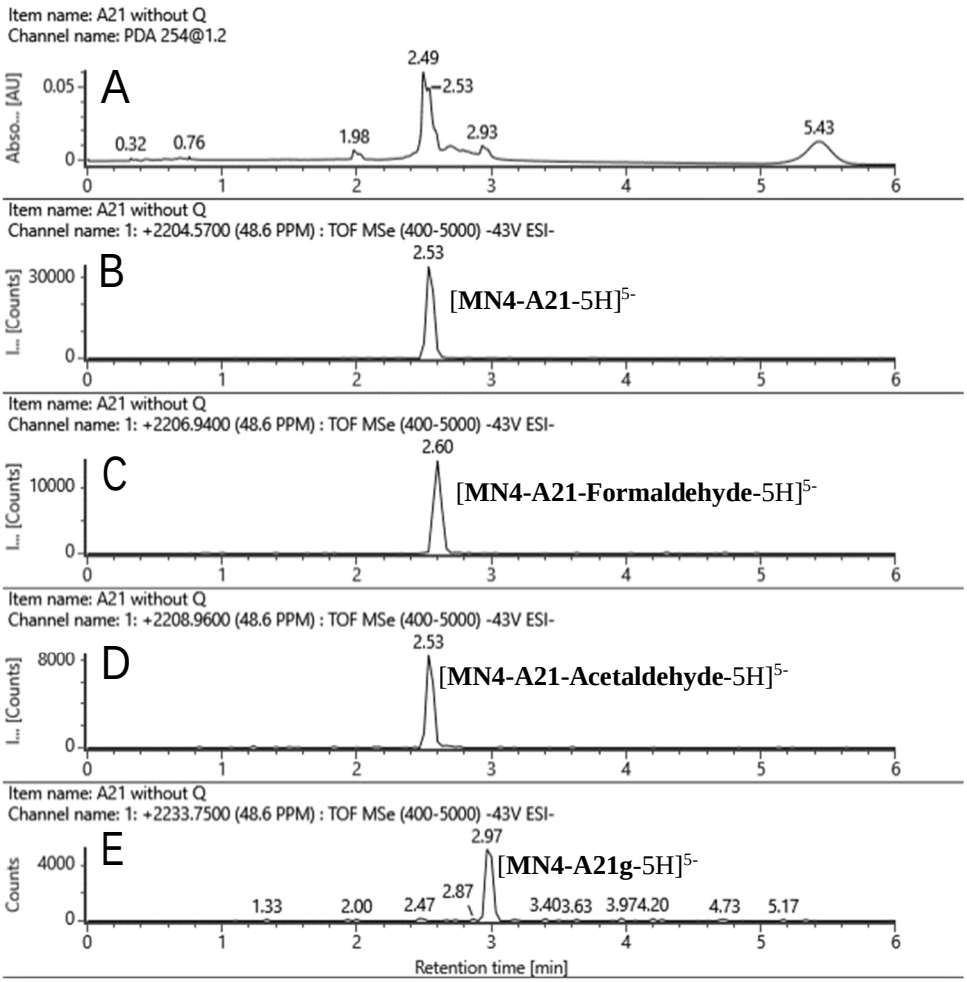


Figure S31. UV (A) and extracted ion (B—E) UPLC traces of **MN4-A21** with aldehyde mixture 2 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

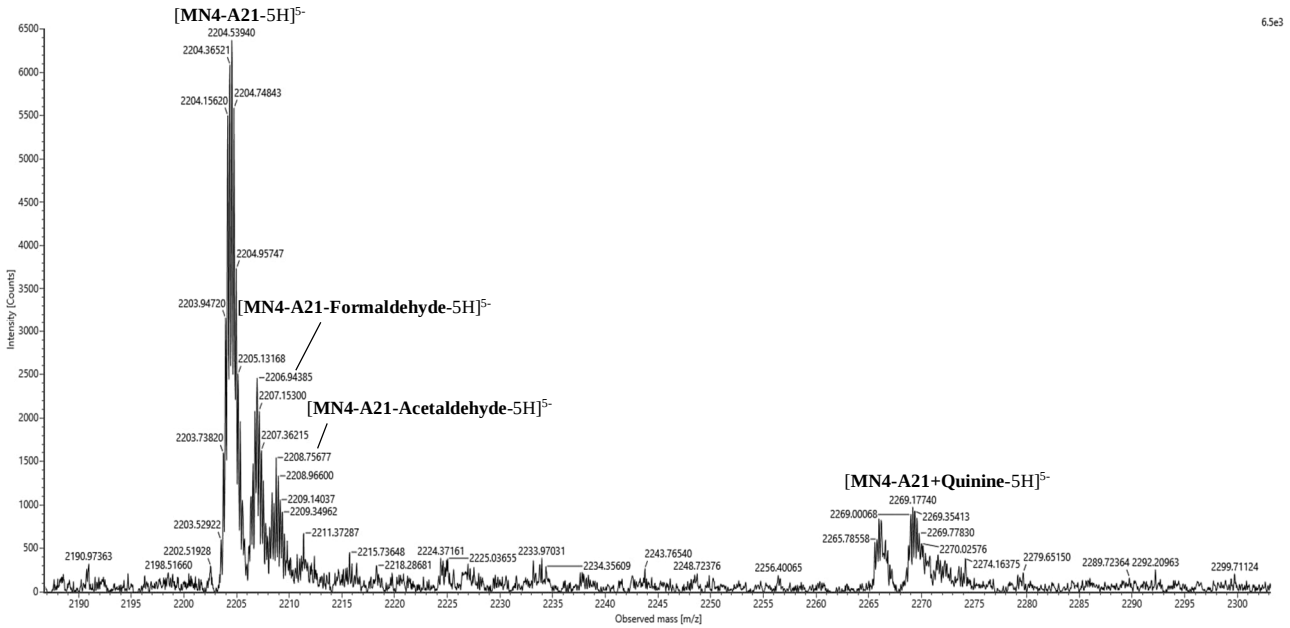


Figure S32. Mass spectrum of **MN4-A21** with aldehyde mixture 2 in the presence of quinine.

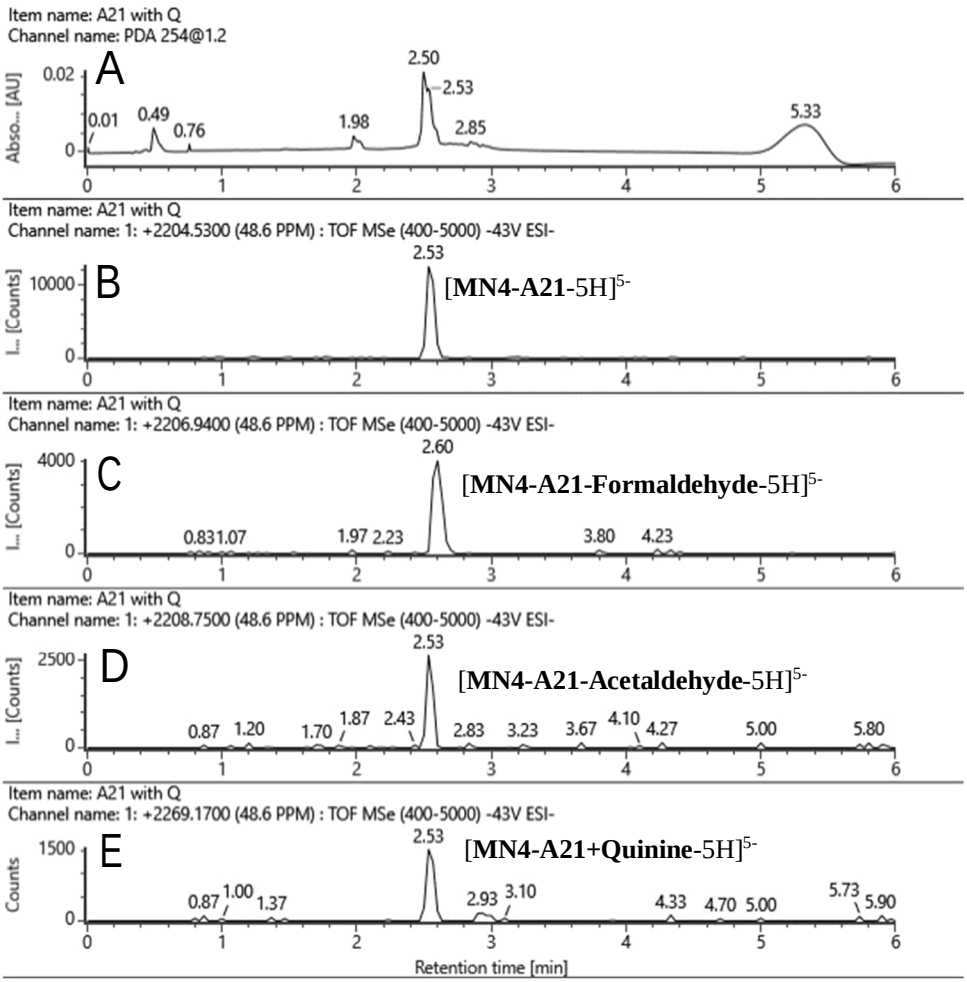


Figure S33. UV (A) and extracted ion (B—E) UPLC traces of **MN4-A21** with aldehyde mixture 2 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

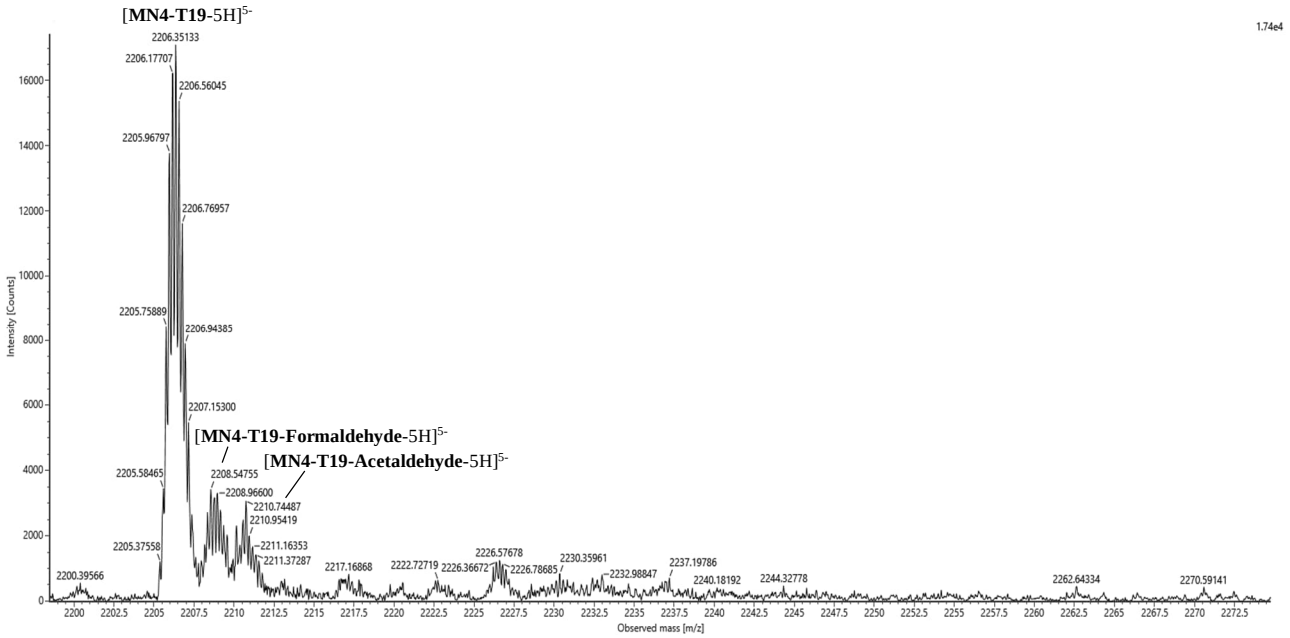


Figure S34. Mass spectrum of **MN4-T19** with aldehyde mixture 3.1 in the absence of quinine.

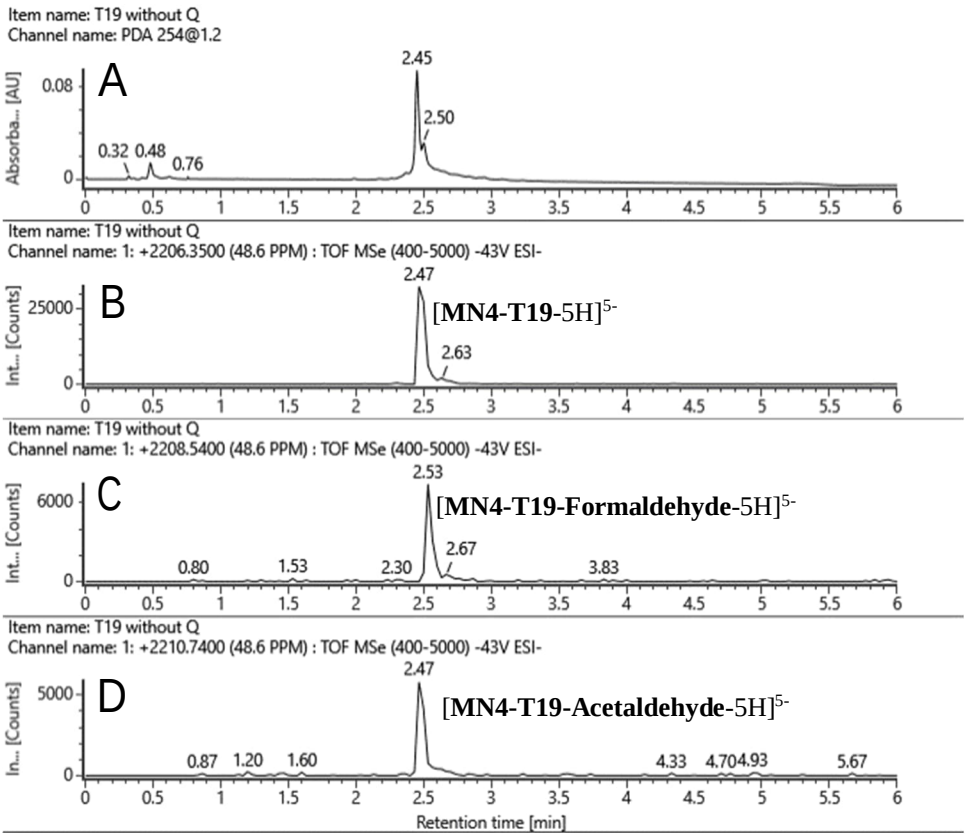


Figure S35. UV (A) and extracted ion (B—D) UPLC traces of **MN4-T19** with aldehyde mixture 3.1 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 μm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

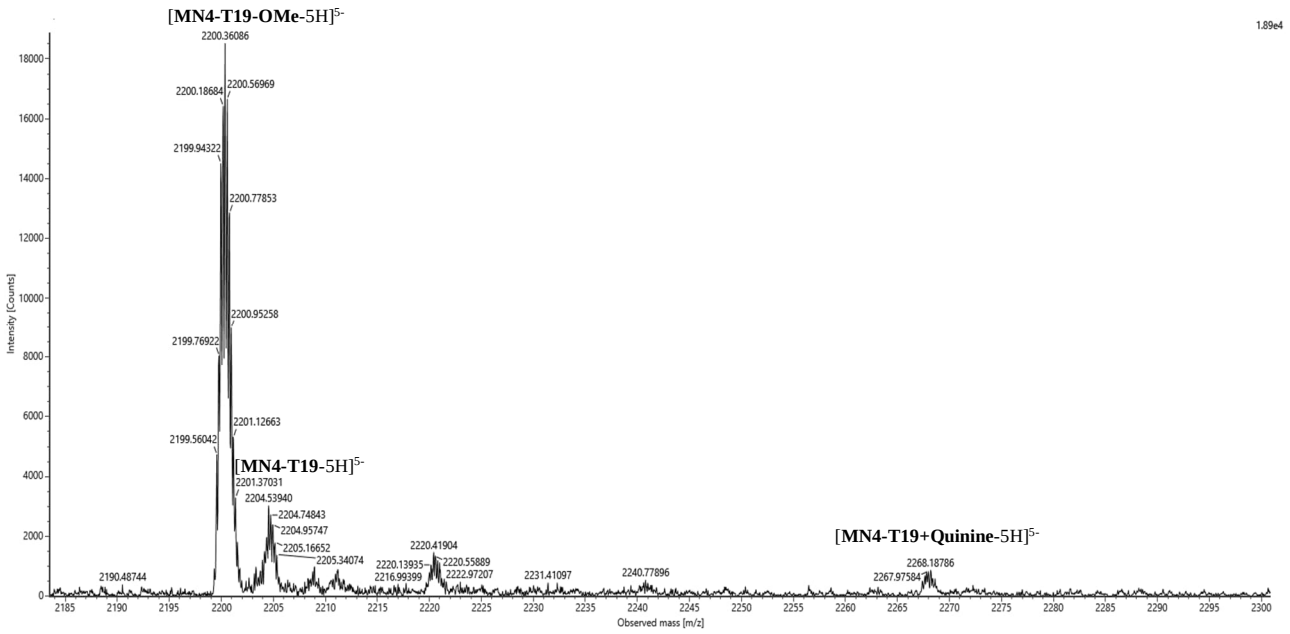


Figure S36. Mass spectrum of **MN4-T19** with aldehyde mixture 3.1 in the presence of quinine.

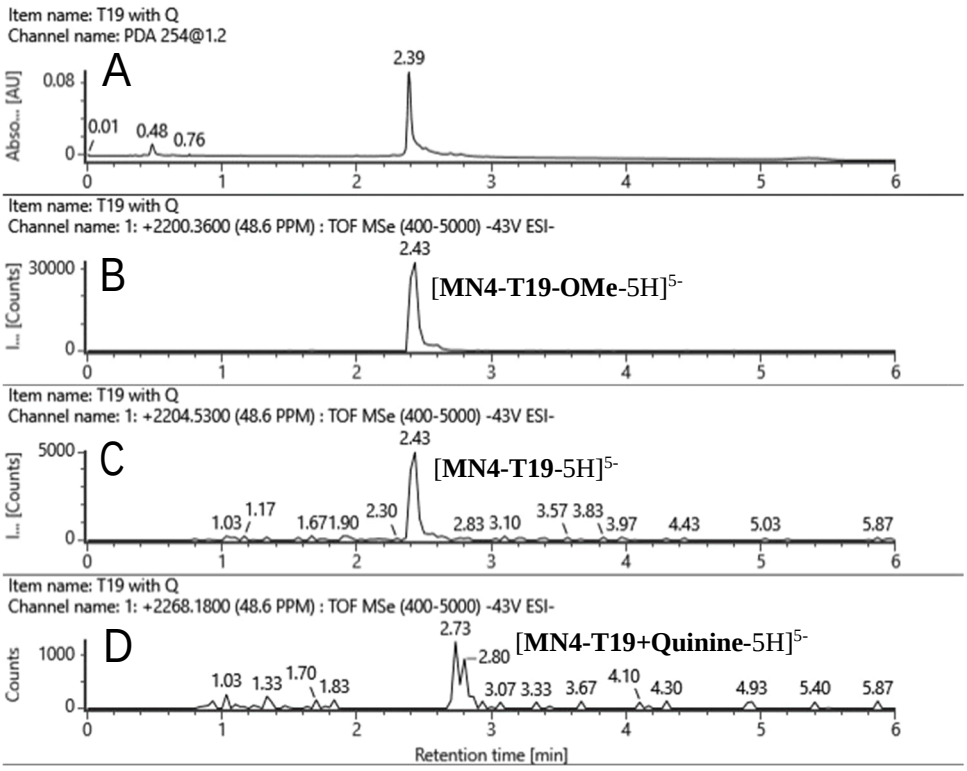


Figure S37. UV (A) and extracted ion (B—D) UPLC traces of **MN4-T19** with aldehyde mixture 3.1 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

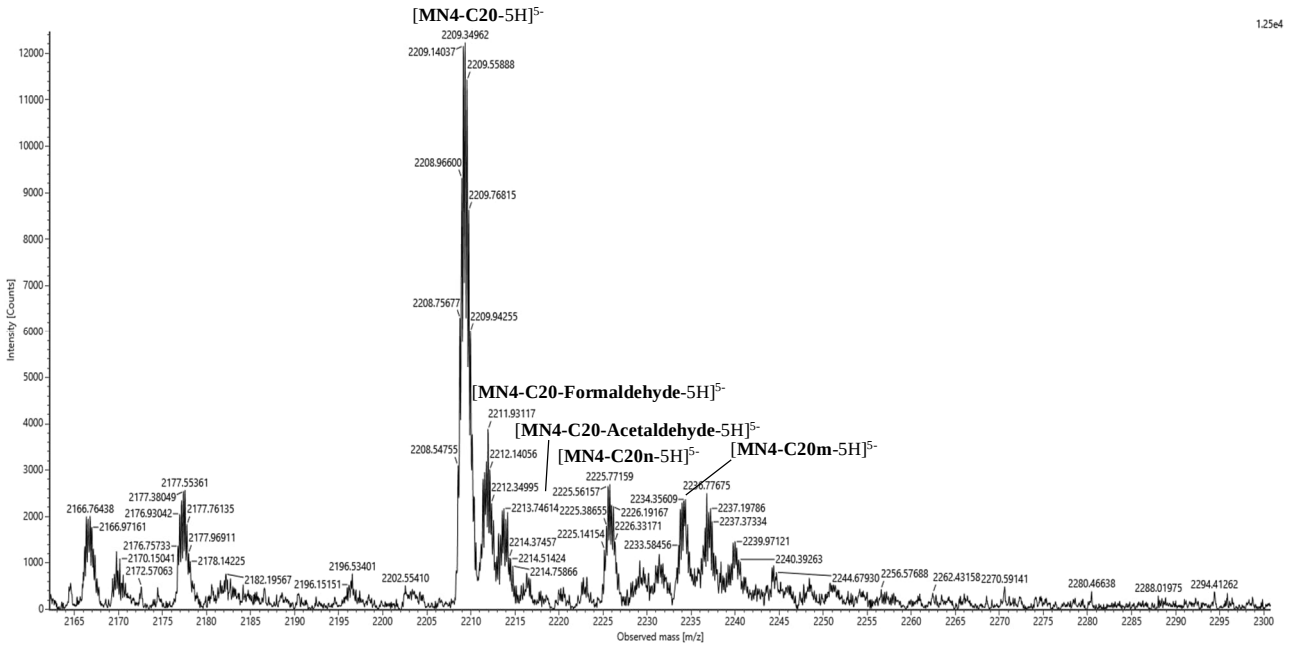


Figure S38. Mass spectrum of **MN4-C20** with aldehyde mixture 3.1 in the absence of quinine.

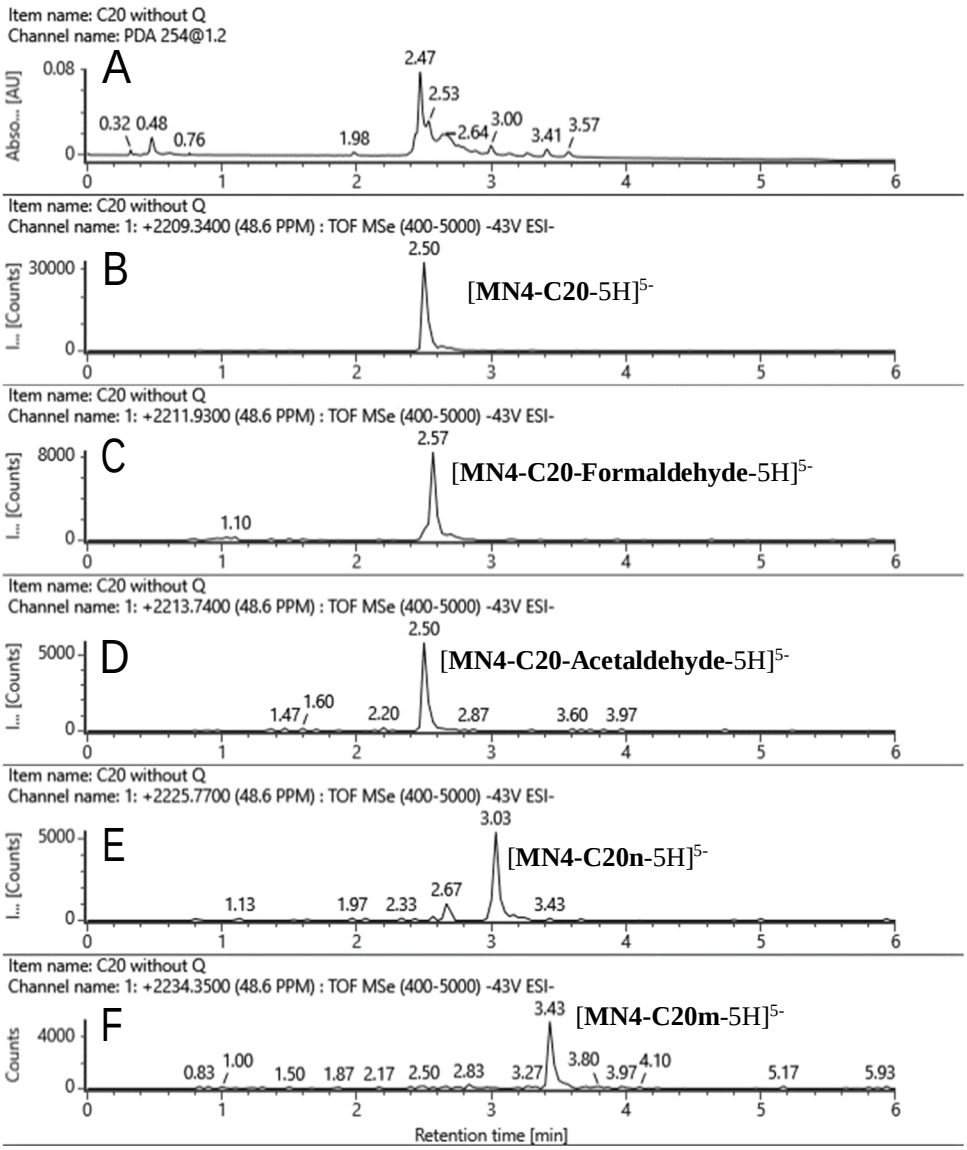


Figure S39. UV (A) and extracted ion (B—F) UPLC traces of **MN4-C20** with aldehyde mixture 3.1 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

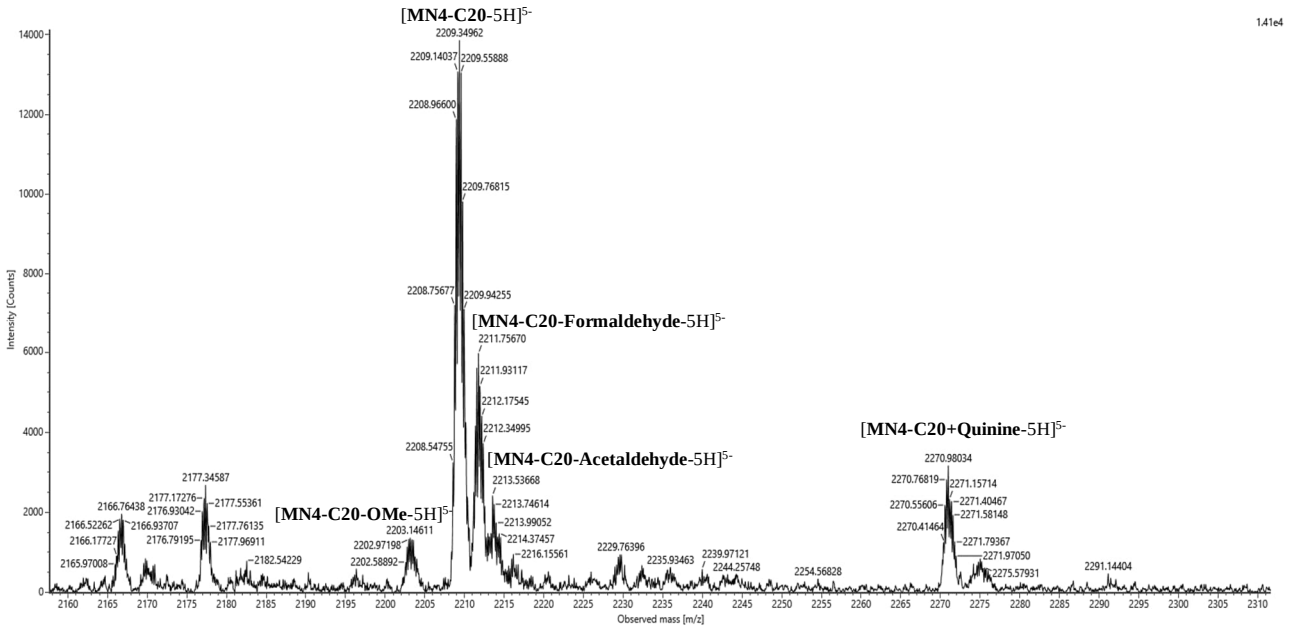


Figure S40. Mass spectrum of **MN4-C20** with aldehyde mixture 3.1 in the presence of quinine.

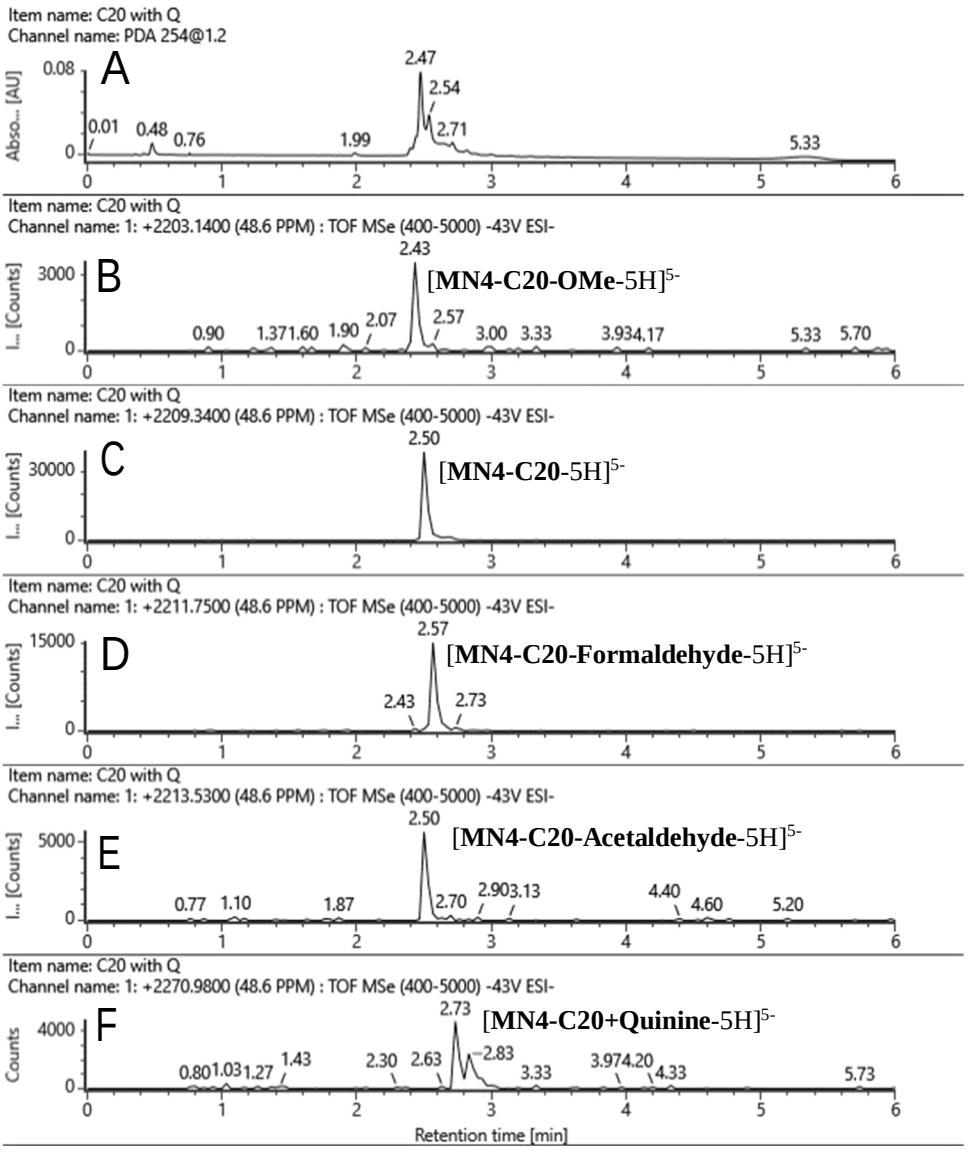


Figure S41. UV (A) and extracted ion (B—F) UPLC traces of **MN4-C20** with aldehyde mixture 3.1 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

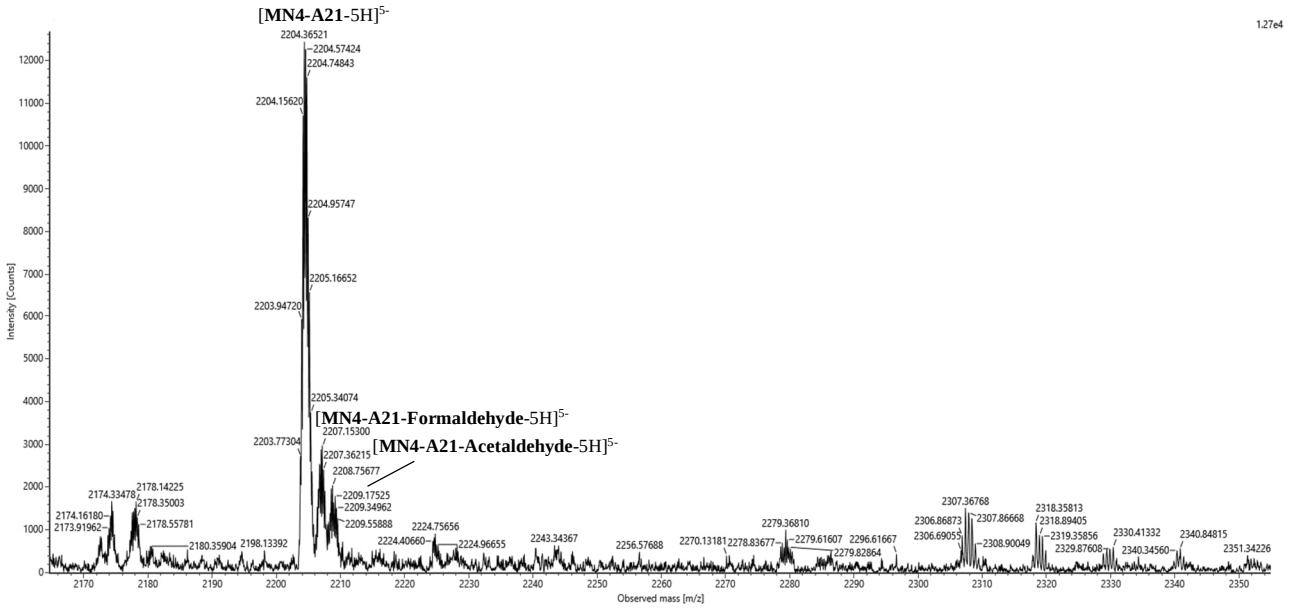


Figure S42. Mass spectrum of **MN4-A21** with aldehyde mixture 3.1 in the absence of quinine.

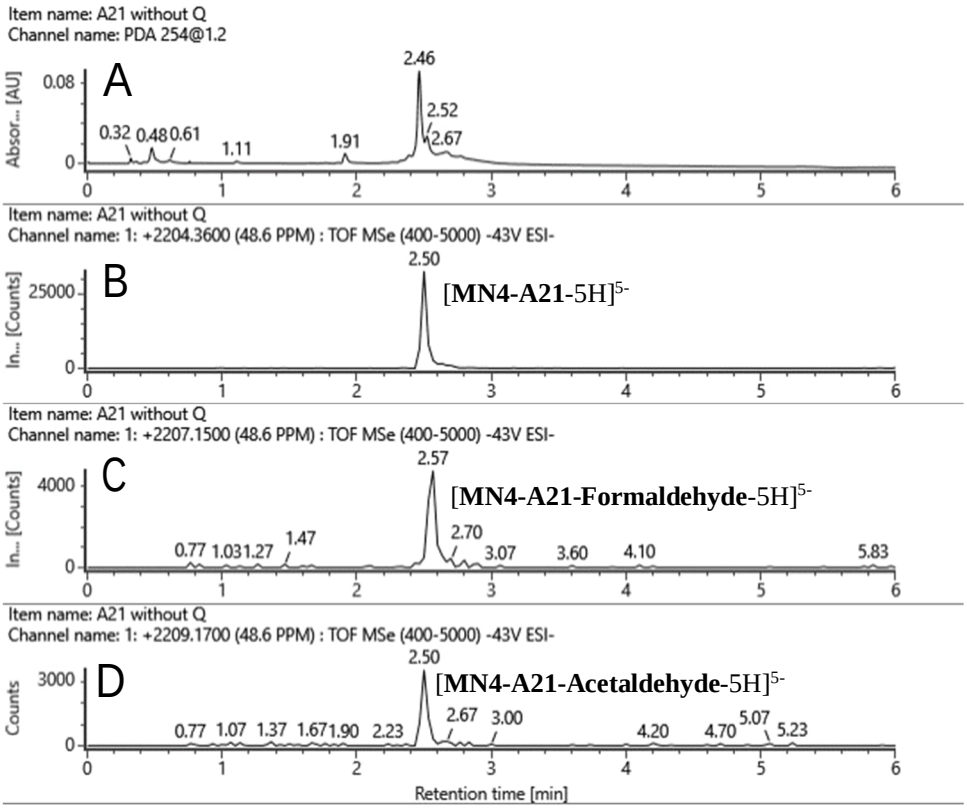


Figure S43. UV (A) and extracted ion (B—D) UPLC traces of **MN4-A21** with aldehyde mixture 3.1 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

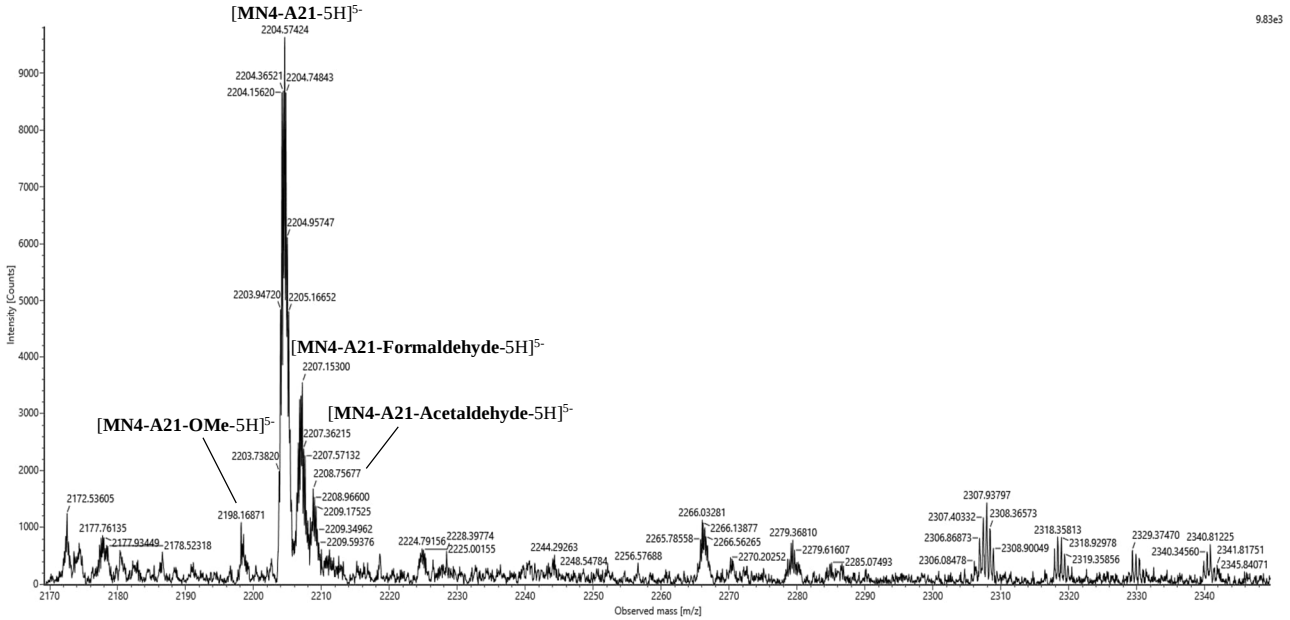


Figure S44. Mass spectrum of **MN4-A21** with aldehyde mixture 3.1 in the presence of quinine.

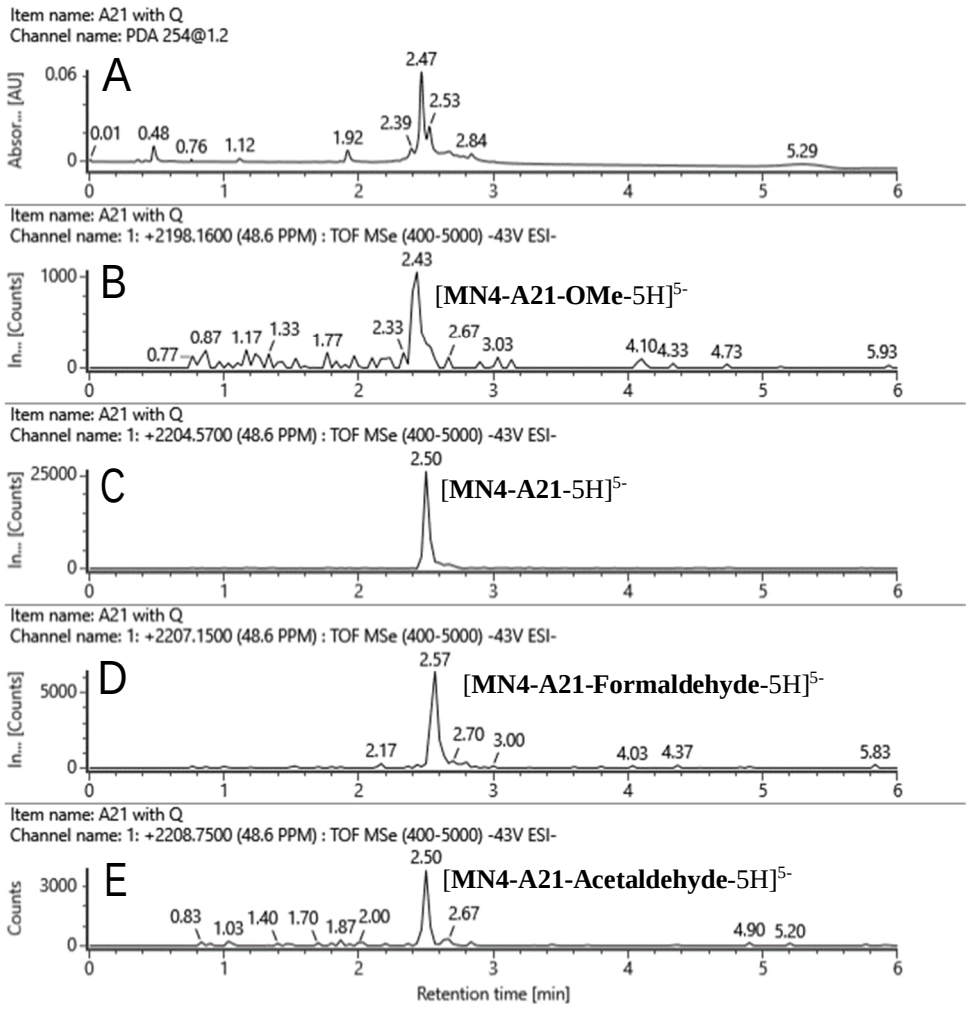


Figure S45. UV (A) and extracted ion (B—E) UPLC traces of **MN4-A21** with aldehyde mixture 3.1 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

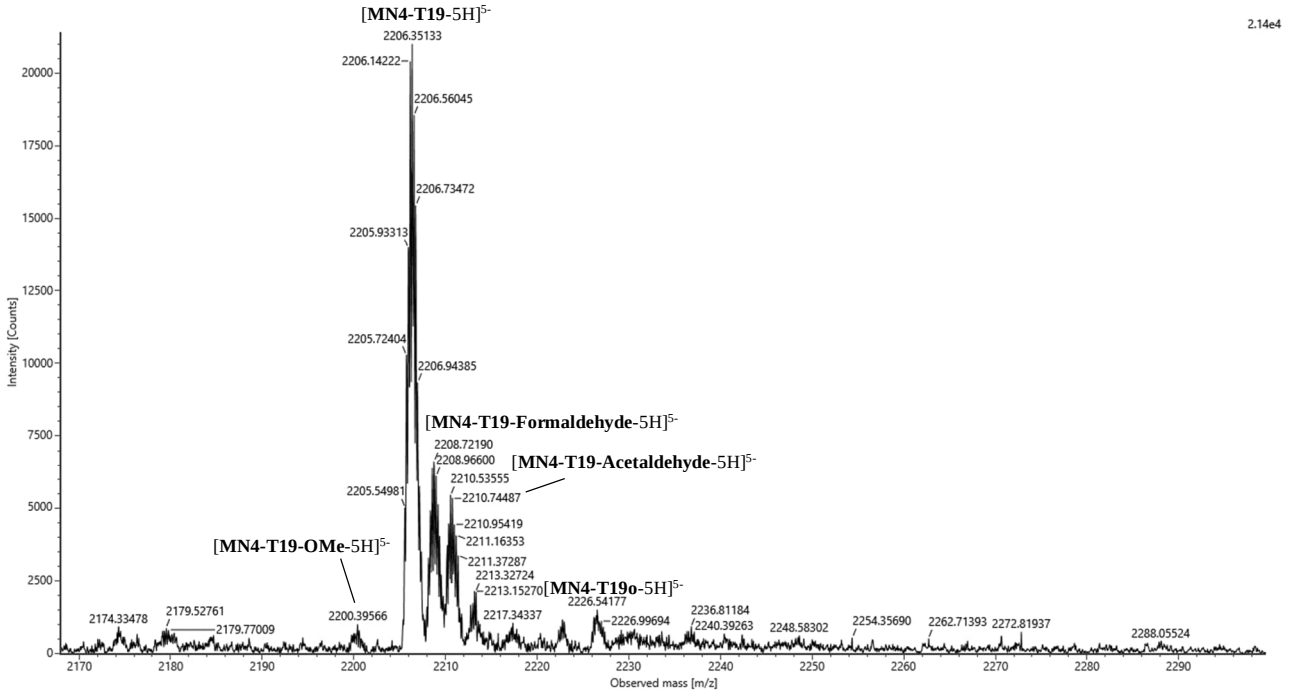


Figure S46. Mass spectrum of **MN4-T19** with aldehyde mixture 3.2 in the absence of quinine.

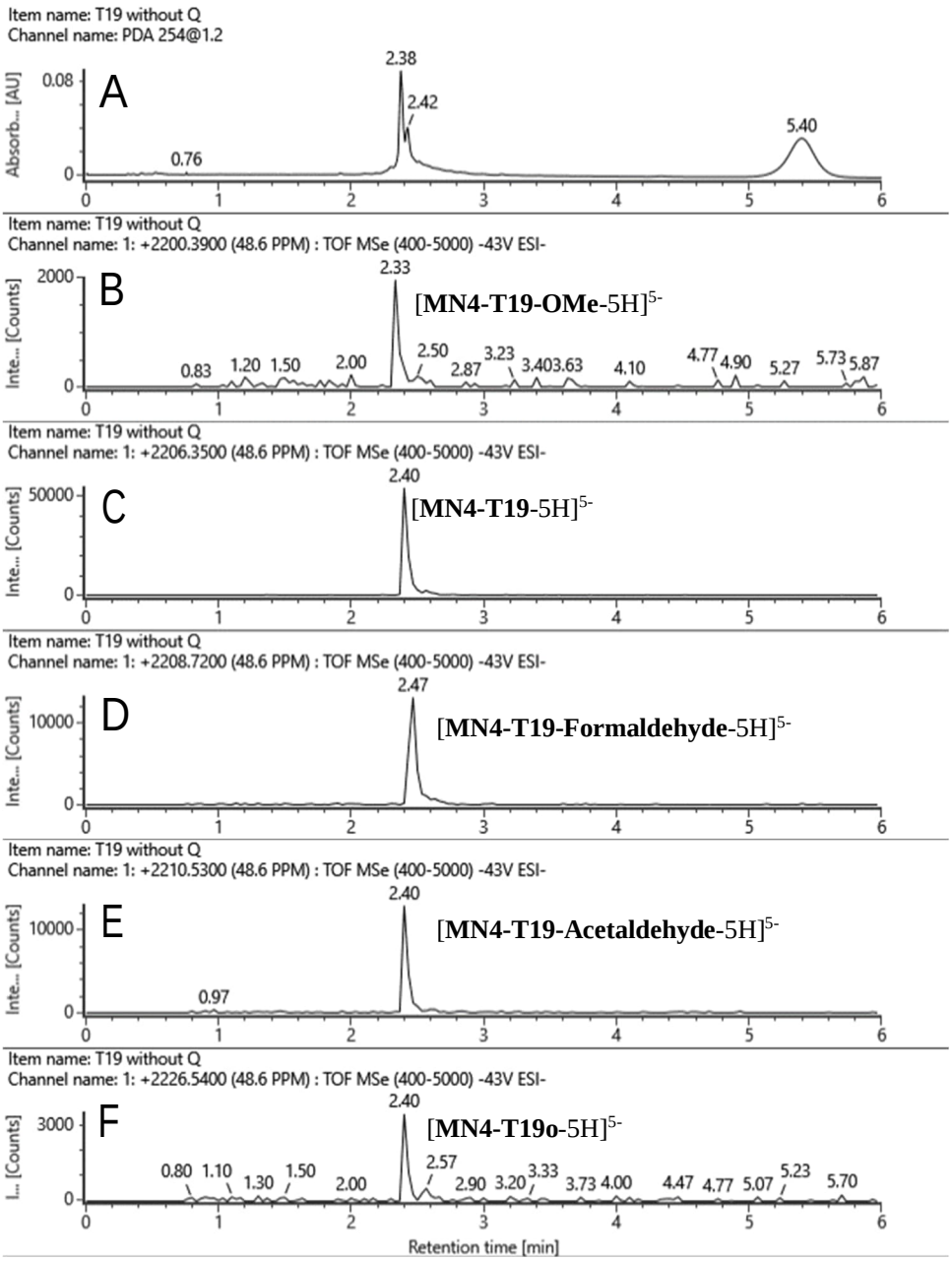


Figure S47. UV (A) and extracted ion (B—F) UPLC traces of **MN4-T19** with aldehyde mixture 3.2 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

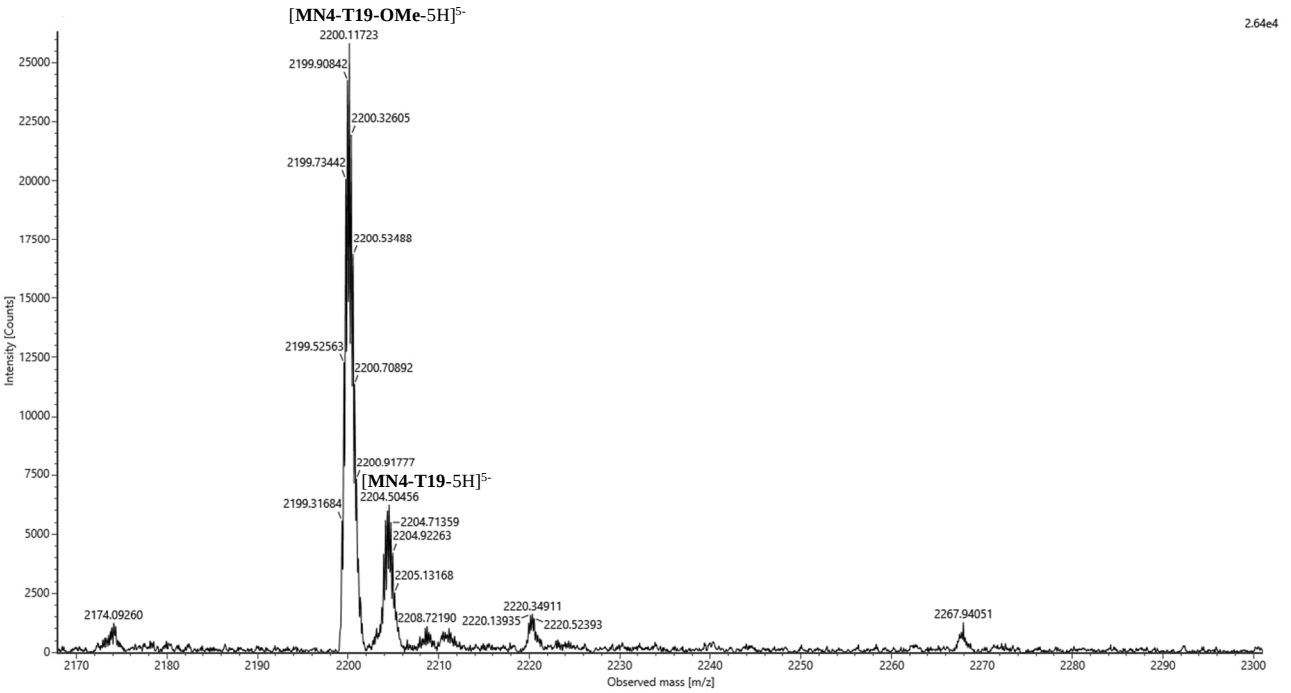


Figure S48. Mass spectrum of **MN4-T19** with aldehyde mixture 3.2 in the presence of quinine.

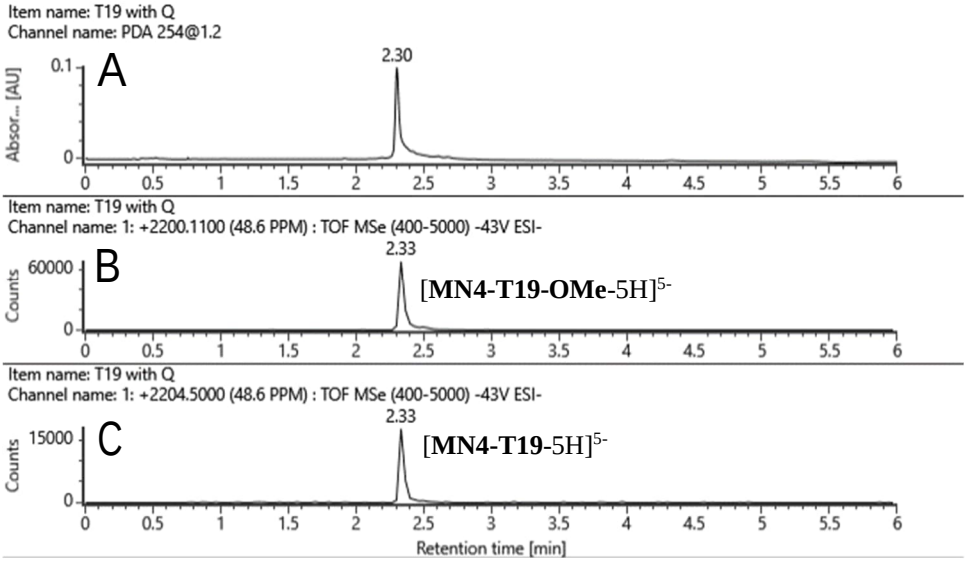


Figure S49. UV (A) and extracted ion (B and C) UPLC traces of **MN4-T19** with aldehyde mixture 3.2 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

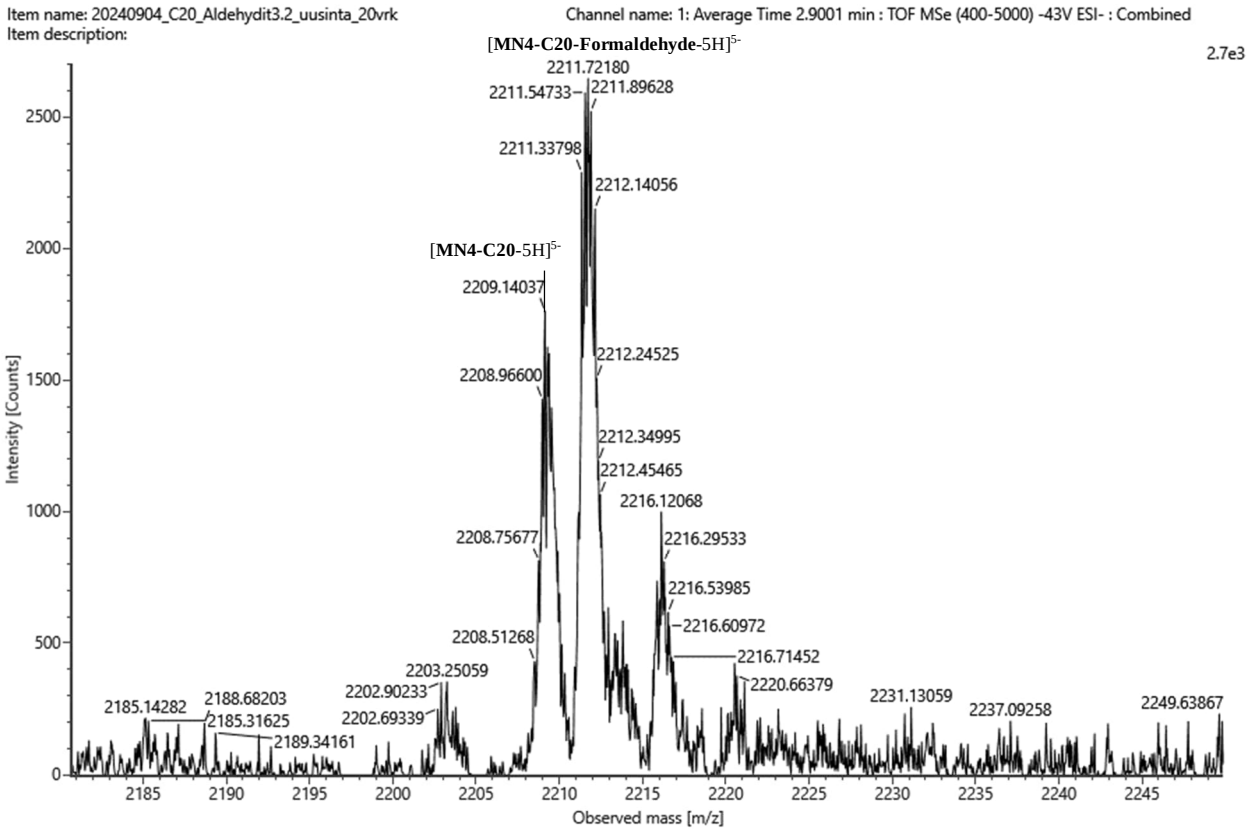


Figure S50. Mass spectrum of **MN4-C20** with aldehyde mixture 3.2 in the absence of quinine.

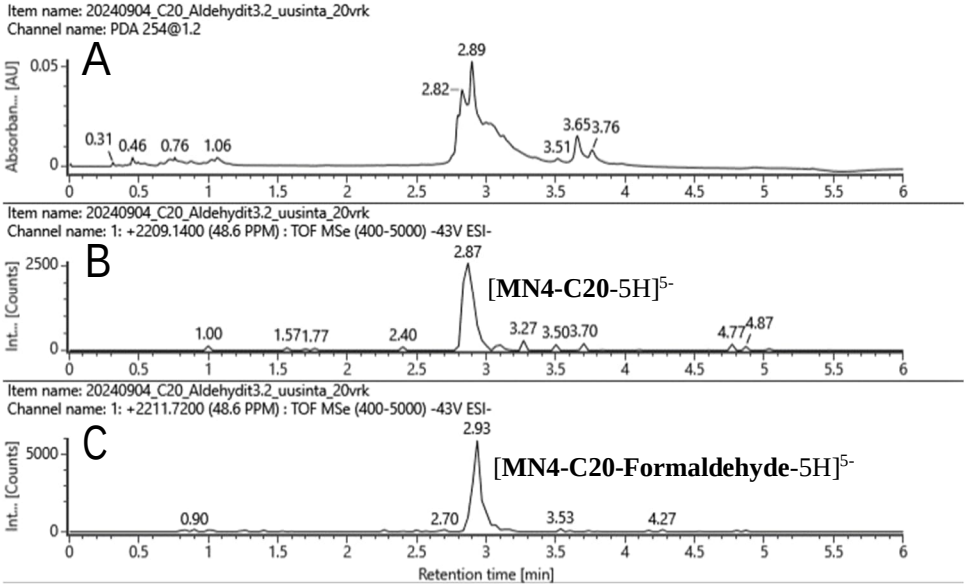


Figure S51. UV (A) and extracted ion (B and C) UPLC traces of **MN4-C20** with aldehyde mixture 3.2 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

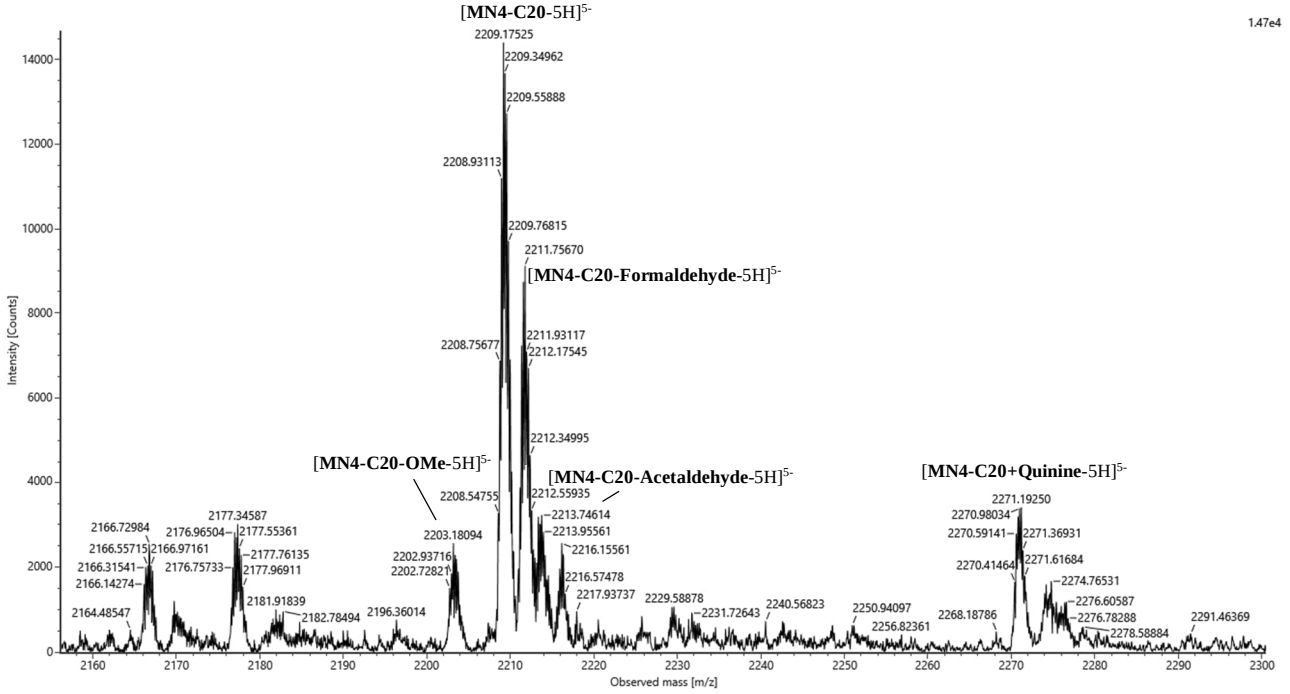


Figure S52. Mass spectrum of **MN4-C20** with aldehyde mixture 3.2 in the presence of quinine.

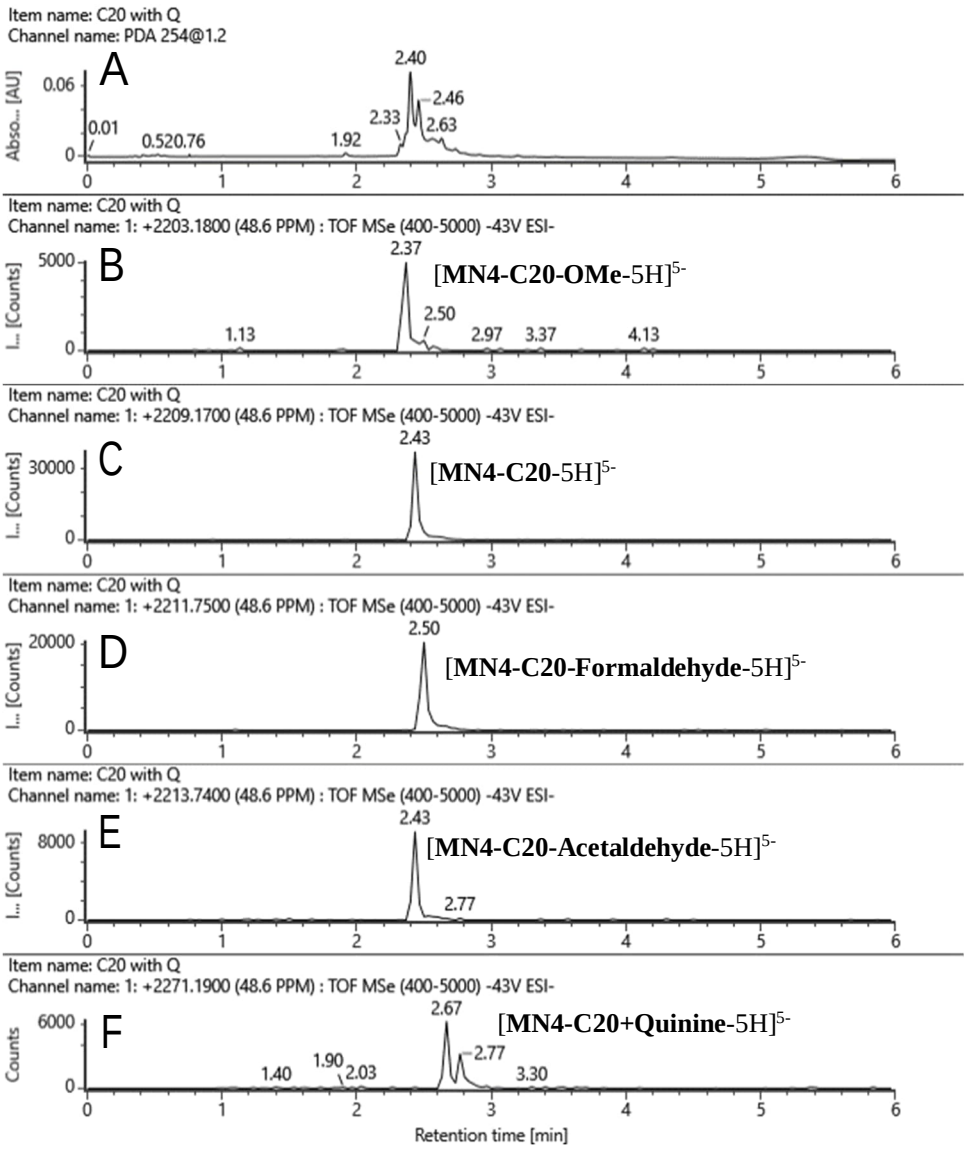


Figure S53. UV (A) and extracted ion (B—F) UPLC traces of **MN4-C20** with aldehyde mixture 3.2 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

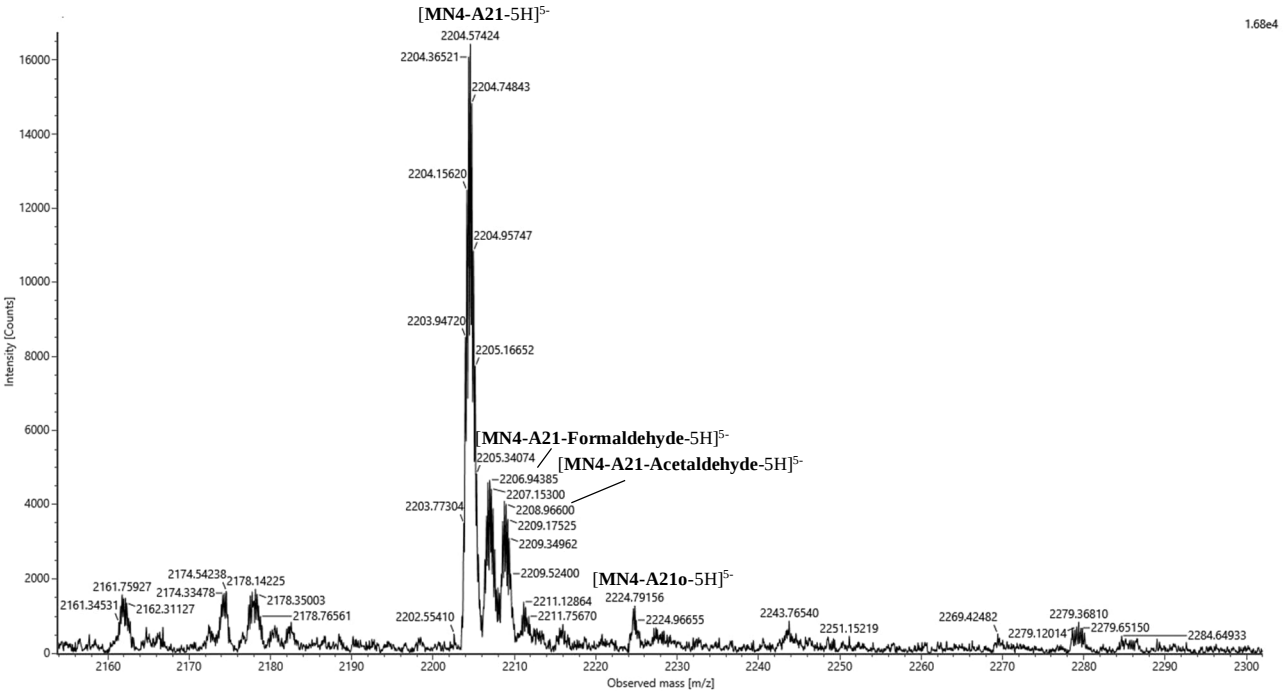


Figure S54. Mass spectrum of **MN4-A21** with aldehyde mixture 3.2 in the absence of quinine.

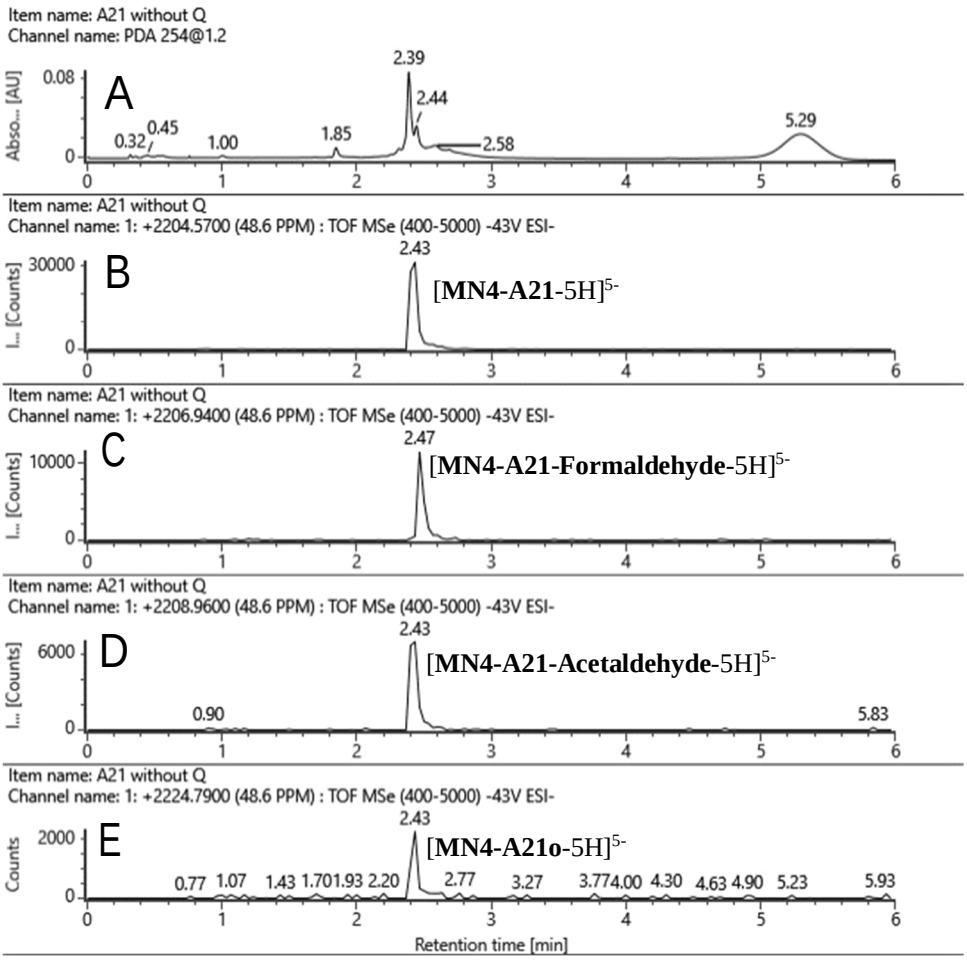


Figure S55. UV (A) and extracted ion (B—E) UPLC traces of **MN4-A21** with aldehyde mixture 3.2 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

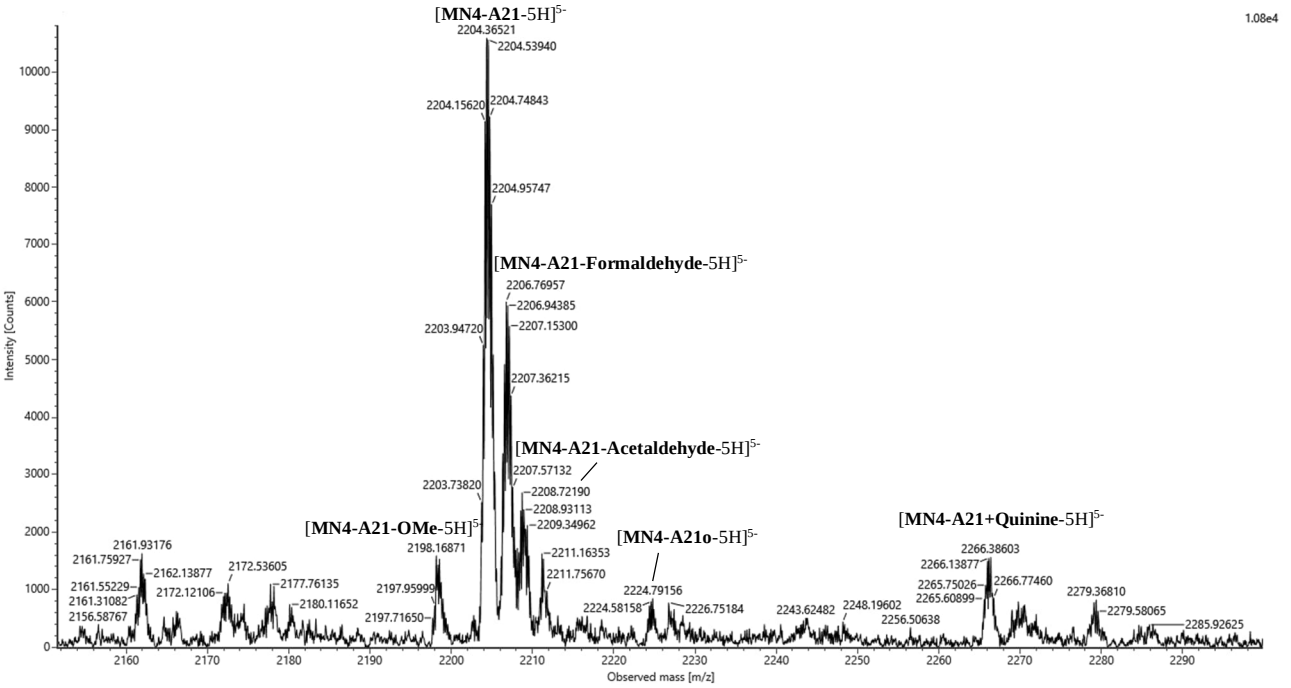


Figure S56. Mass spectrum of **MN4-A21** with aldehyde mixture 3.2 in the presence of quinine.

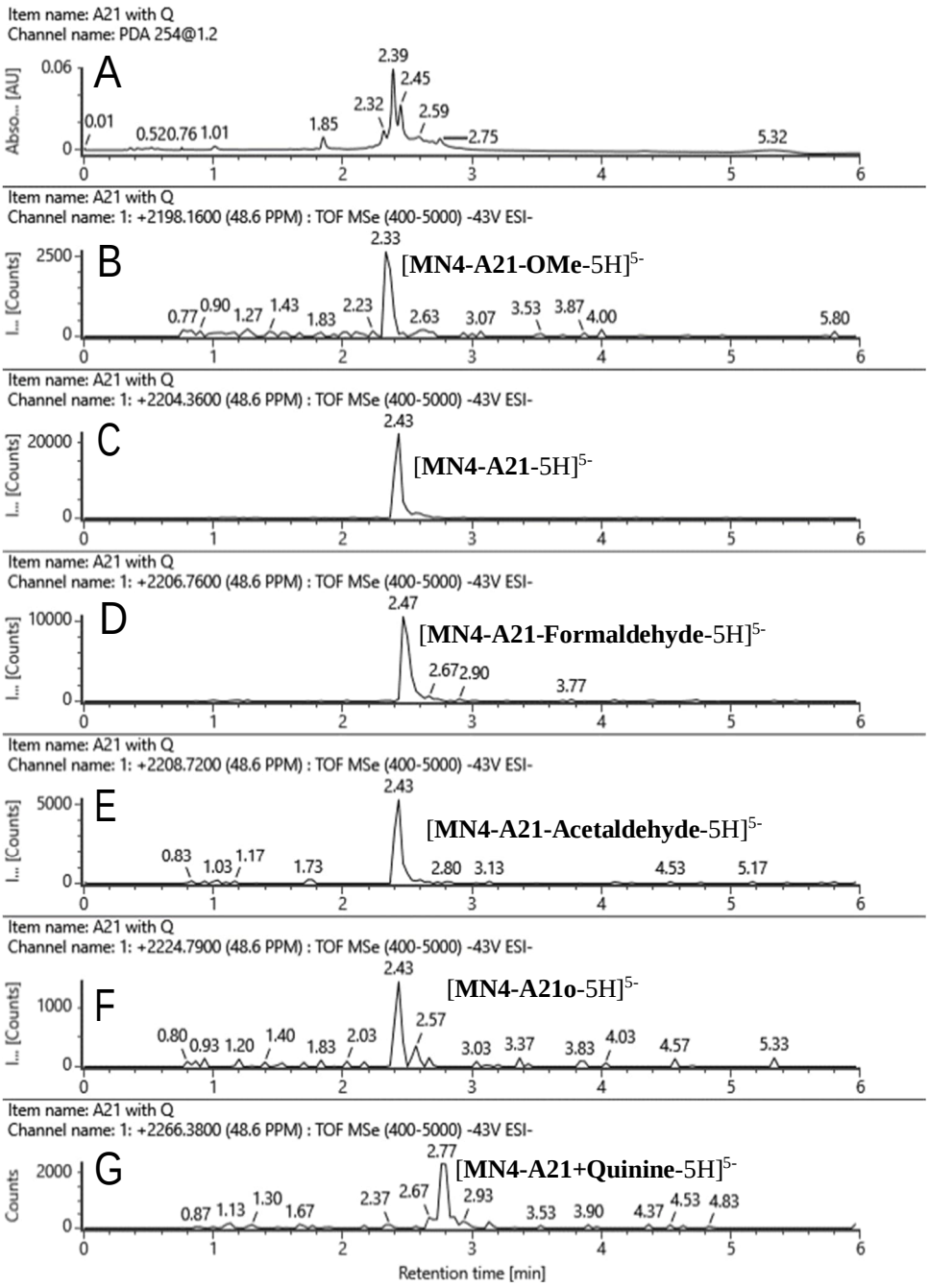


Figure S57. UV (A) and extracted ion (B—G) UPLC traces of **MN4-A21** with aldehyde mixture 3.2 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

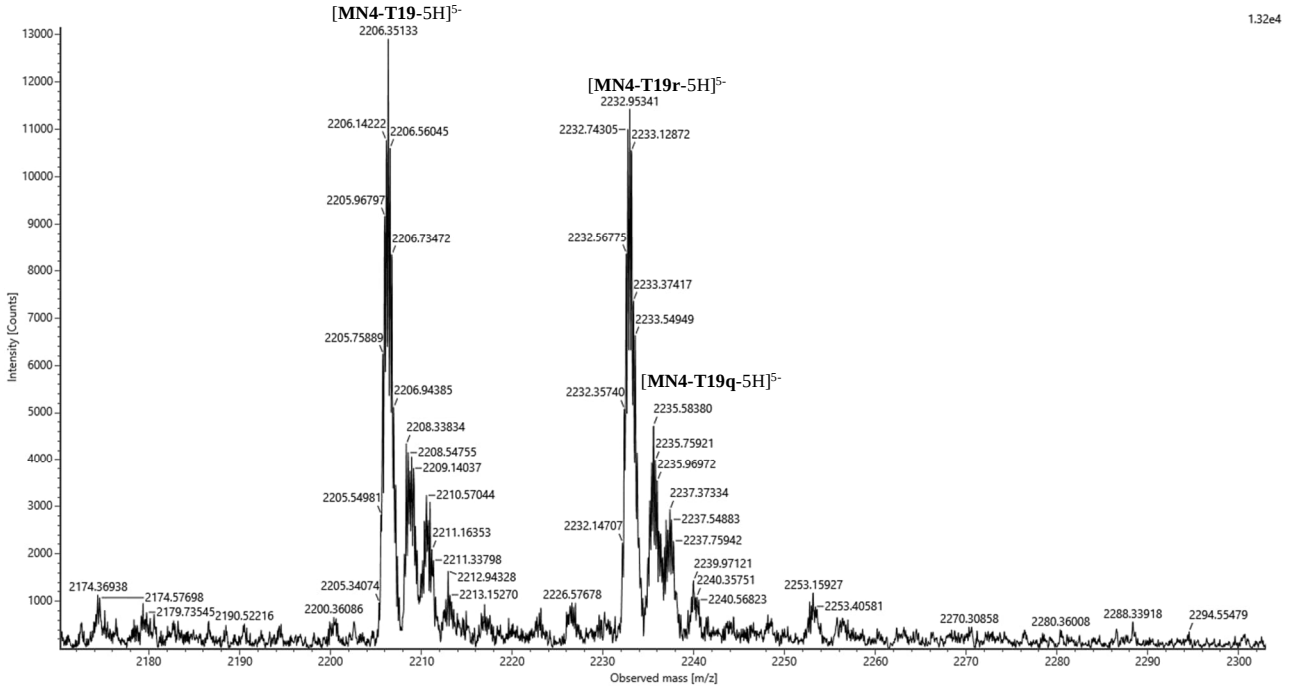


Figure S58. Mass spectrum of **MN4-T19** with aldehyde mixture 3.3 in the absence of quinine.

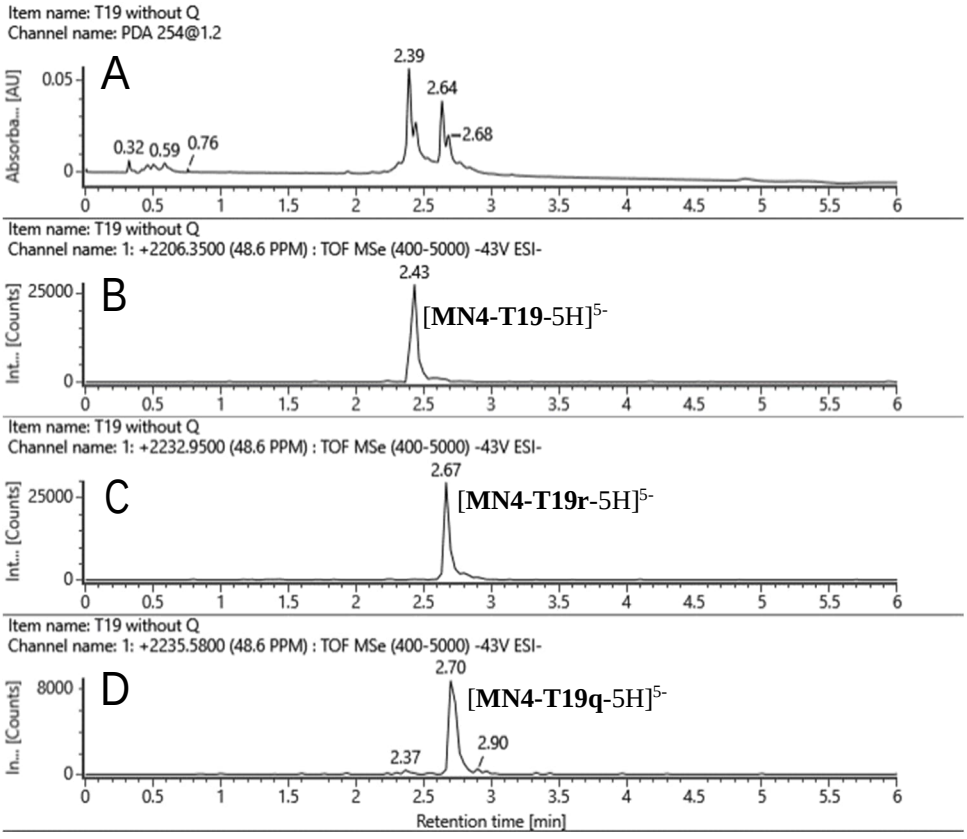


Figure S59. UV (A) and extracted ion (B—D) UPLC traces of **MN4-T19** with aldehyde mixture 3.3 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

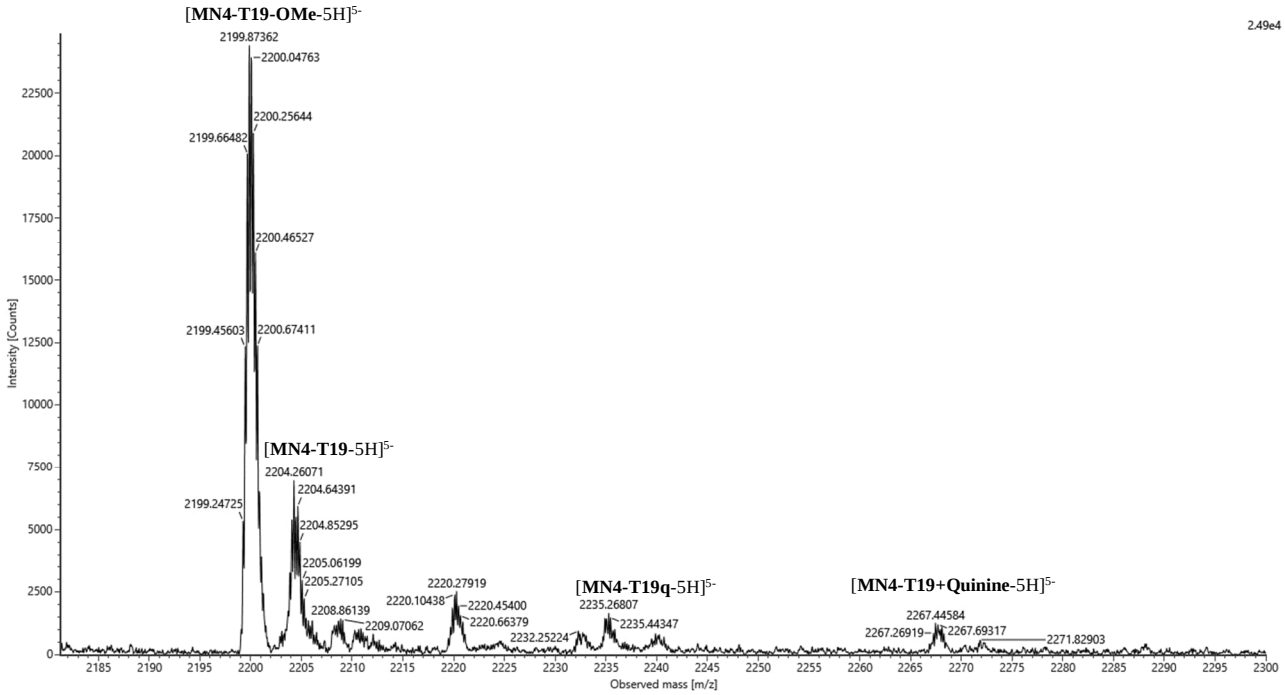


Figure S60. Mass spectrum of **MN4-T19** with aldehyde mixture 3.3 in the presence of quinine.

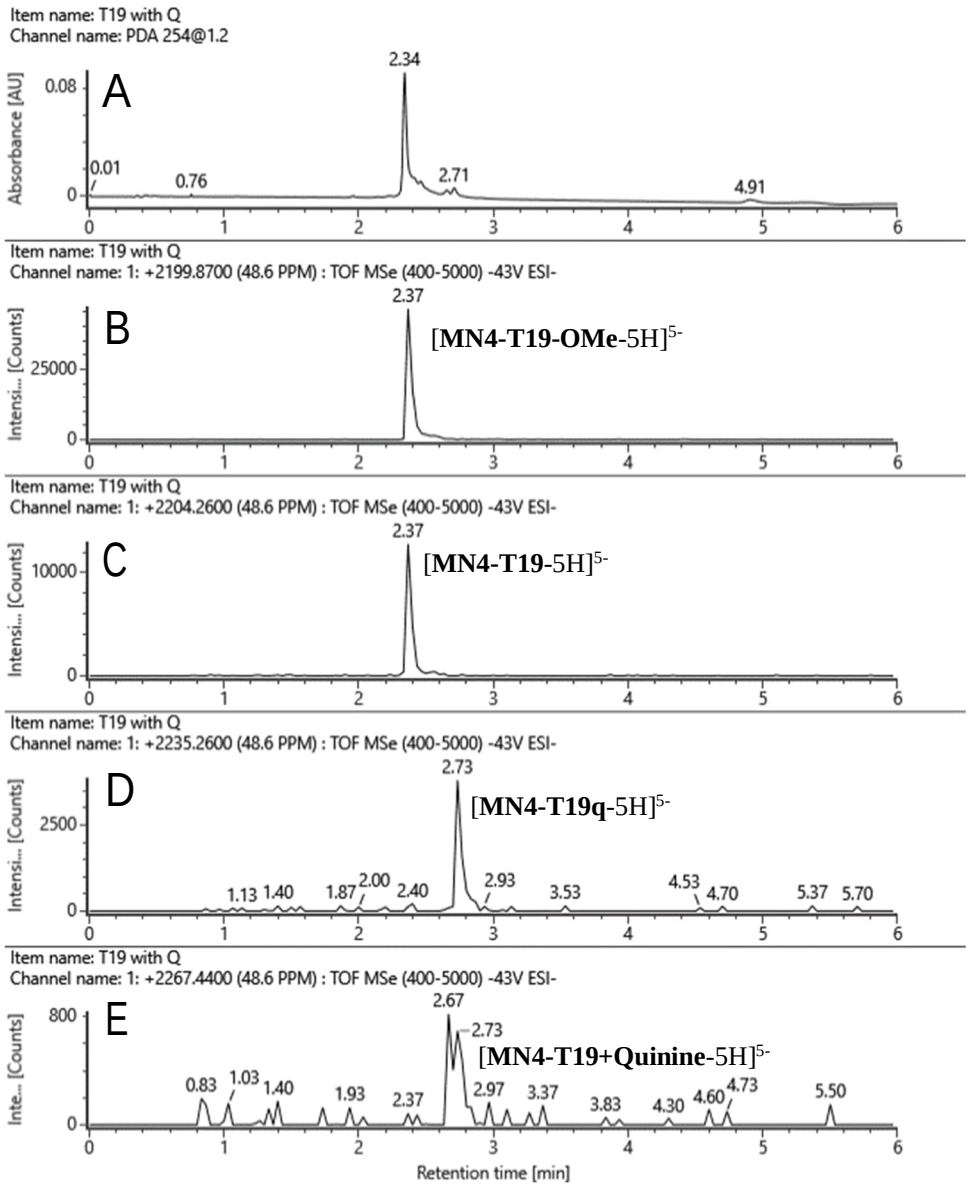


Figure S61. UV (A) and extracted ion (B—E) UPLC traces of **MN4-T19** with aldehyde mixture 3.3 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

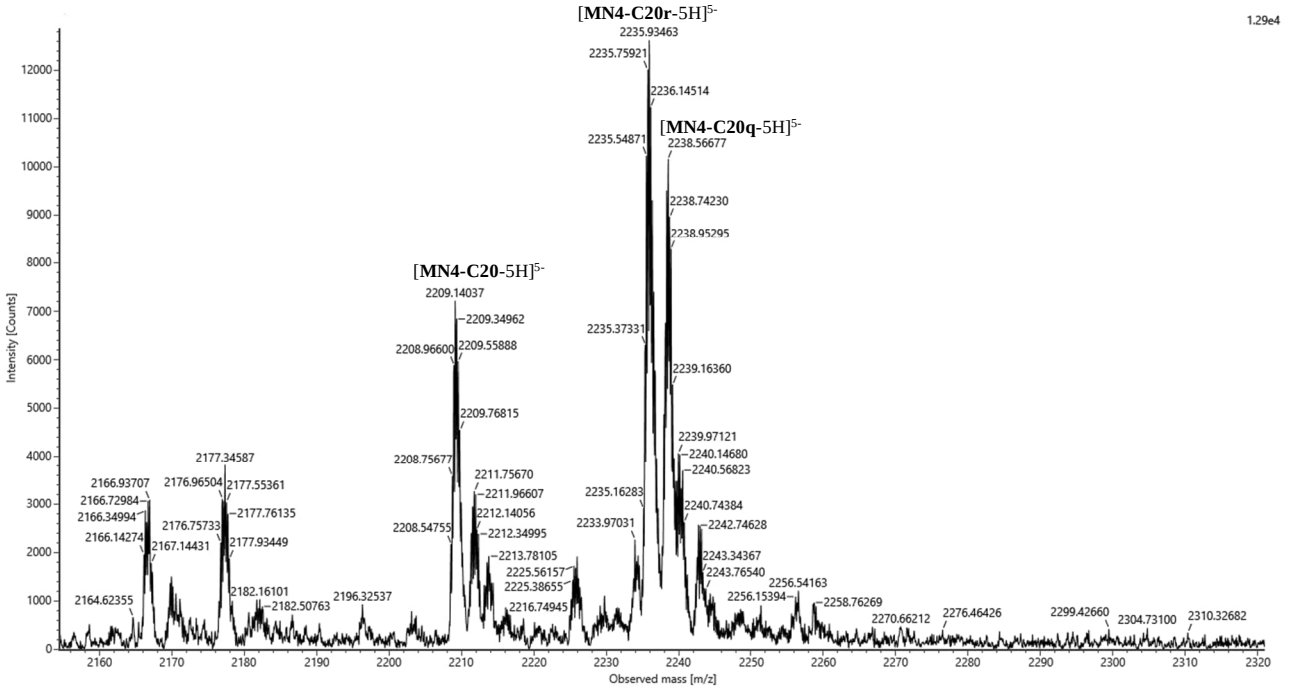


Figure S62. Mass spectrum of **MN4-C20** with aldehyde mixture 3.3 in the absence of quinine.

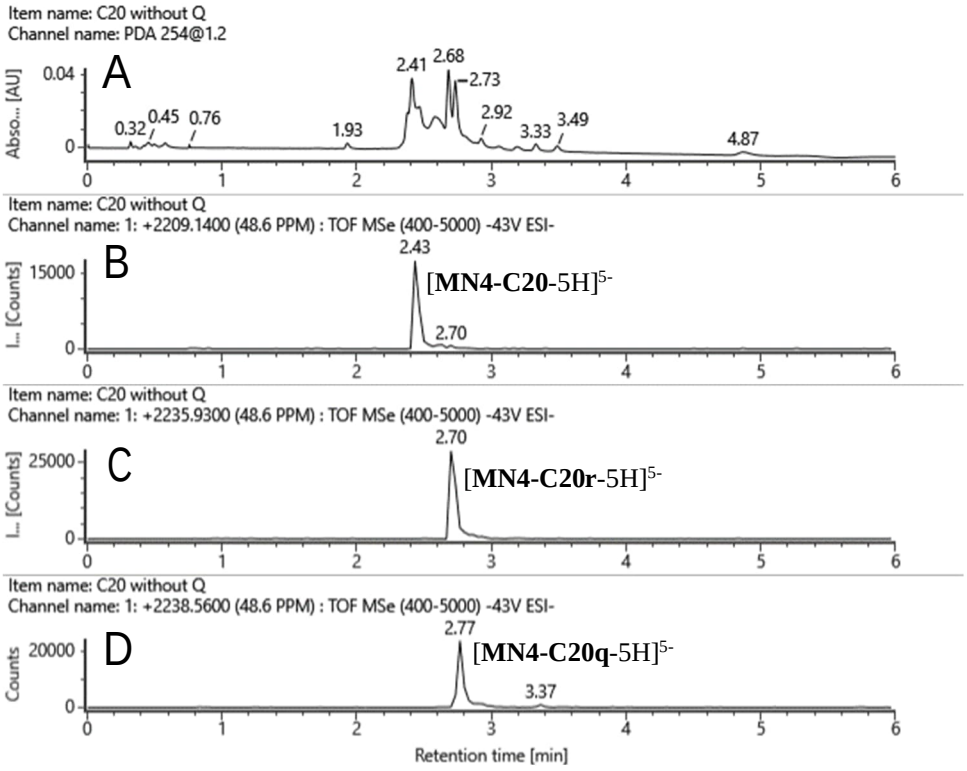


Figure S63. UV (A) and extracted ion (B—D) UPLC traces of **MN4-C20** with aldehyde mixture 3.3 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

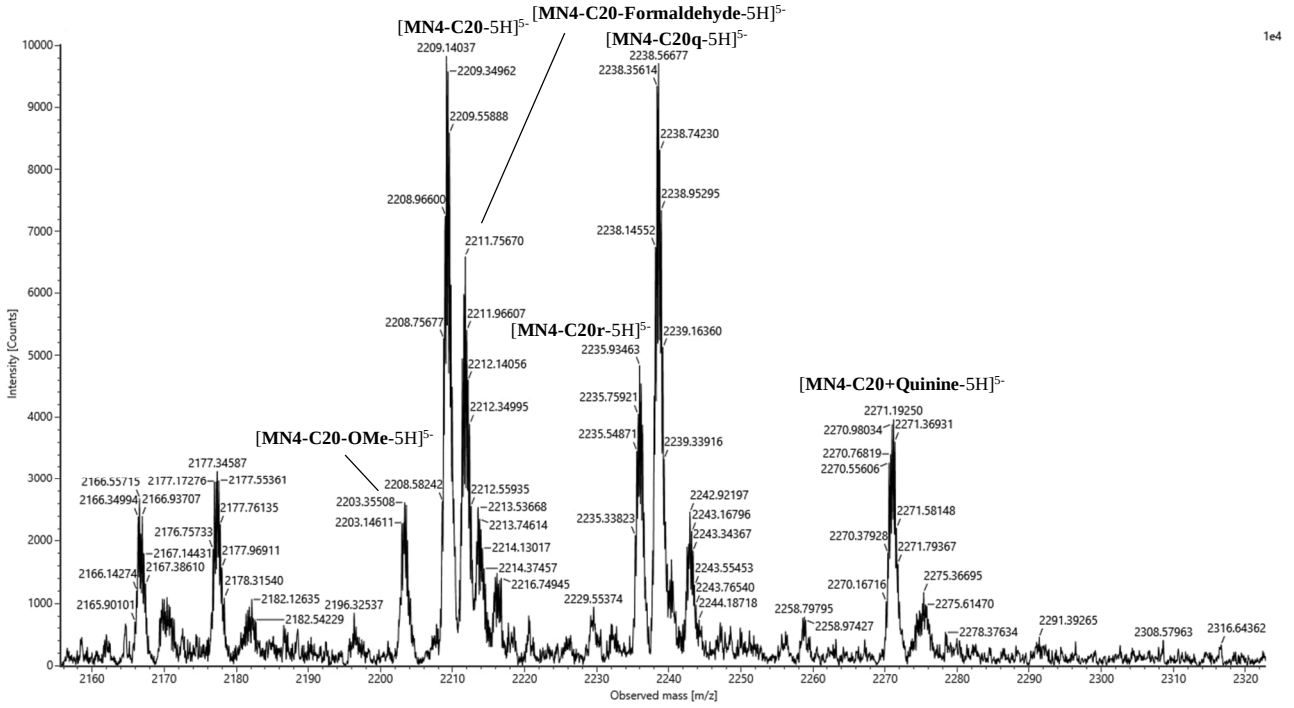


Figure S64. Mass spectrum of **MN4-C20** with aldehyde mixture 3.3 in the presence of quinine.

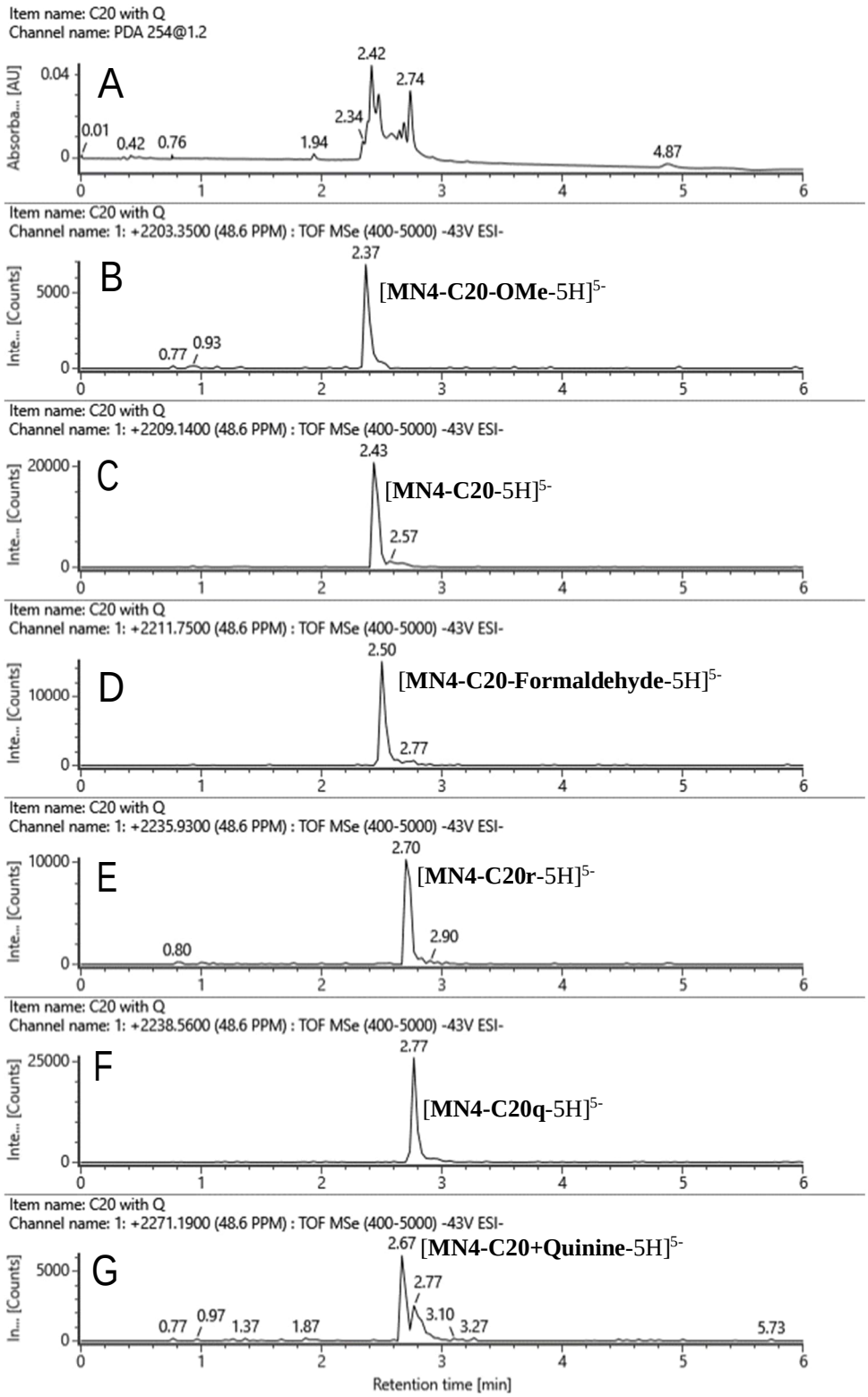


Figure S65. UV (A) and extracted ion (B—G) UPLC traces of **MN4-C20** with aldehyde mixture 3.3 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

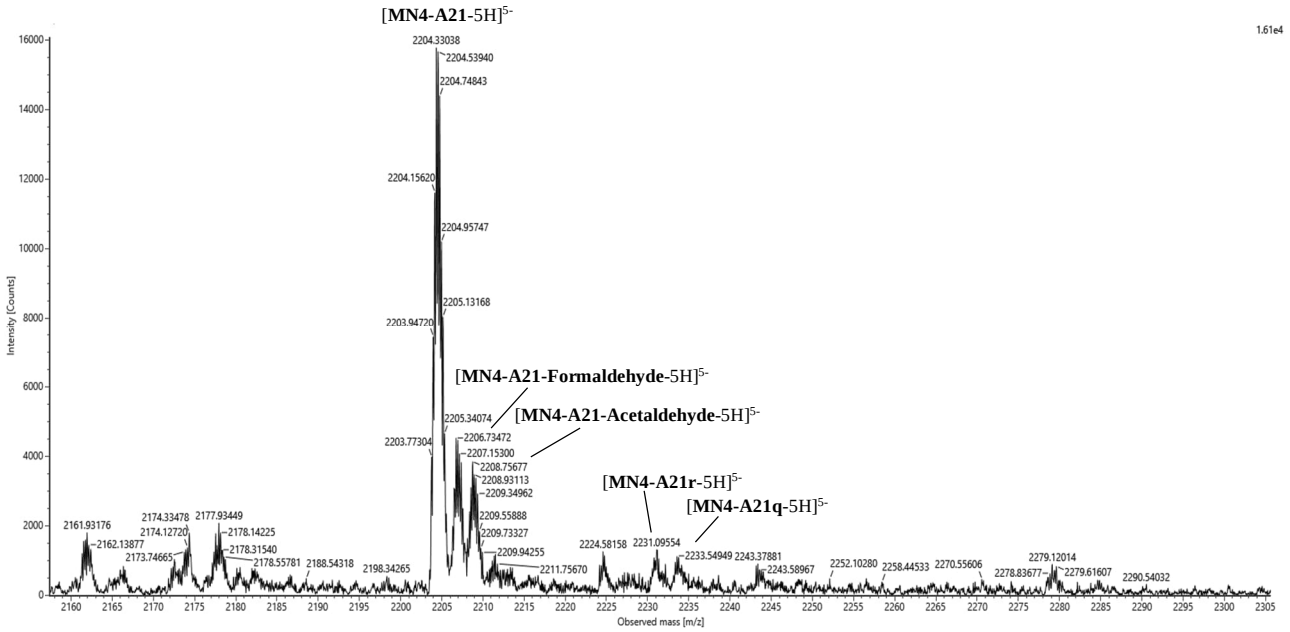


Figure S66. Mass spectrum of **MN4-A21** with aldehyde mixture 3.3 in the absence of quinine.

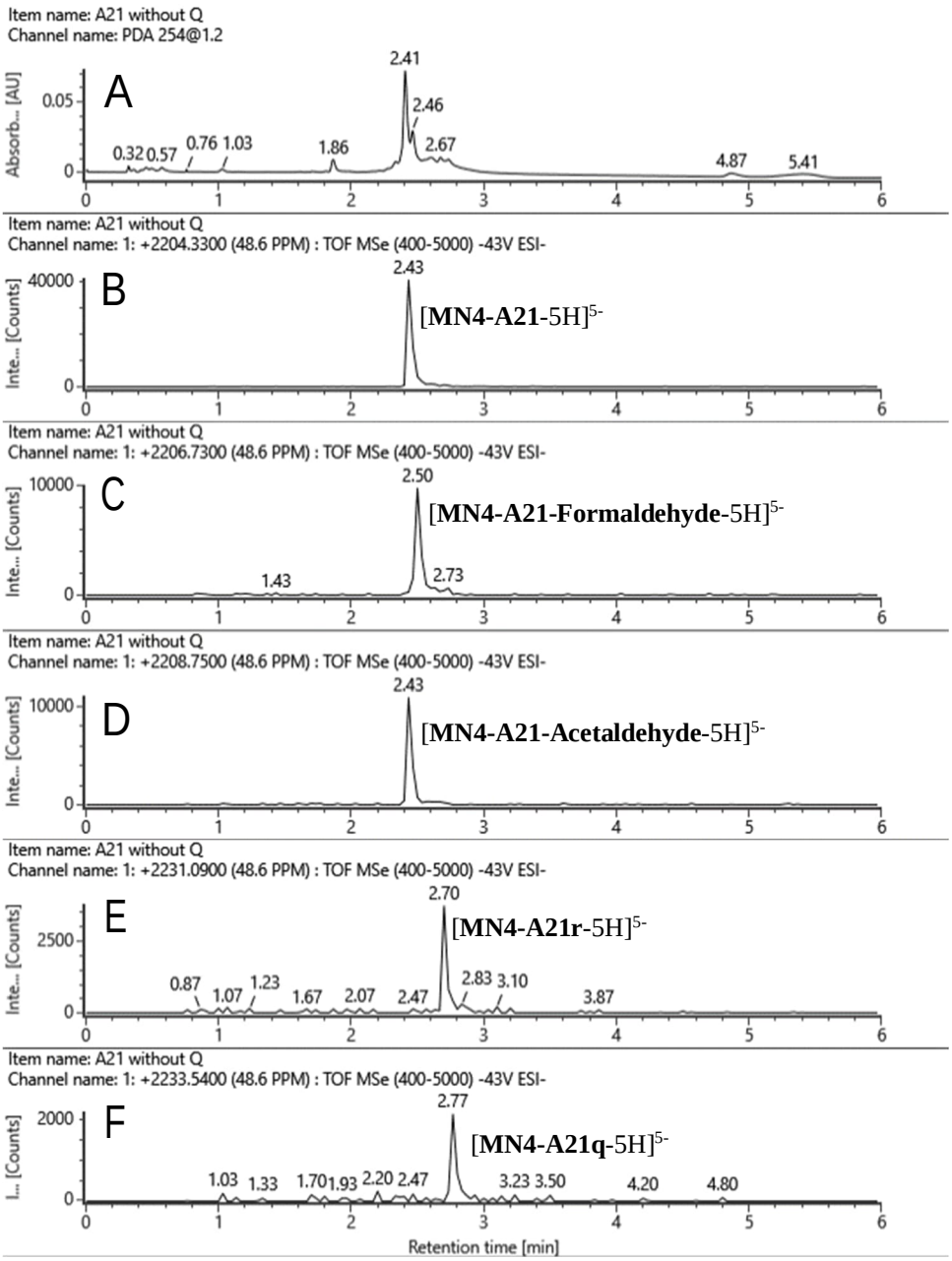


Figure S67. UV (A) and extracted ion (B—F) UPLC traces of **MN4-A21** with aldehyde mixture 3.3 in the absence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

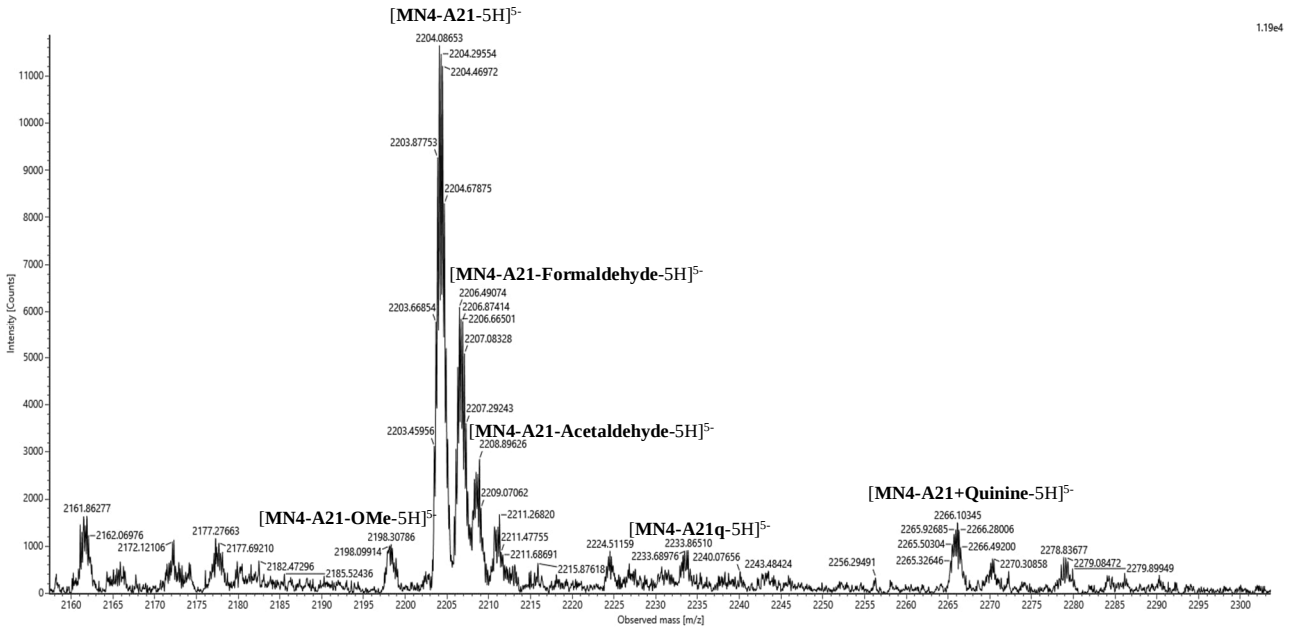


Figure S68. Mass spectrum of **MN4-A21** with aldehyde mixture 3.3 in the presence of quinine.

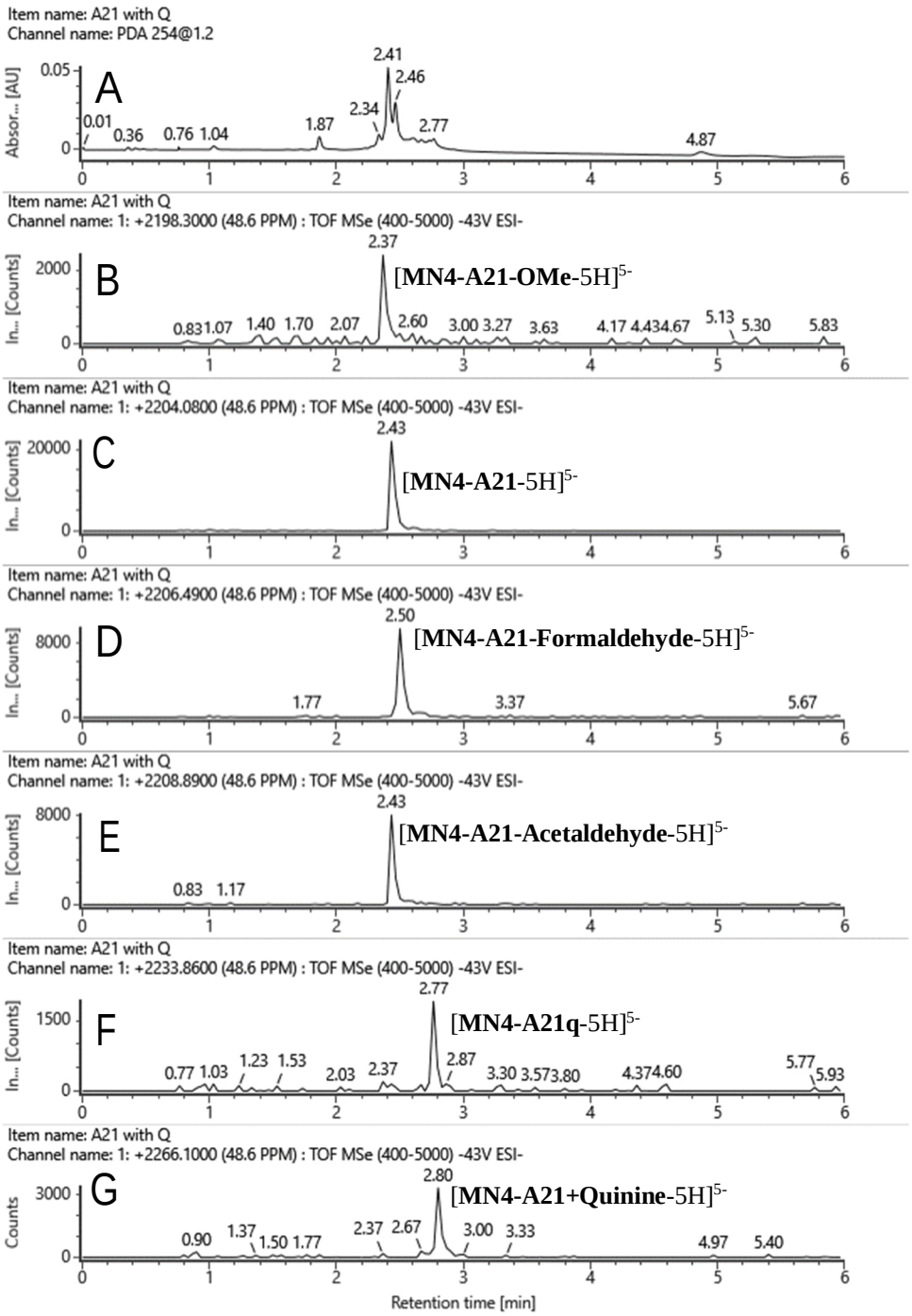


Figure S69. UV (A) and extracted ion (B—G) UPLC traces of **MN4-A21** with aldehyde mixture 3.3 in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

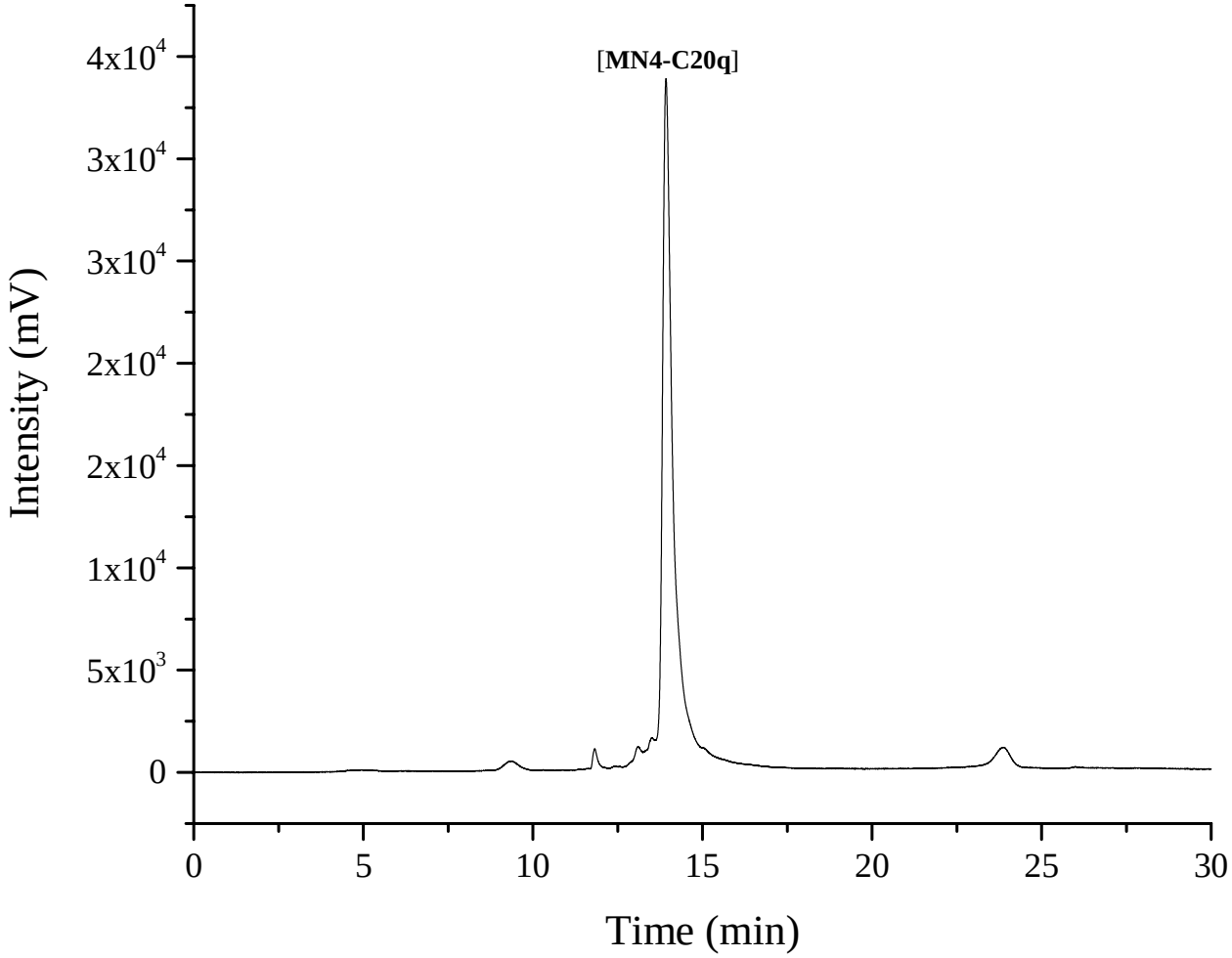


Figure S70. RP-HPLC traces of crude **MN4-C20q**; bioZen oligo LC column (2.6 µm, 150 x 4.6 mm); flow rate = 0.6 ml min-1; linear gradient (5-25 % over 20 min) of 50 mM aqueous TEAA in ACN (pH 7.0); λ = 260 nm.

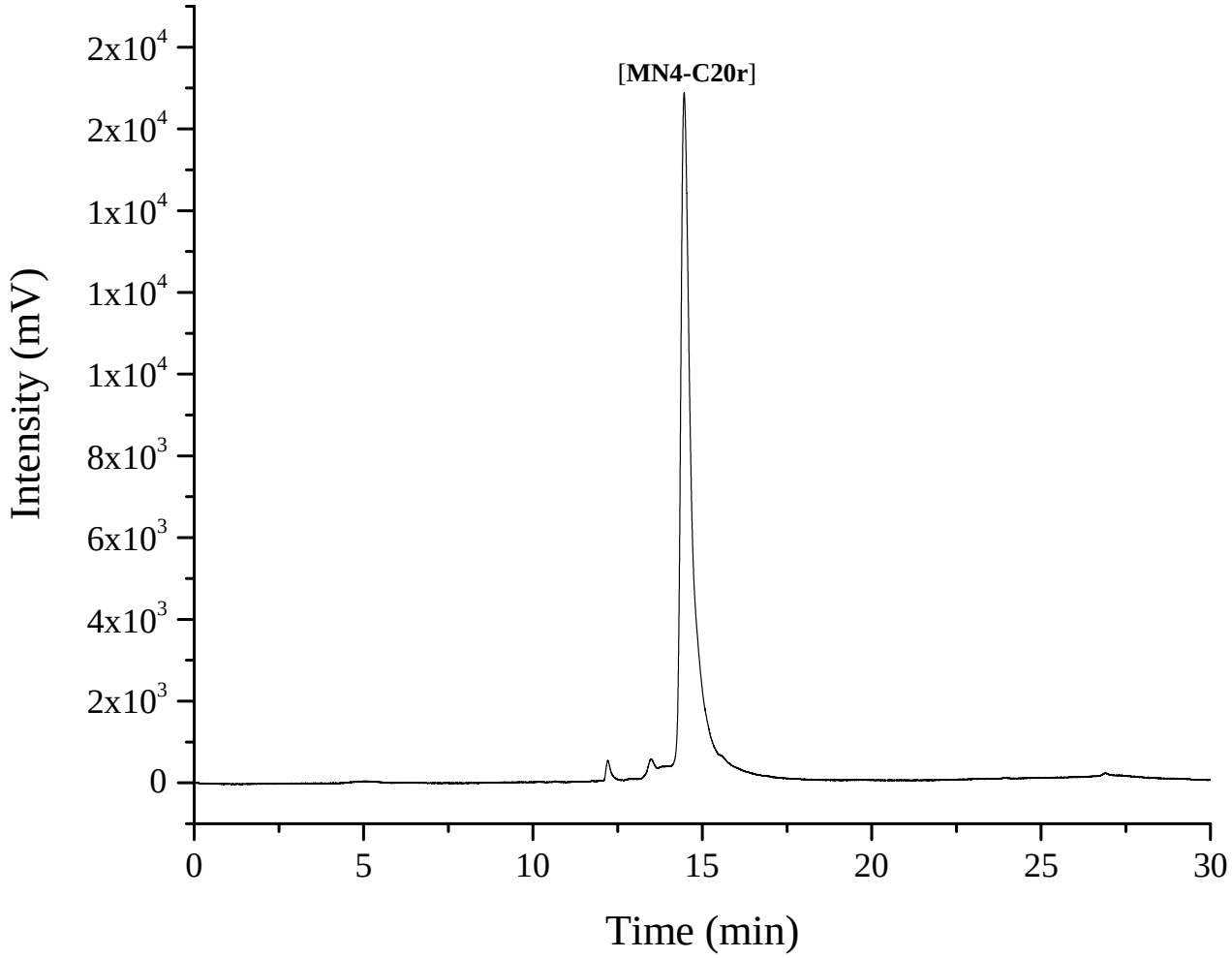


Figure S71. RP-HPLC traces of crude **MN4-C20r**; bioZen oligo LC column (2.6 µm, 150 x 4.6 mm); flow rate = 0.6 ml min^{-}1; linear gradient (5-25 % over 20 min) of 50 mM aqueous TEAA in ACN (pH 7.0); λ = 260 nm.

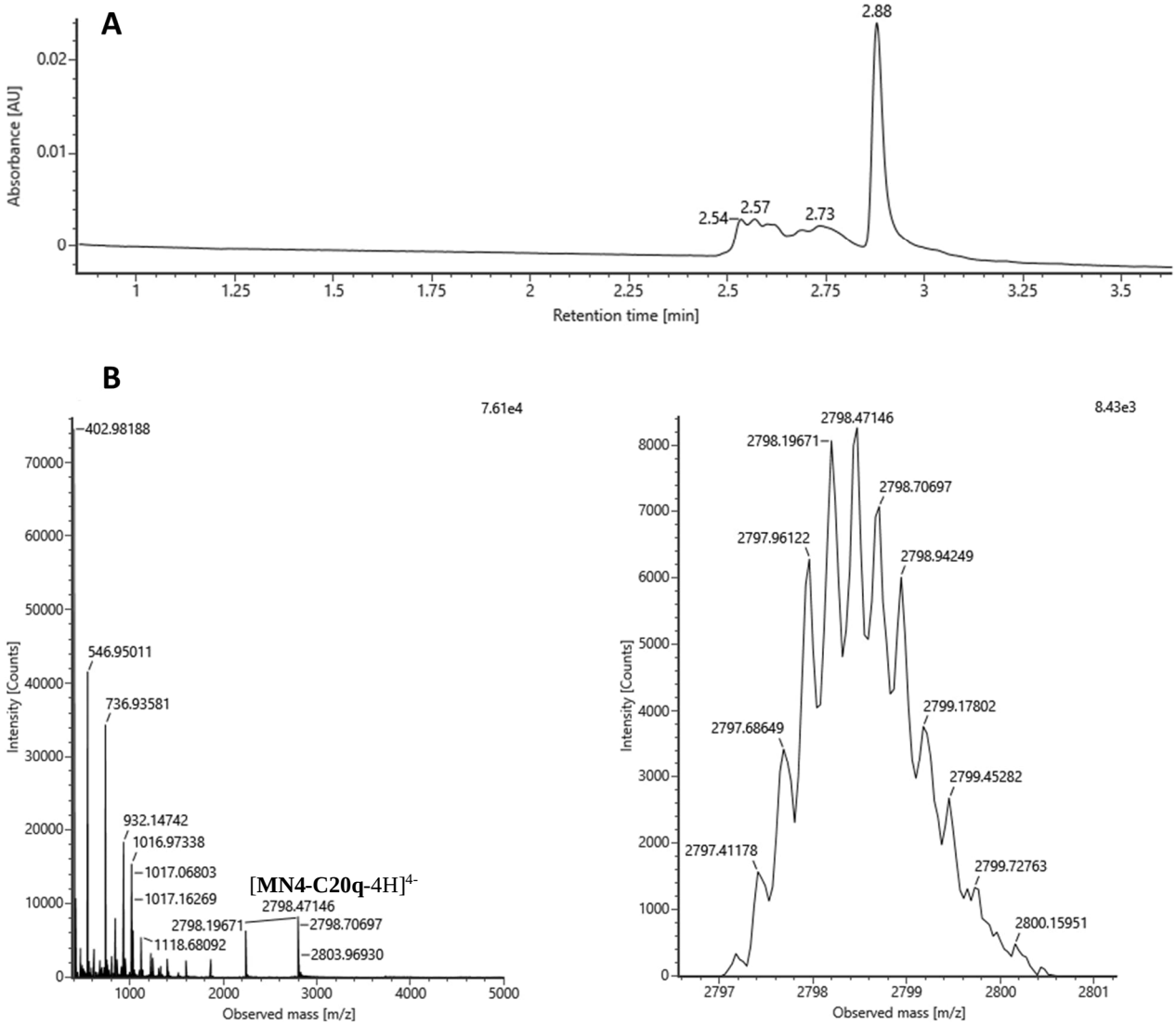


Figure S72. UV (A) and mass spectrum of purified **MN4-C20q** (B); ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5— 25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

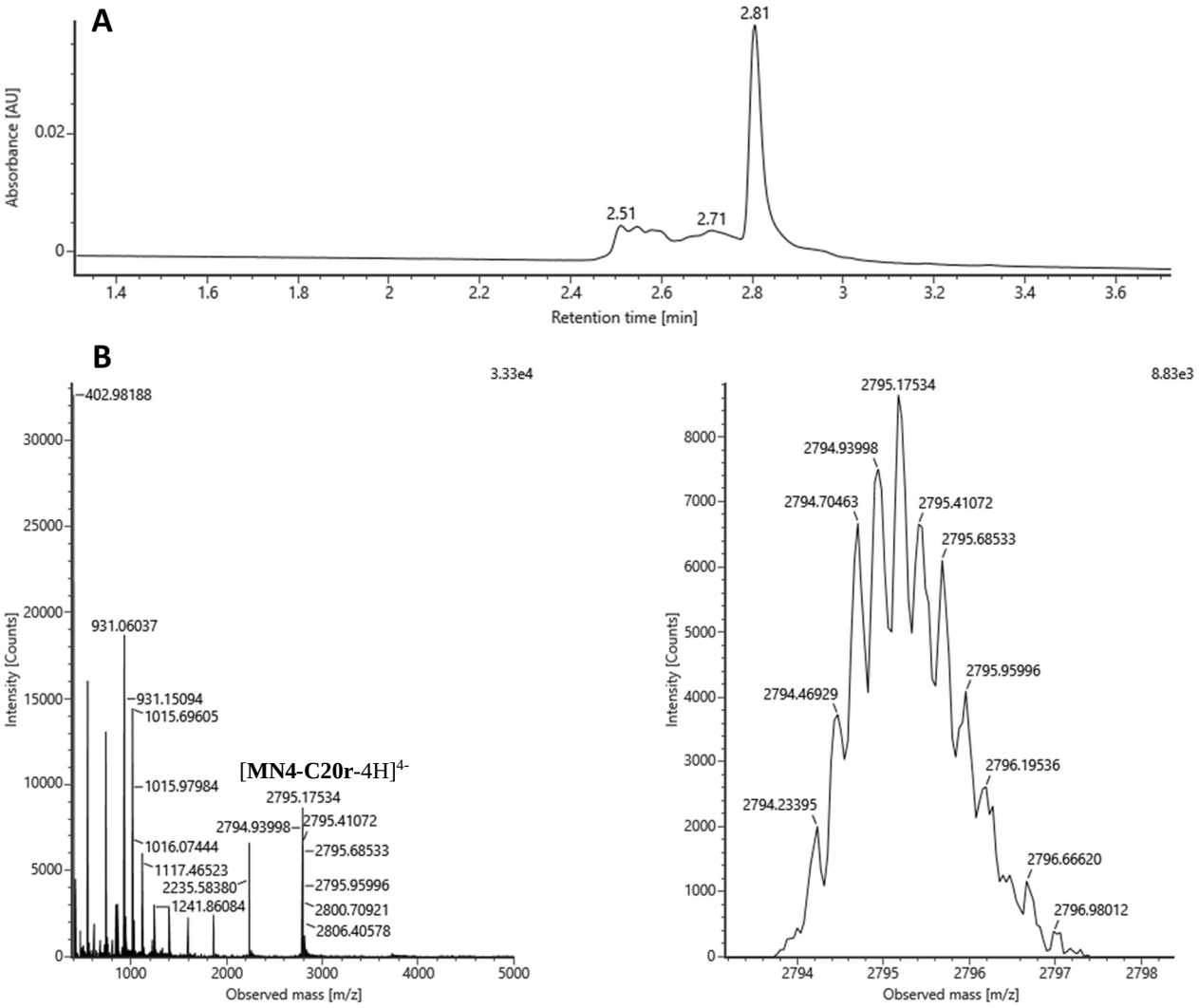


Figure S73. UV (A) and mass spectrum of purified **MN4-C20r** (B); ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5— 25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

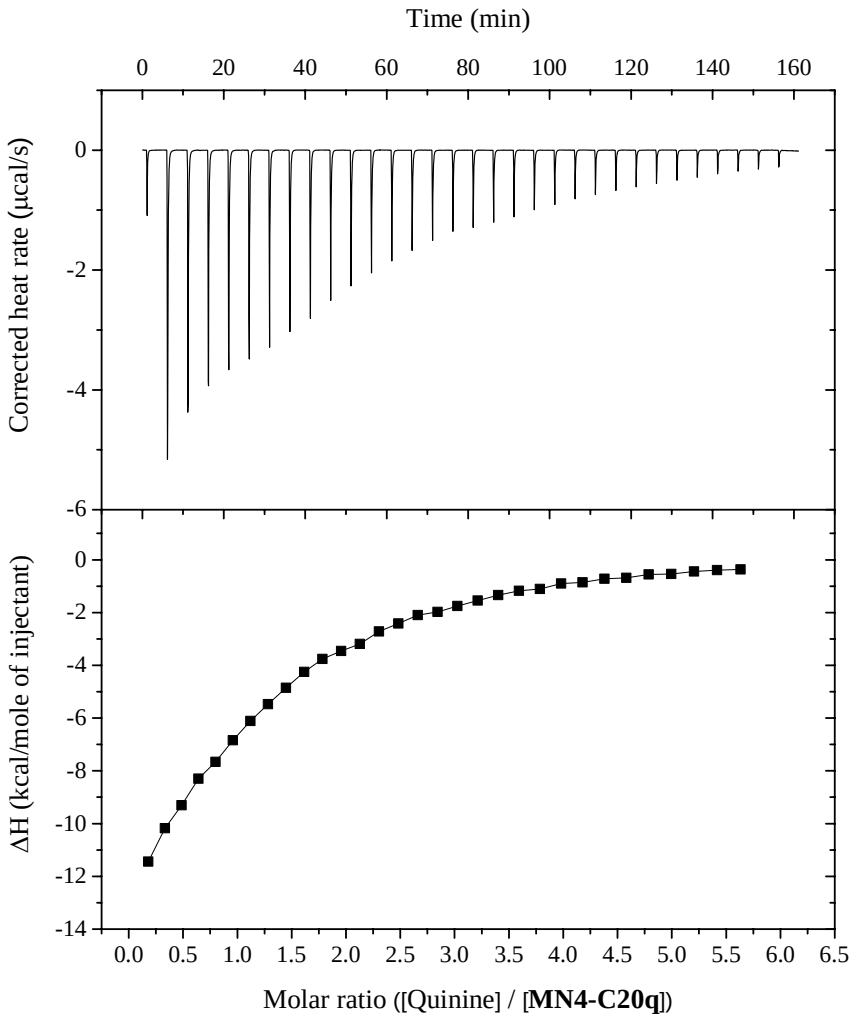


Figure S74. Corrected heat rate and the mean of resulting isotherms obtained from two **MN4-C20q** replicates titrated with quinine at 25 °C in 20 mM Tris•HCl buffer (pH 7.4) with 140 mM NaCl and 5 mM KCl. The first injection is excluded from the data set to remove the effect of titrant diffusion during the equilibration.

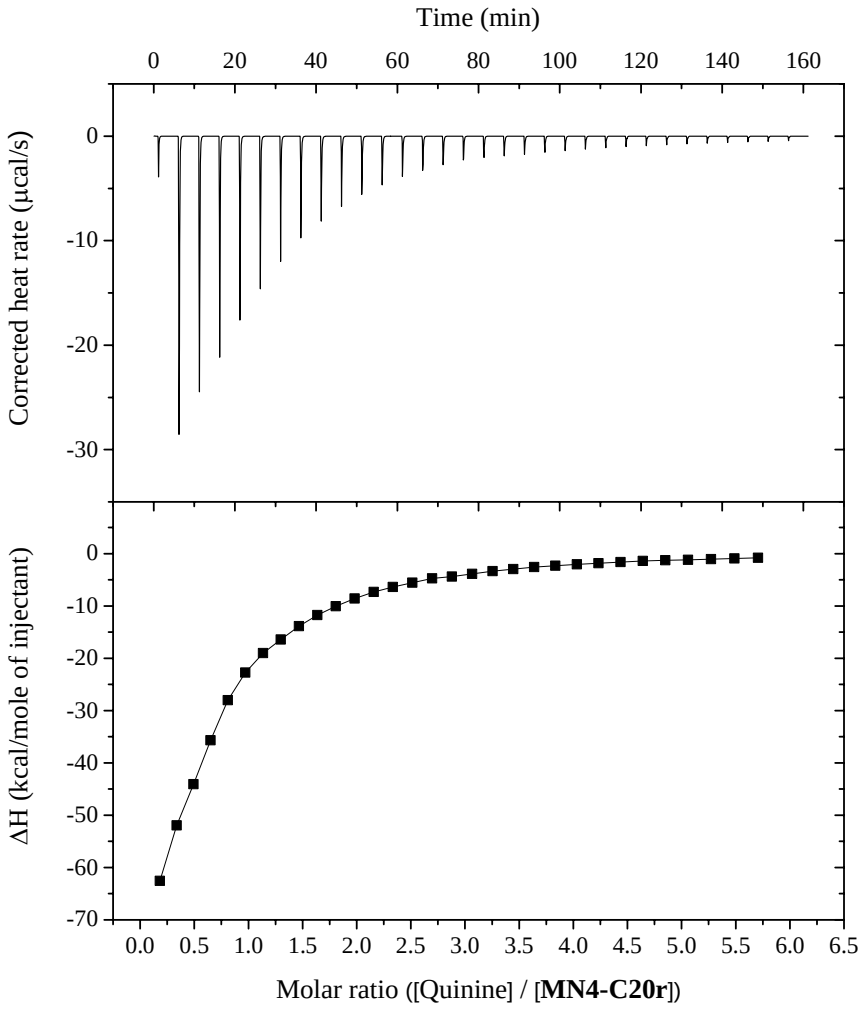


Figure S75. Corrected heat rate and the mean of resulting isotherms obtained from two **MN4-C20r** replicates titrated with quinine at 25 °C in 20 mM Tris•HCl buffer (pH 7.4) with 140 mM NaCl and 5 mM KCl. The first injection is excluded from the data set to remove the effect of titrant diffusion during the equilibration.

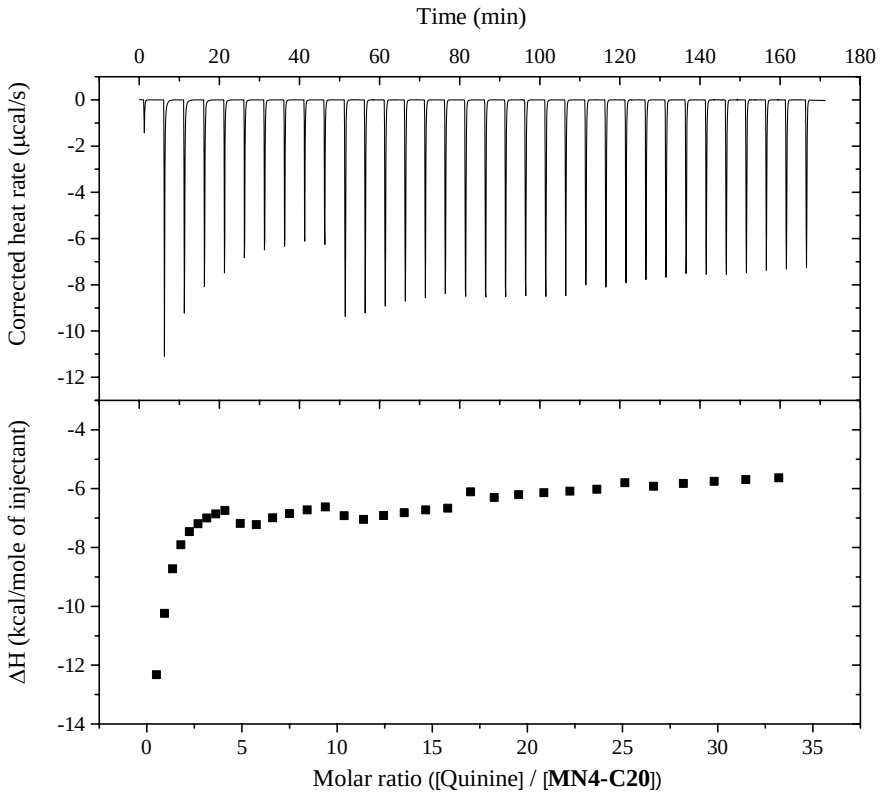


Figure S76. Corrected heat rate and the resulting isotherm obtained from the naked **MN4-C20** titrated with quinine at 20 °C in 20 mM Tris•HCl buffer (pH 7.4) with 140 mM NaCl and 5 mM KCl. The first injection is excluded from the data set to remove the effect of titrant diffusion during the equilibration.

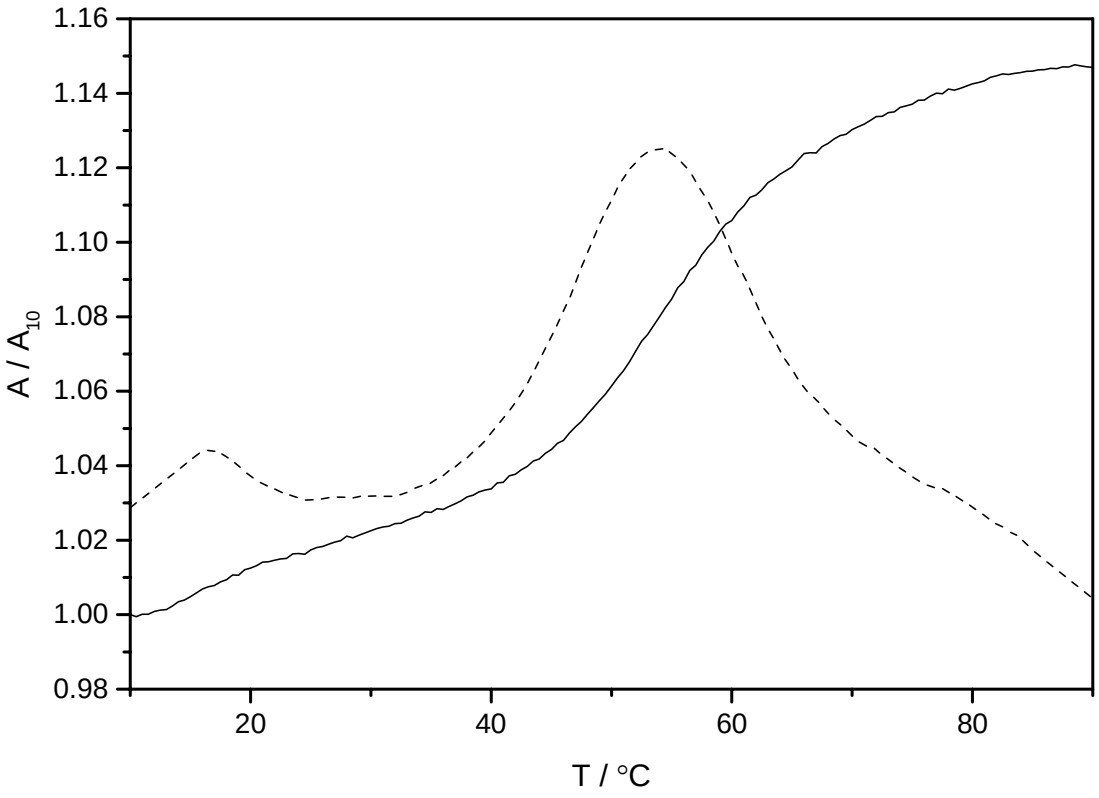


Figure S77. UV melting curve (solid line) and its first derivative (dashed line) of **MN4-C20q**; pH 7.4 (20 mM Tris•HCl, 140 mM NaCl, 5 mM KCl buffer); [**MN4-C20q**]= 0.8 μM.

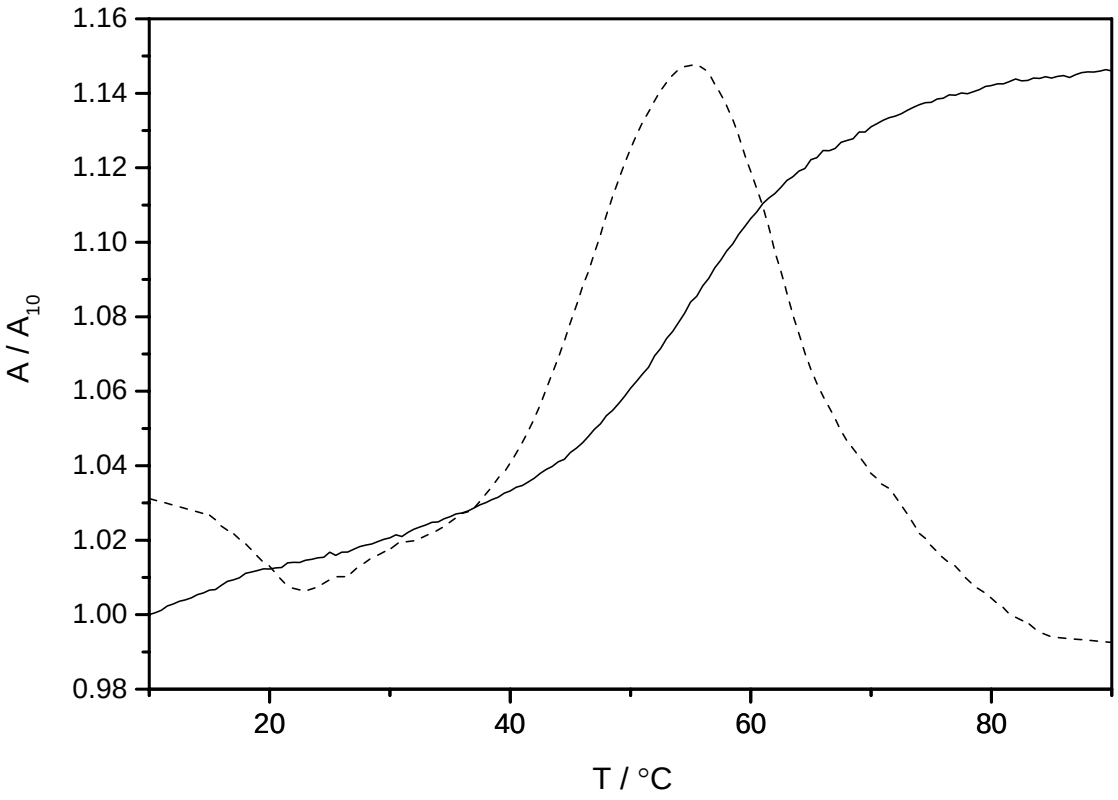


Figure S78. UV melting curve (solid line) and its first derivative (dashed line) of **MN4-C20r**; pH 7.4 (20 mM Tris•HCl, 140 mM NaCl, 5 mM KCl buffer); [**MN4-C20r**]= 0.8 μM.

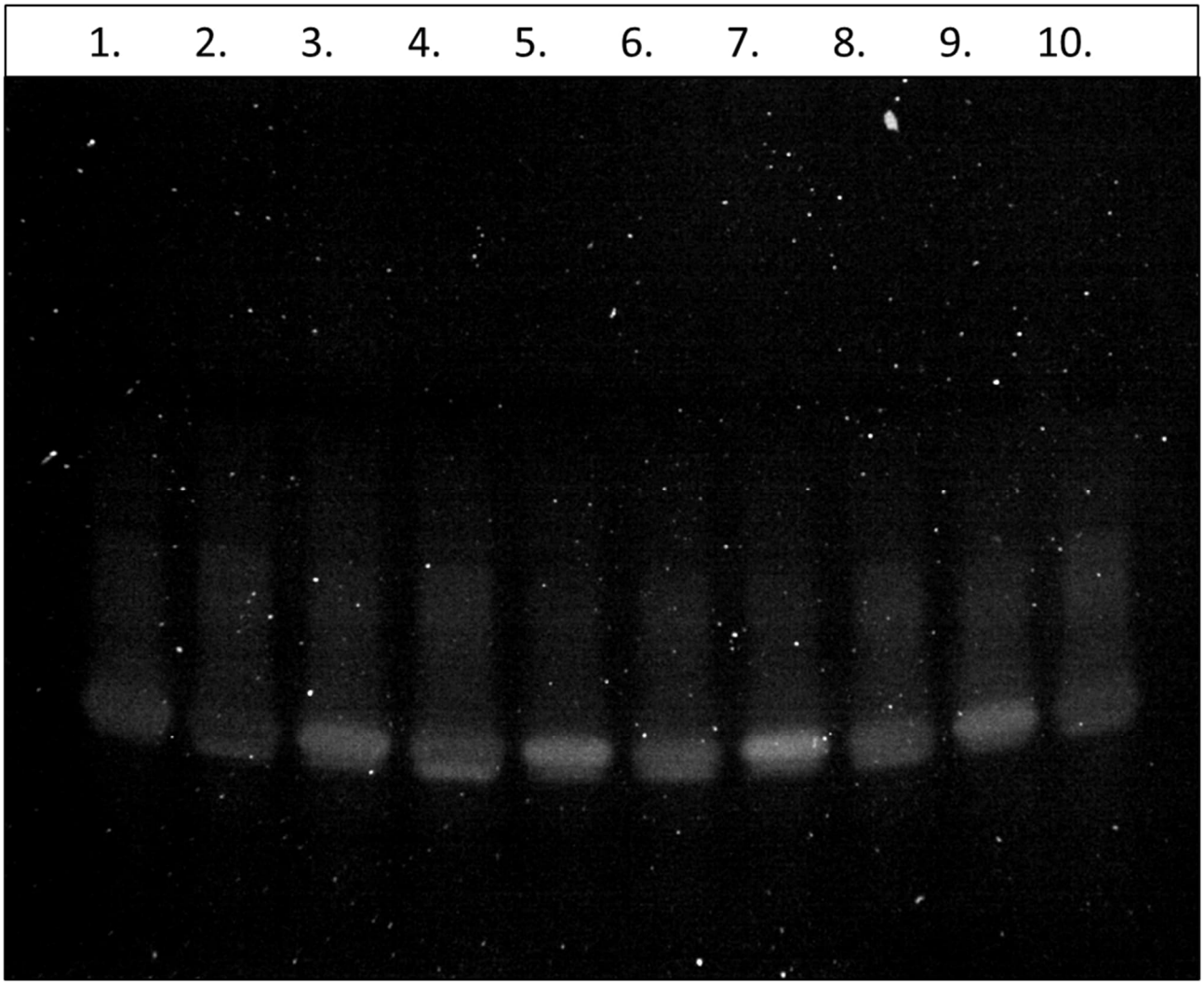


Figure S79. PAGE of **MN4-C20q** and **MN4-C20r** with varying quinine concentrations. 1) **MN4-C20q**; 2) **MN4-C20r**; 3) **MN4-C20q** with 4 µM quinine; 4) **MN4-C20r** with 4 µM quinine; 5) **MN4-C20q** with 200 µM quinine; 6) **MN4-C20r** with 200 µM quinine; 7) **MN4-C20q** with 500 µM quinine; 8) **MN4-C20r** with 500 µM quinine; 9) **MN4-C20q** with 1 mM quinine; 10) **MN4-C20r** with 1 mM quinine.

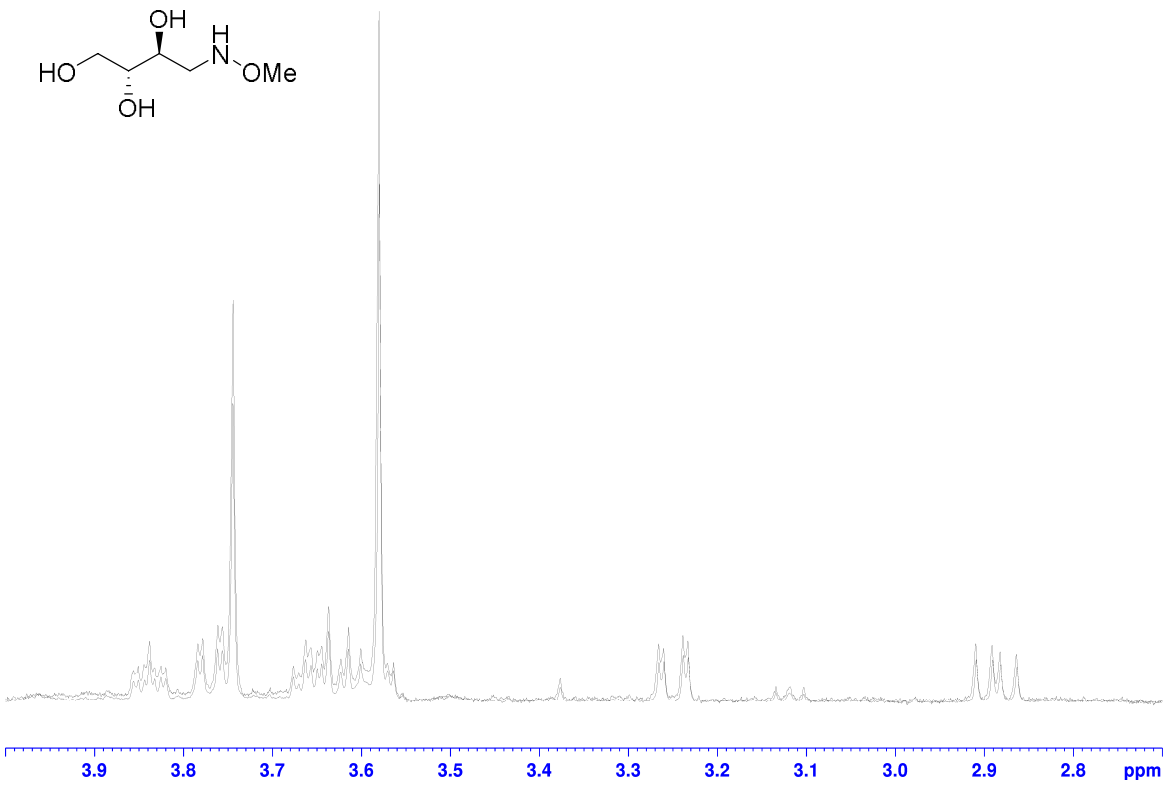


Figure S80. ^{1}H NMR (500 MHz, deuterated acetate buffer) spectrum of (2*R*,3*S*)-4-(methoxyamino)butane-1,2,3-triol (overlaid spectra for day 1 and day 13).

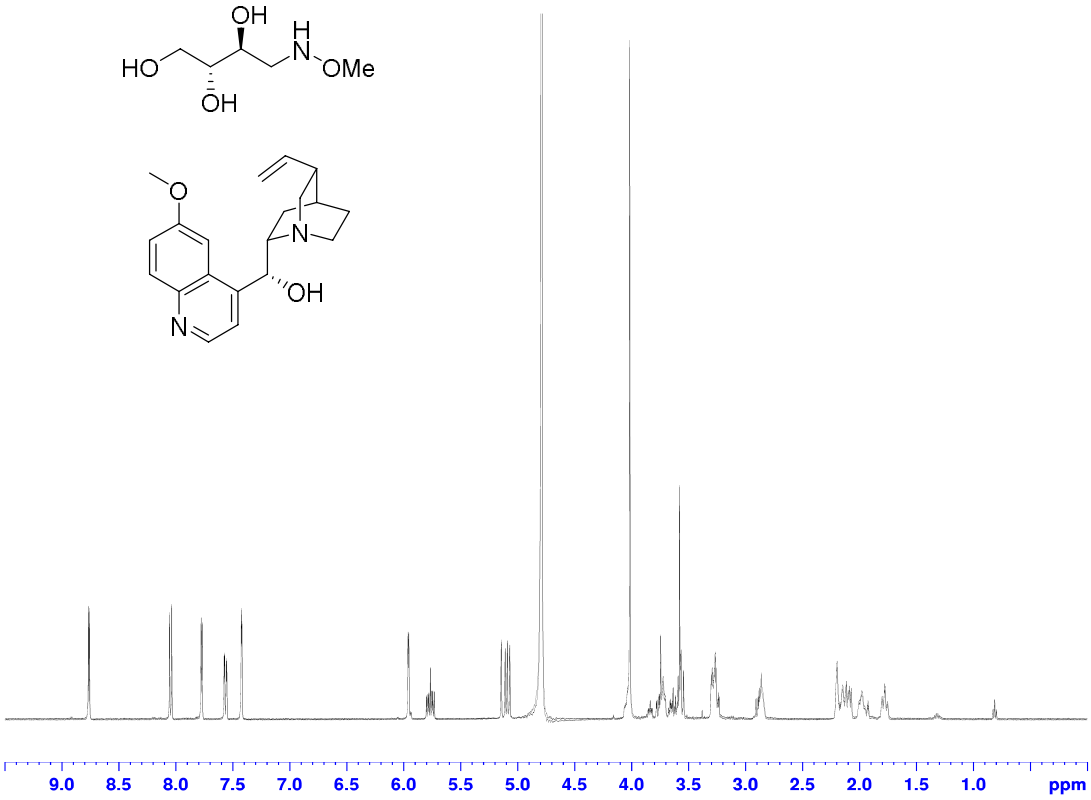


Figure S81. ^{1}H NMR (500 MHz, deuterated acetate buffer) spectrum of (2*R*,3*S*)-4-(methoxyamino)butane-1,2,3-triol and quinine (overlaid spectra for day 1 and day 13).

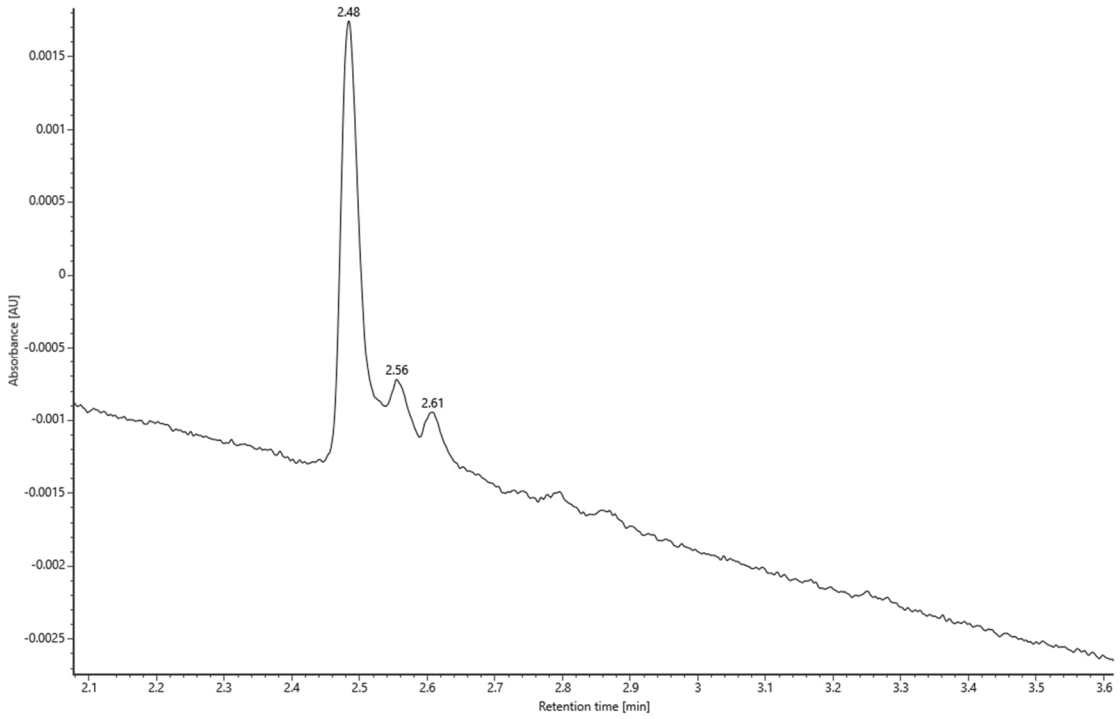


Figure S82. UV spectrum of **MN4-T19-OMe** obtained in the absence of aldehydes, but in the presence of quinine; ACQUITY Premier BEH C18 column (1.7 µm, 2.1 x 50 mm); flow rate = 0.4 ml min^{-1}; linear gradient (5—25 % over 4 min) of MeOH in an aqueous solution of hexafluoroisopropanol (40 mM) and triethylamine (7 mM); λ = 254 nm; T = 60 °C.

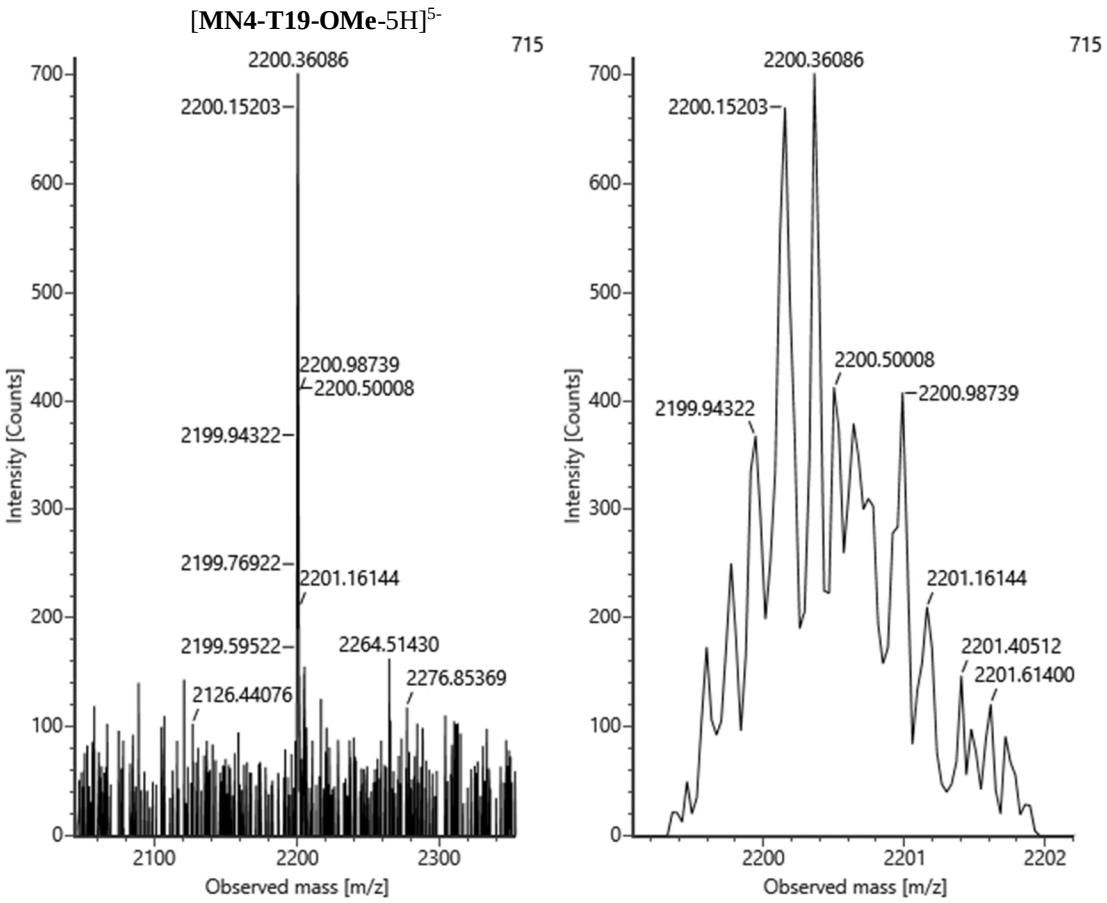


Figure S83. Mass spectrum of **MN4-T19-OMe** obtained in the absence of aldehydes, but in the presence of quinine.

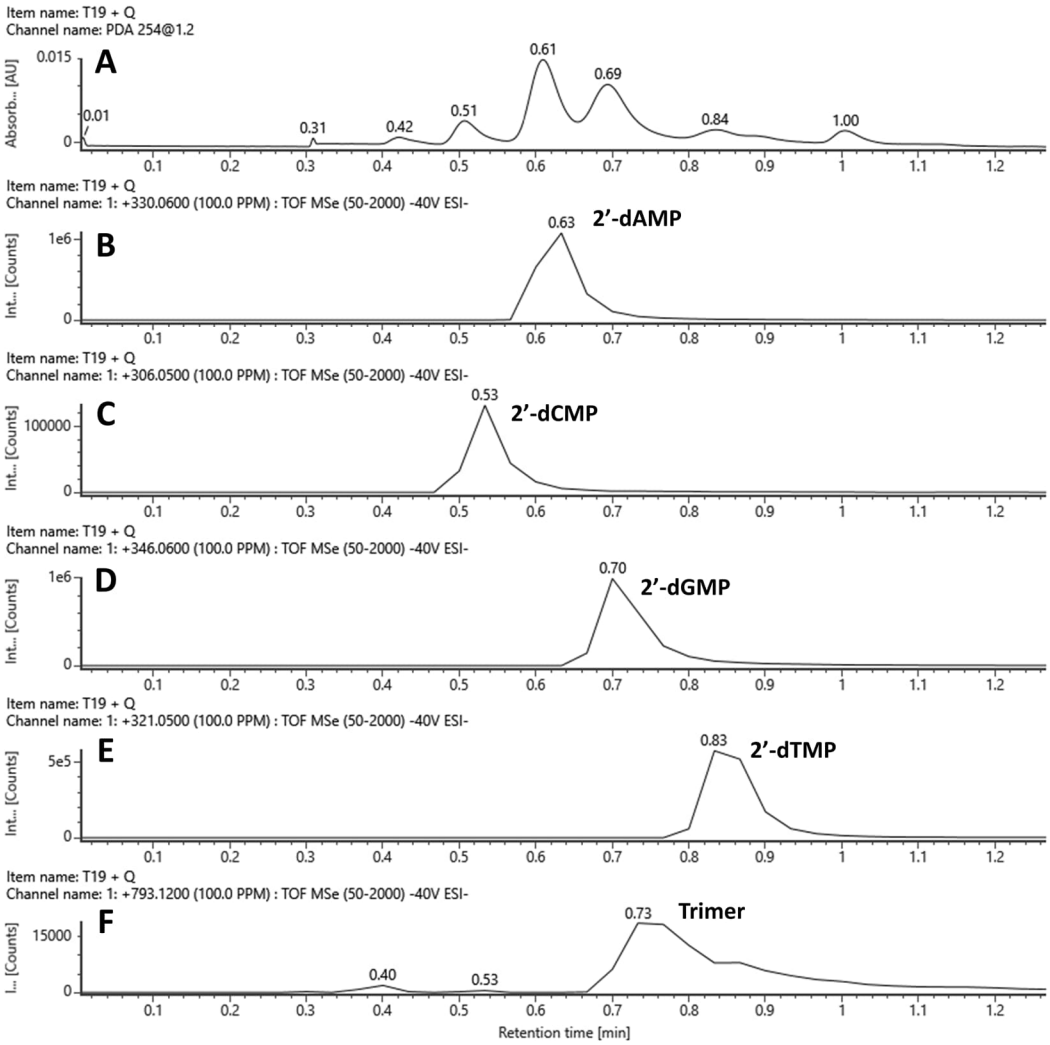


Figure S84. UV (A) and extracted ion UPLC traces (B—F) of **MN4-T19-OMe** digestion products; XBridge C18 column (5 µm, 4.6 x 30 mm); linear gradient of 0.1 % formic acid in MeOH (5–100 % over 2.2 min); flow rate of 0.8 ml min^{-1}; λ = 254 nm. Besides canonical nucleotides (B—E) a trimer consisting of phosphodiester-linked (2*R*,3*S*)-4-aminobutane-1,2,3-triol, thymidine and deoxycytidine (F) was found.

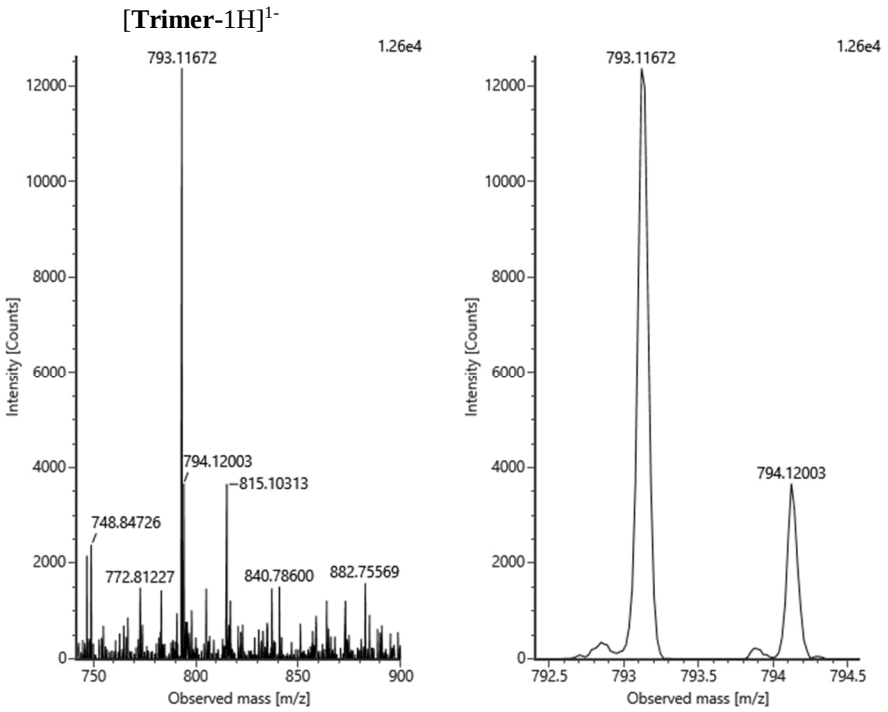


Figure S85. Mass spectrum of trimer consisting of phosphodiester-linked (2*R*,3*S*)-4-aminobutane-1,2,3-triol, thymidine and deoxycytidine obtained from **MN4-T19-OMe** digestion with nuclease P_1.