

Supplementary Information

Synthesis of fluorescent 5-heteroarylpyrimidine-containing oligonucleotides by post-synthetic trifluoromethyl conversion

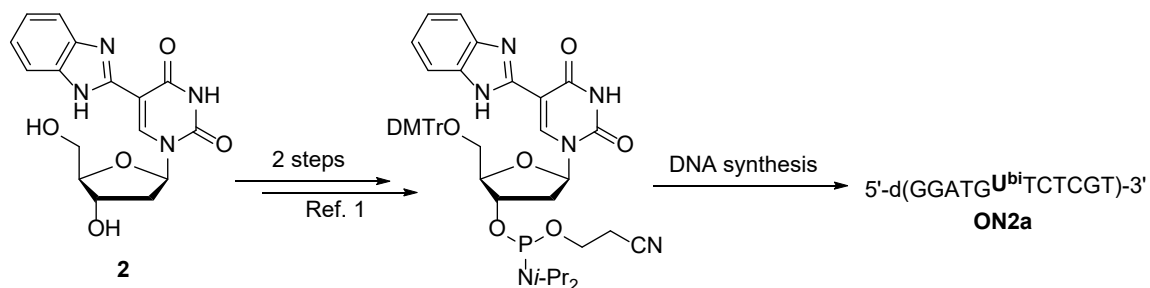
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Tokushima 770-8514, Japan

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1. Alternative synthesis of ON2a from nucleoside 2



Scheme S1 Alternative synthesis of **ON2a** from 5-(benzimidazol-2-yl)-2'-deoxyuridine **2**.

Reference

1. X. Guo, S. A. Ingale, H. Yang, Y. He and F. Seela, *Org. Biomol. Chem.*, 2017, **15**, 870–883.

2. Fluorescence spectra of ON2a and ON6a

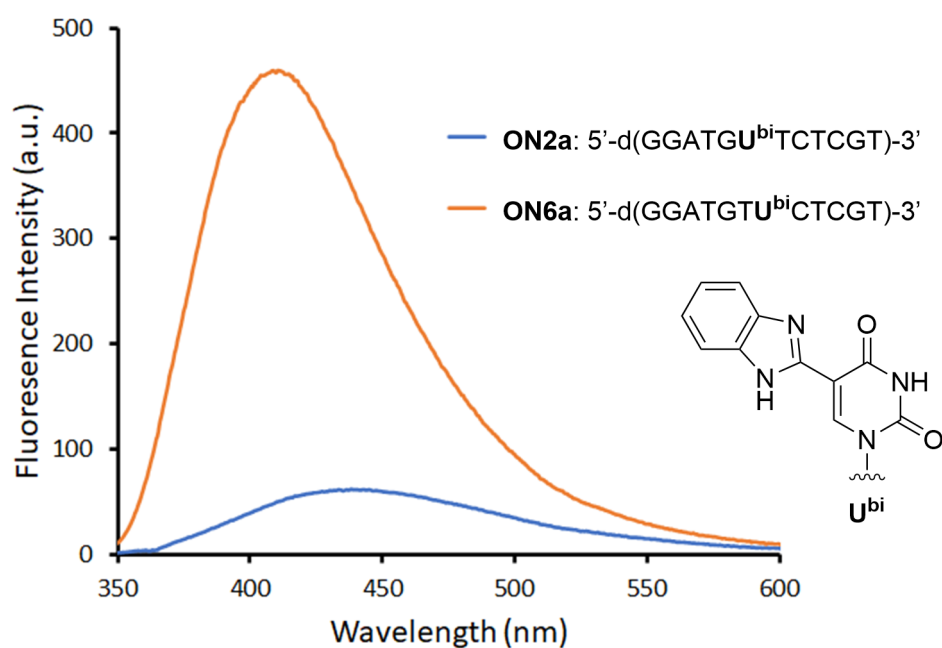
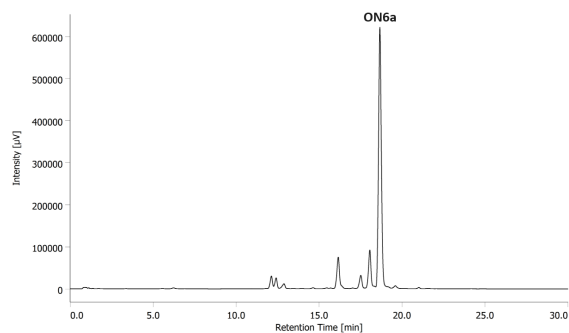


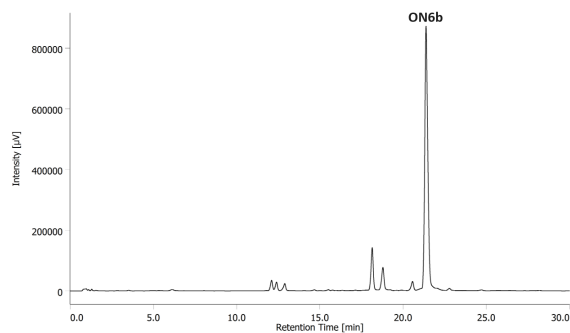
Fig. S1 Fluorescence spectra of **ON2a** and **ON6a**. Conditions: 1 μ M oligonucleotide in 10 mM phosphate buffer (pH 7.0) at 25 $^{\circ}$ C.

3. HPLC chromatograms of crude mixtures of the reactions of ON5

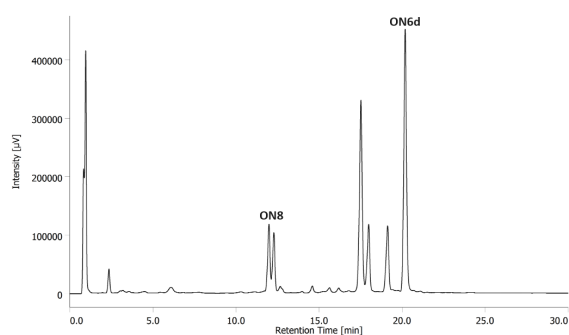
Reaction for ON6a



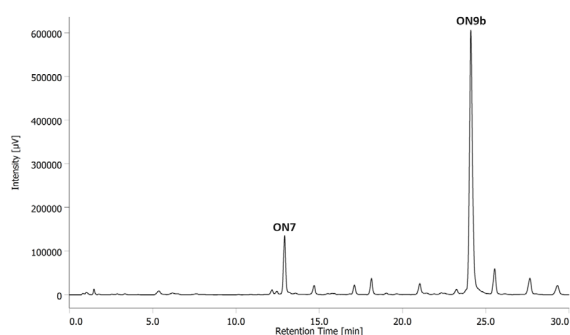
Reaction for ON6b



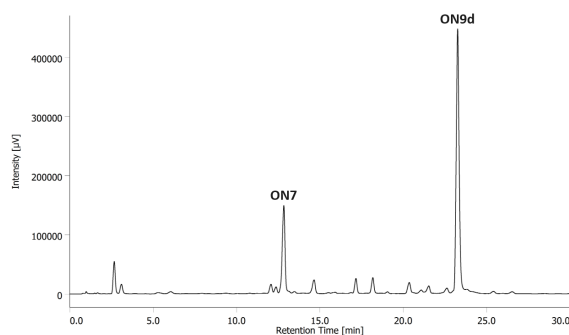
Reaction for ON6d



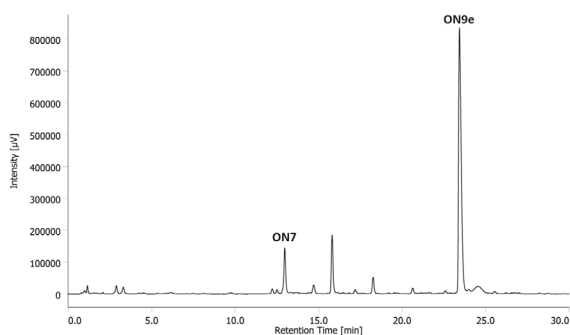
Reaction for ON9b



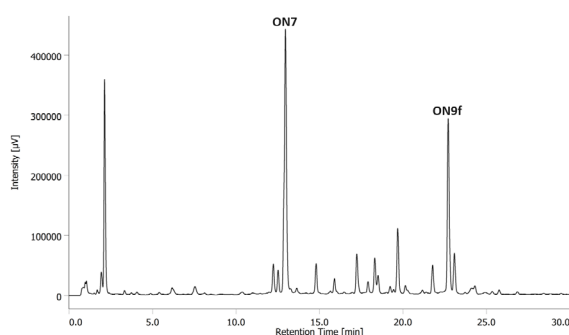
Reaction for ON9d



Reaction for ON9e



Reaction for ON9f

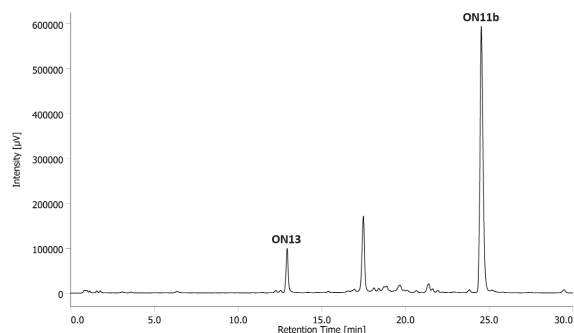


HPLC conditions

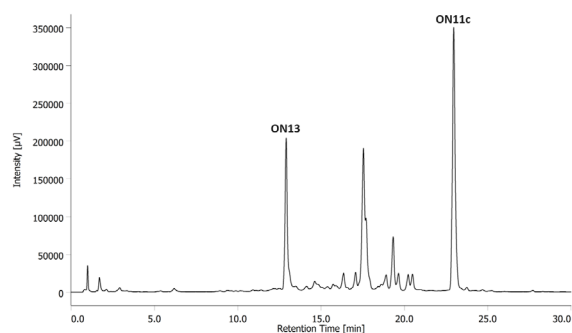
Column: Waters XBridge™ Shield RP18 2.5 μ m, 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 $^{\circ}$ C.

4. HPLC chromatograms of crude mixtures of the reactions of ON10

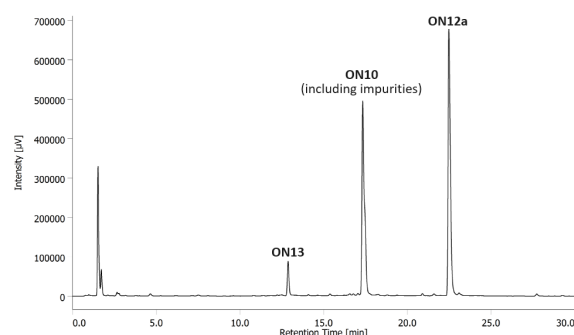
Reaction for ON11b



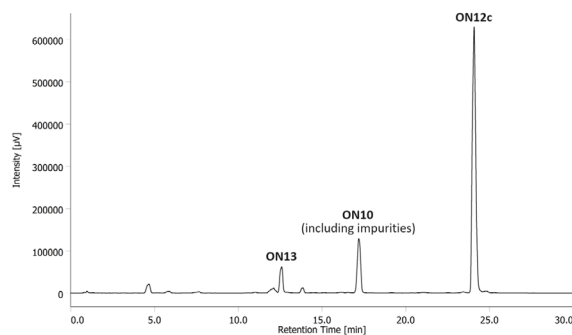
Reaction for ON11c



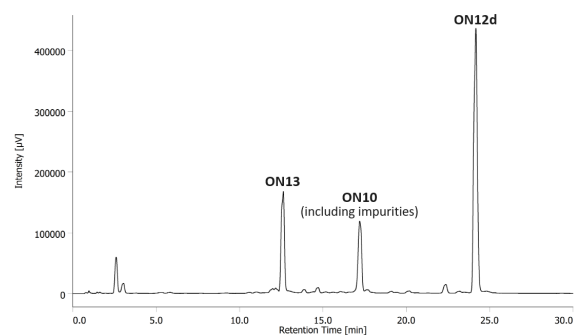
Reaction for ON12a



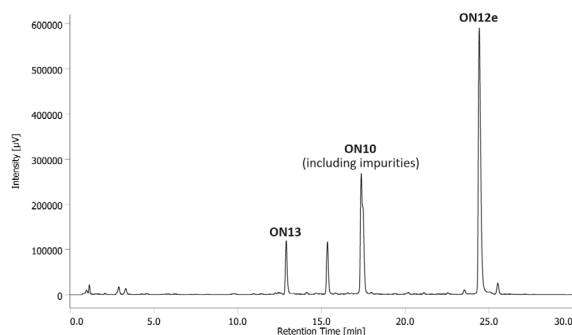
Reaction for ON12c



Reaction for ON12d



Reaction for ON12e

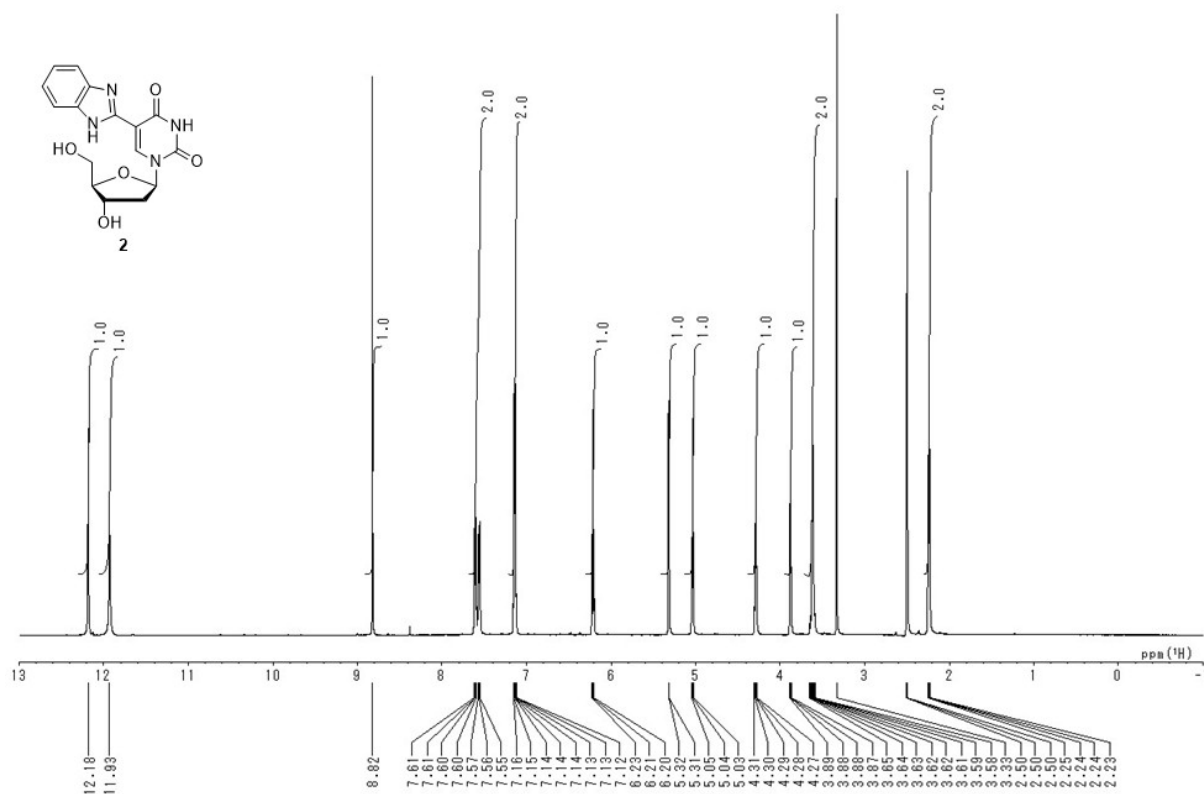


HPLC conditions

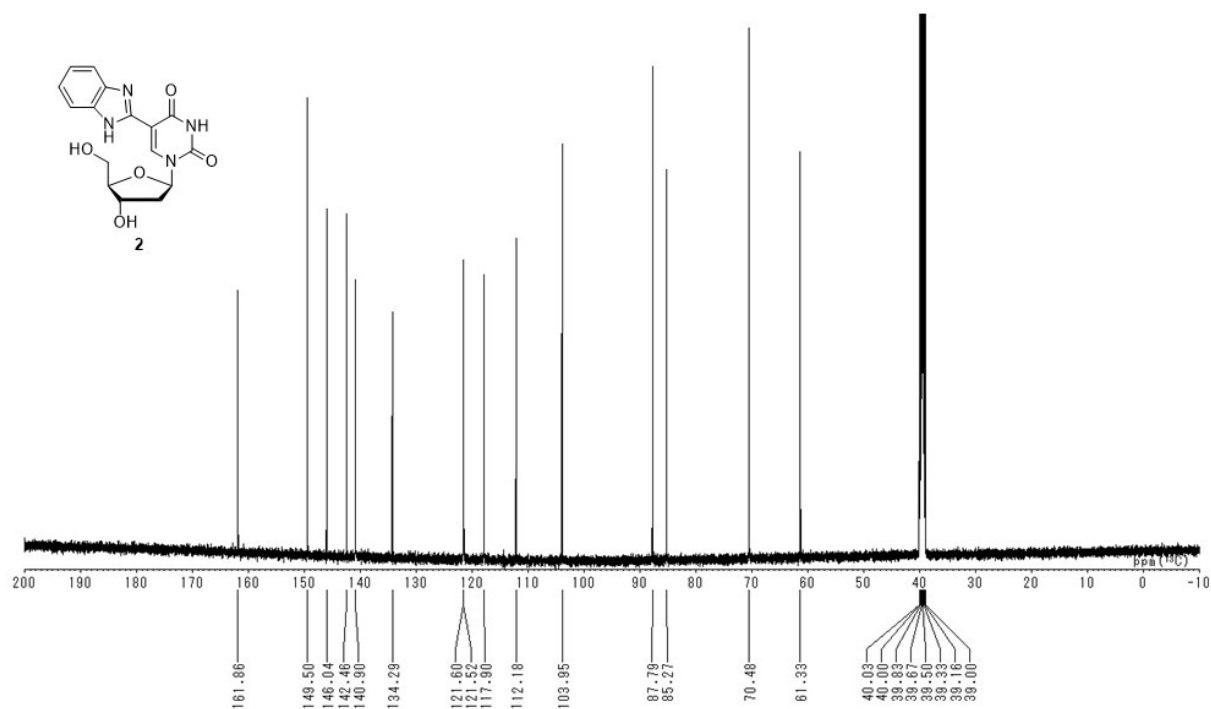
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

5. ^1H and $^{13}\text{C}\{^1\text{H}\}$ spectra of nucleosides 2, 3, 5, and 6

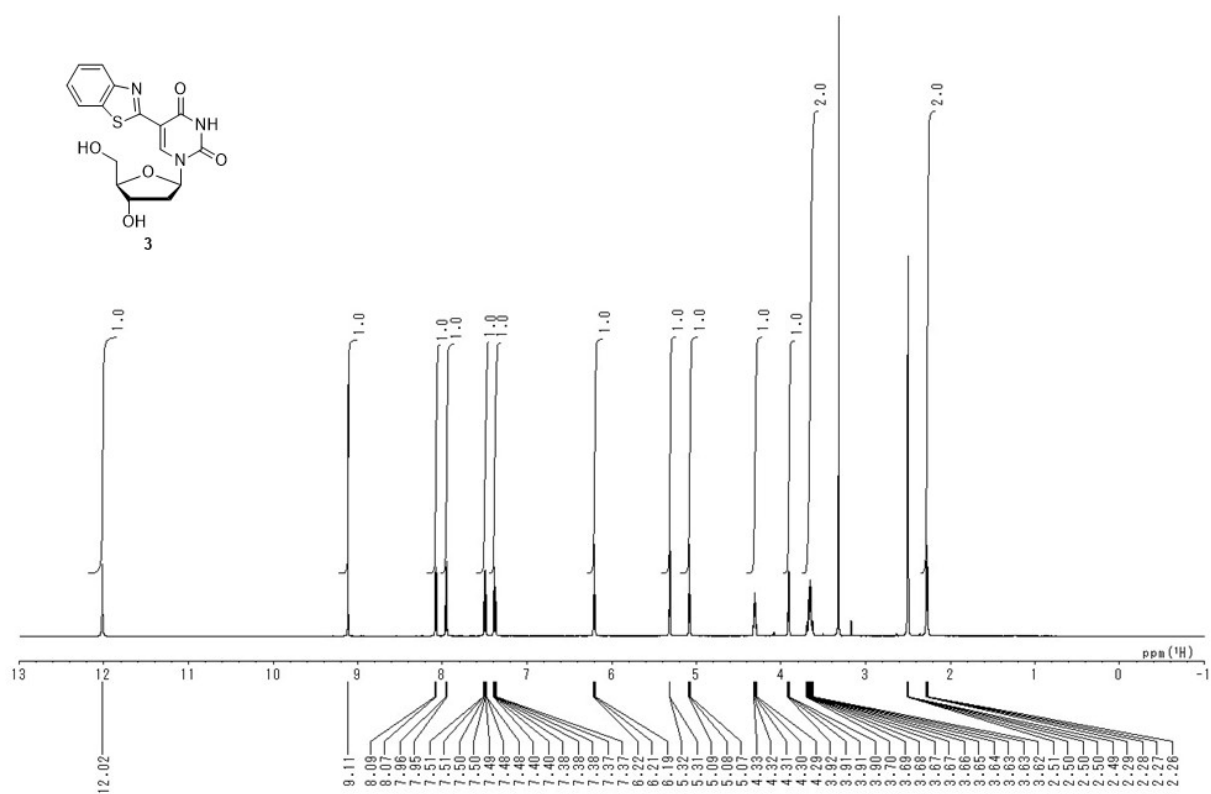
2: ^1H NMR (500 MHz, $\text{DMSO}-d_6$)



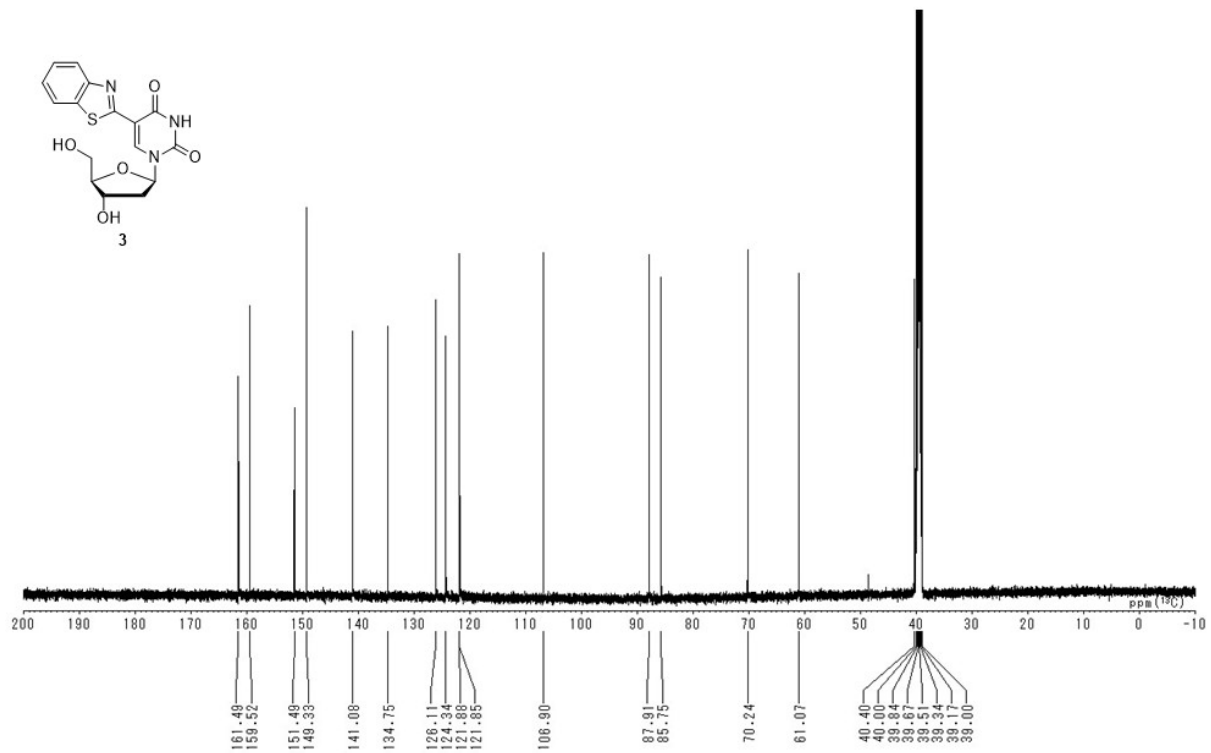
2: $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, $\text{DMSO}-d_6$)



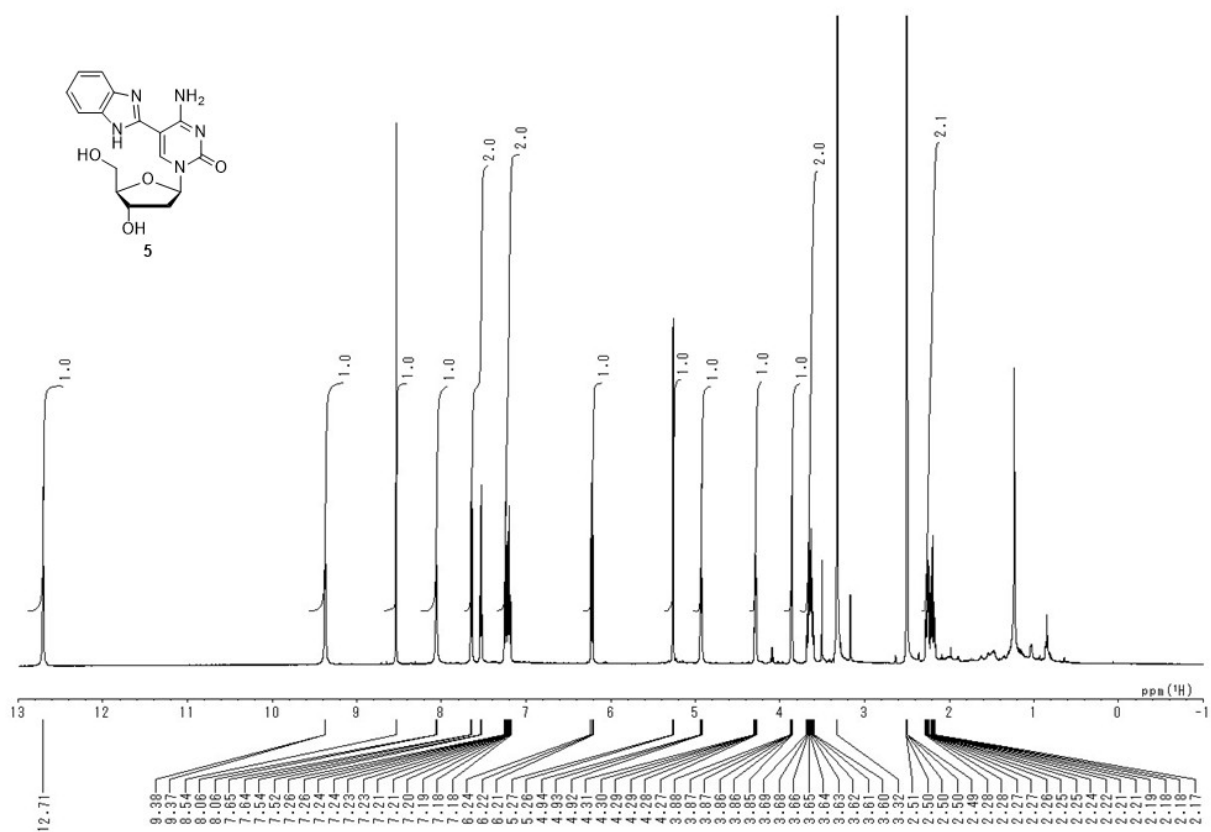
3: ^1H NMR (500 MHz, $\text{DMSO-}d_6$)



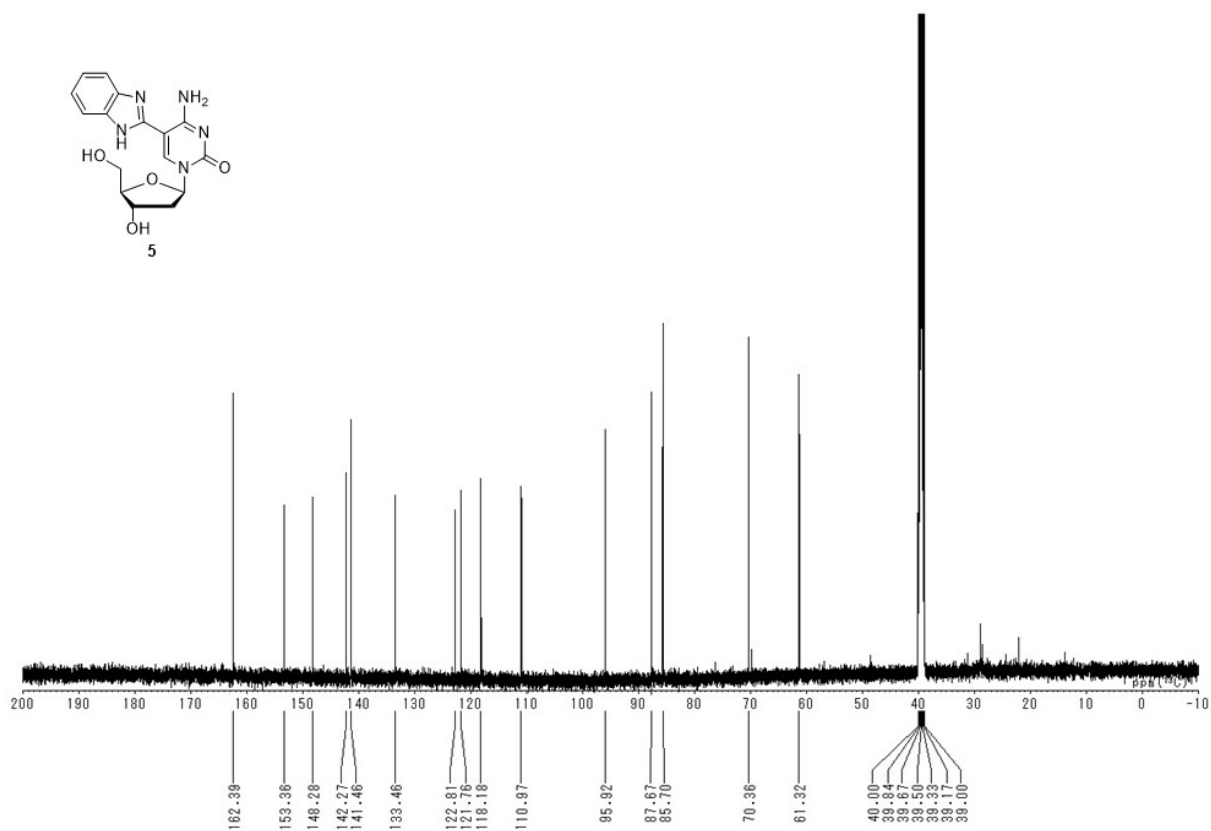
3: $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, $\text{DMSO-}d_6$)



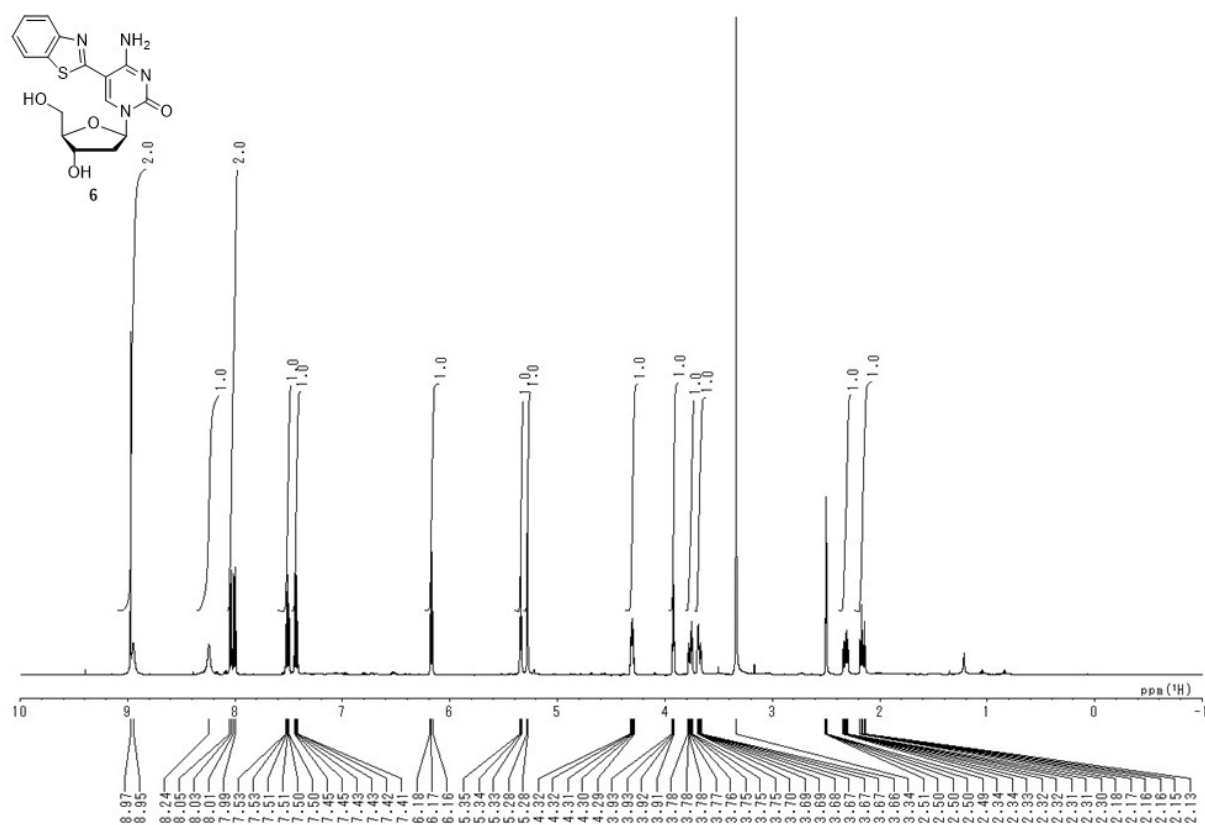
5: ^1H NMR (500 MHz, $\text{DMSO}-d_6$)



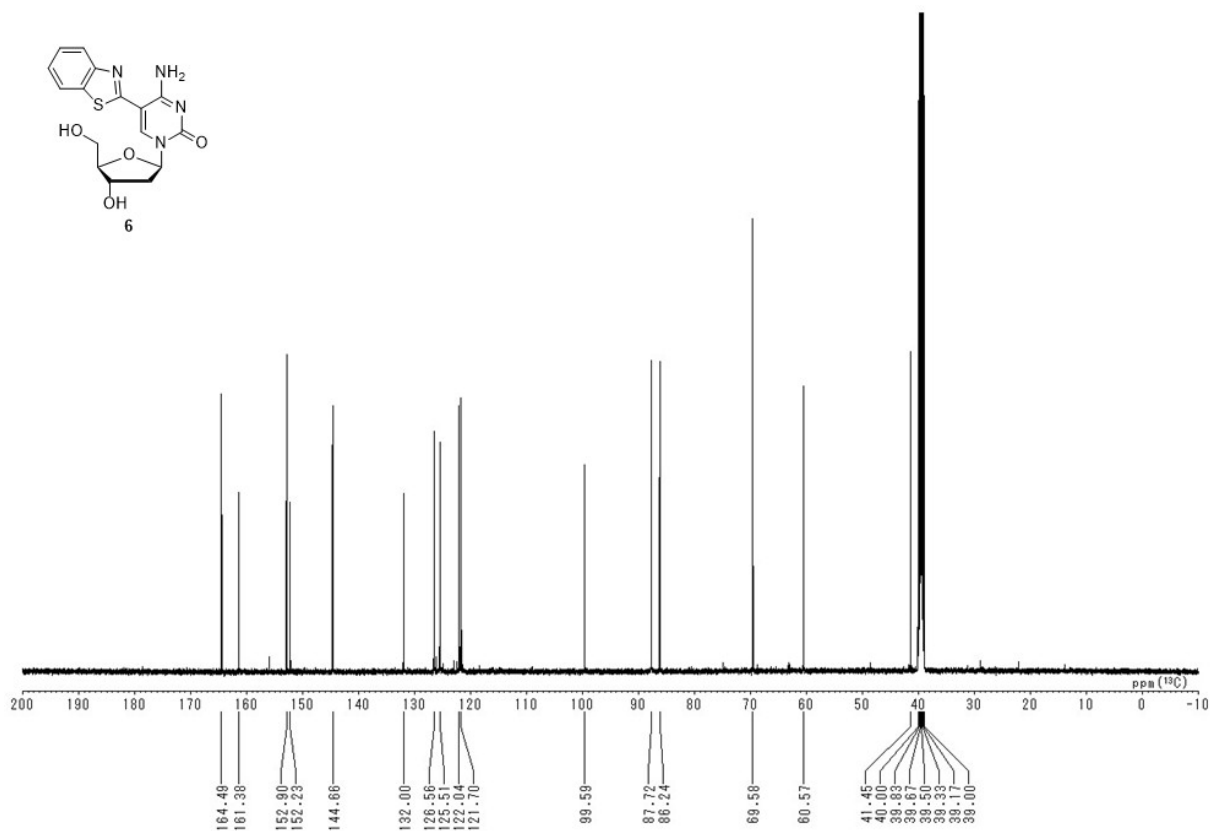
5: $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, $\text{DMSO}-d_6$)



6: ^1H NMR (500 MHz, $\text{DMSO}-d_6$)



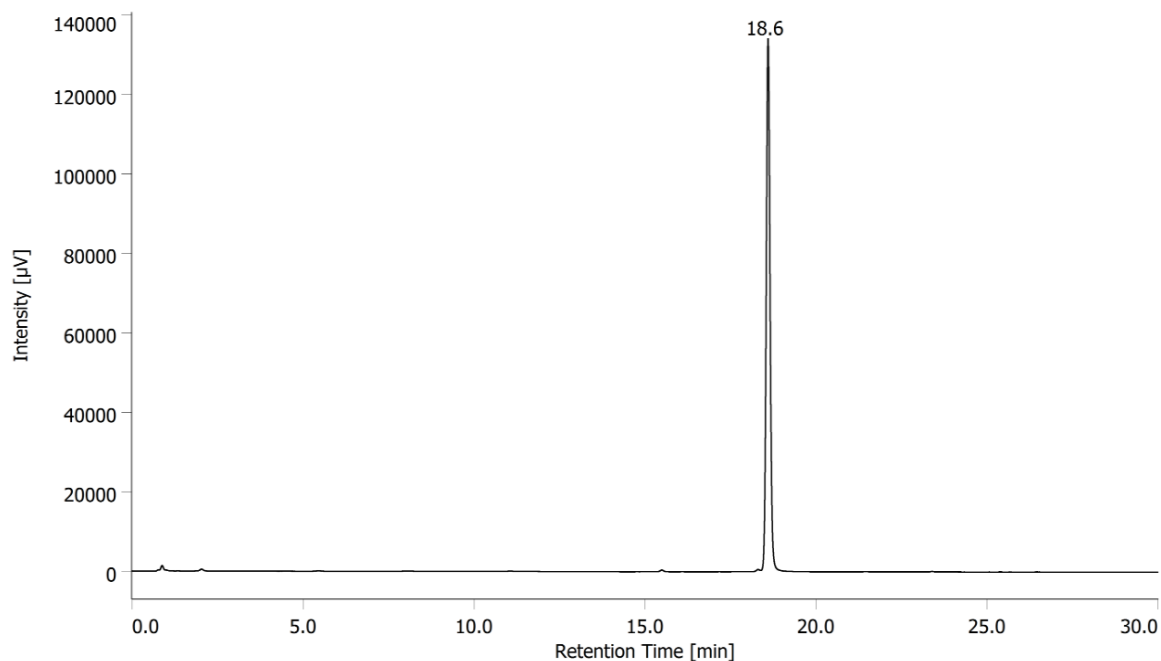
6: $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, $\text{DMSO}-d_6$)



6. HPLC chromatograms and ESI-MS spectra of new oligonucleotides

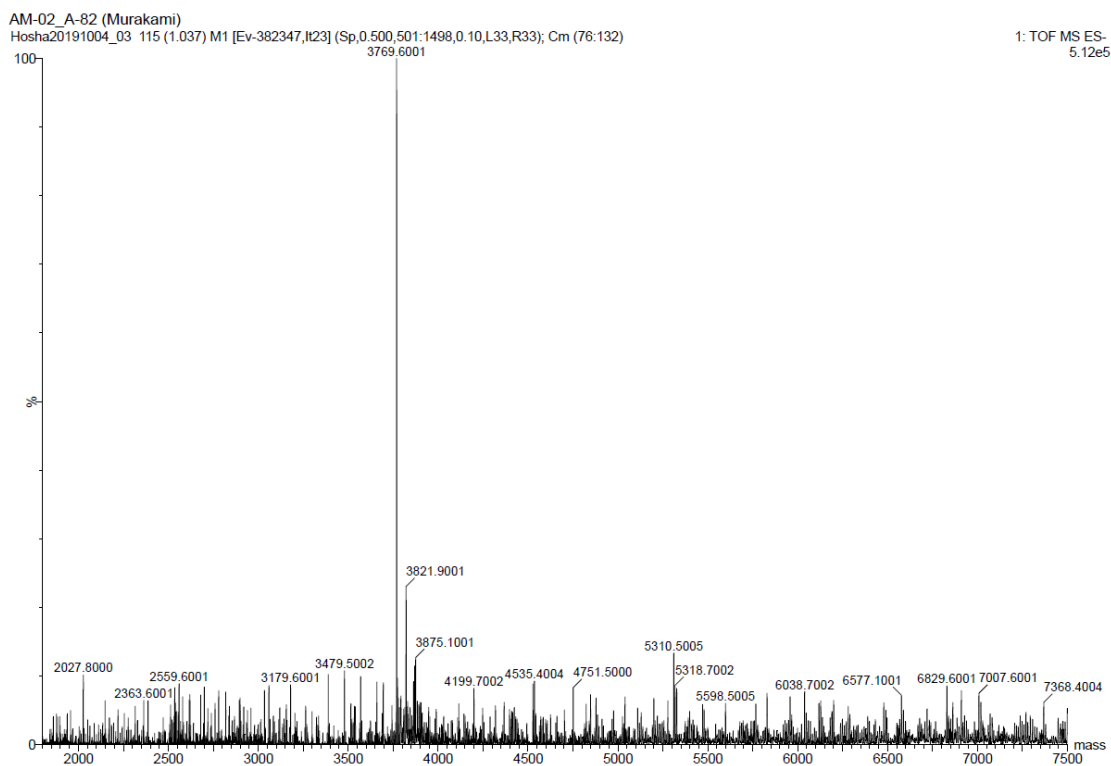
For **ON2a**

HPLC chromatogram of purified **ON2a**



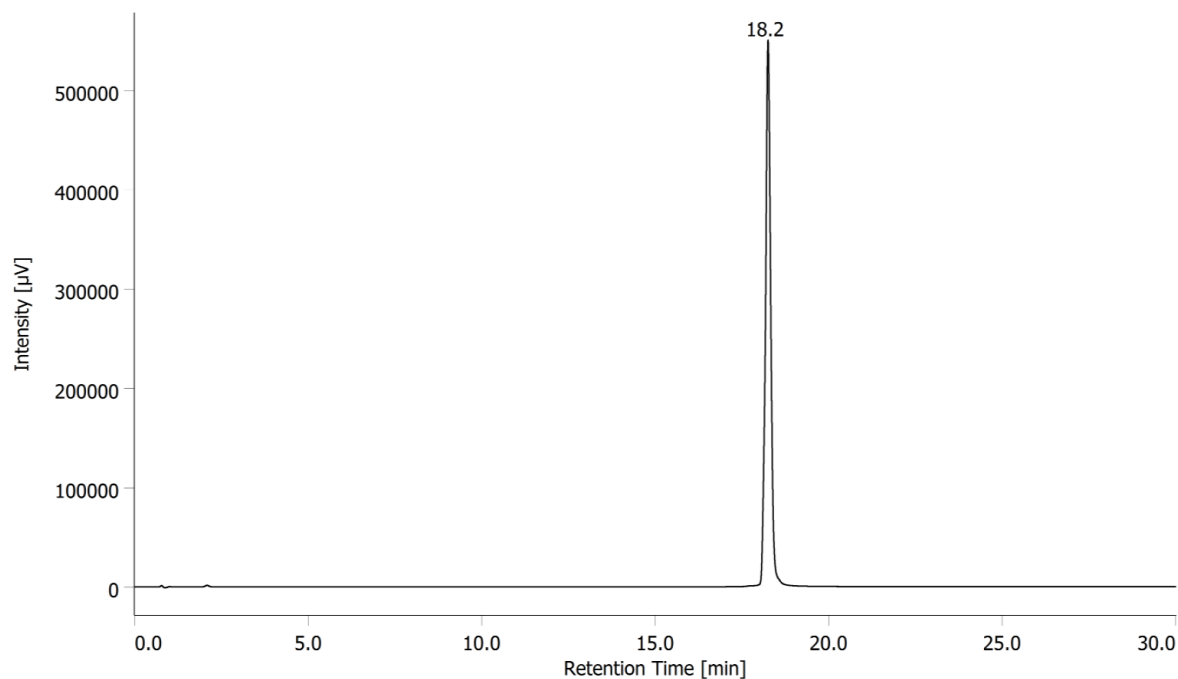
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON2a**



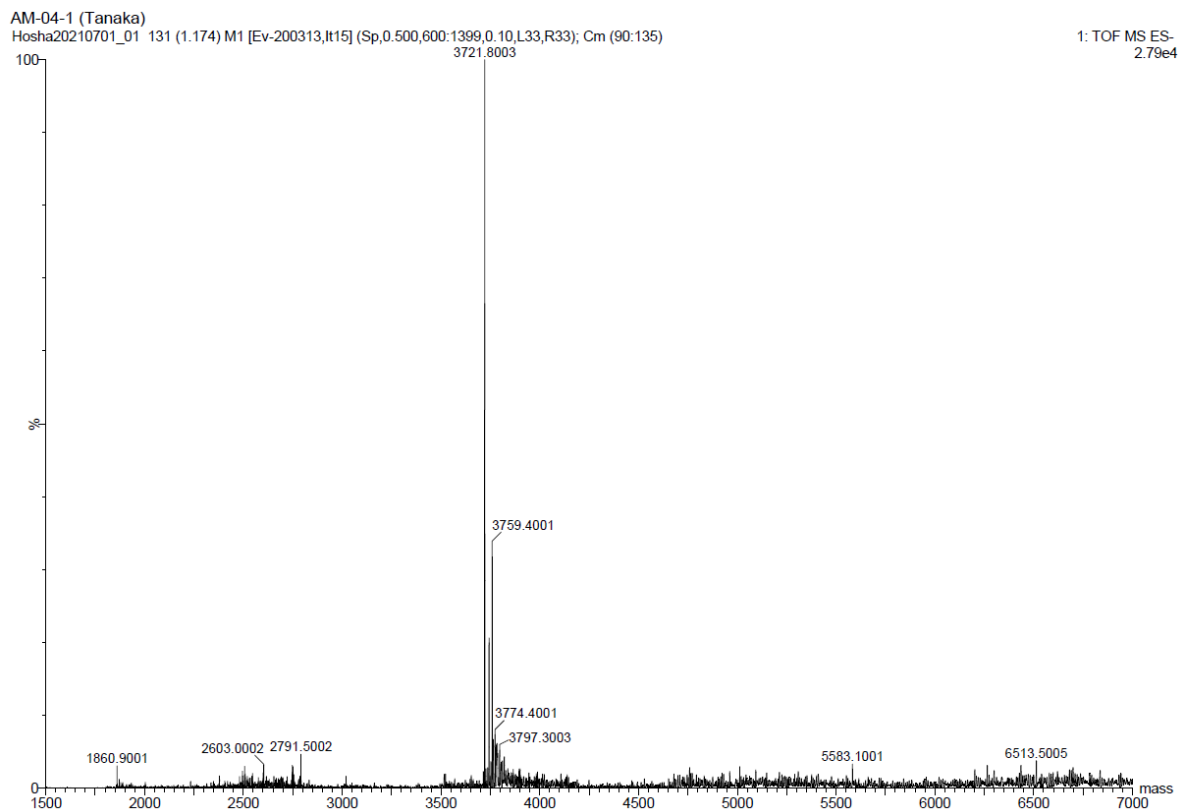
For **ON5**

HPLC chromatogram of purified **ON5**



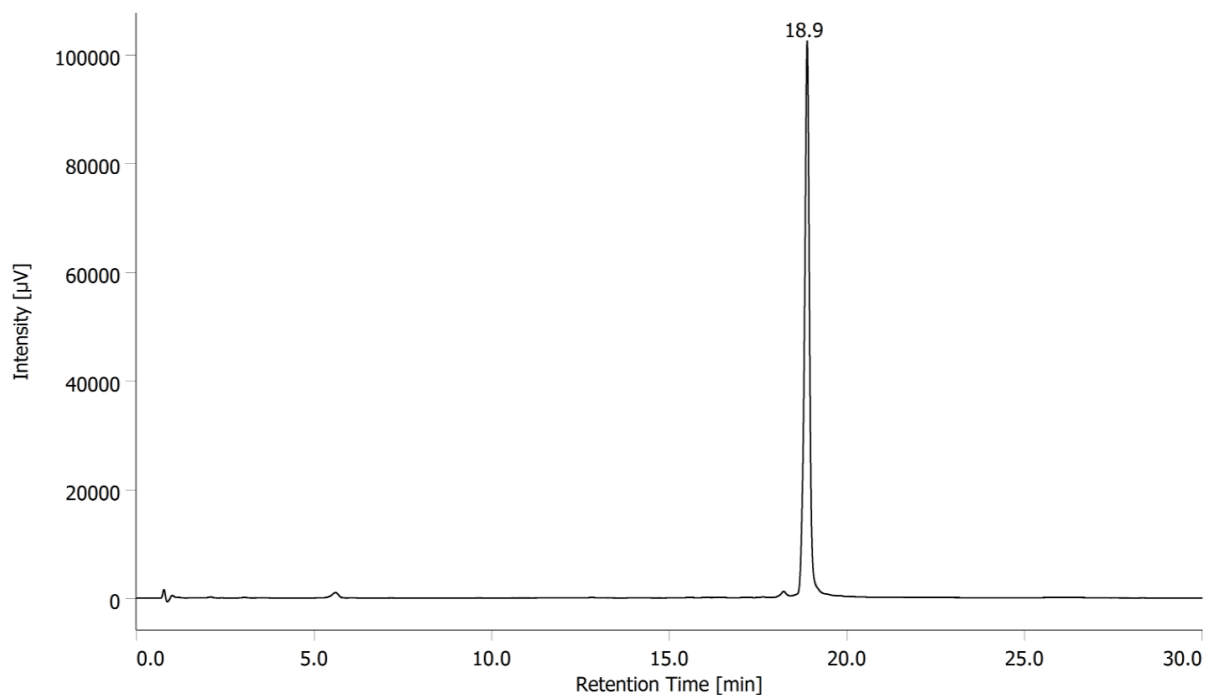
Column: Waters XBridge™ Shield RP18 2.5 μm, 4.6 × 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON5**



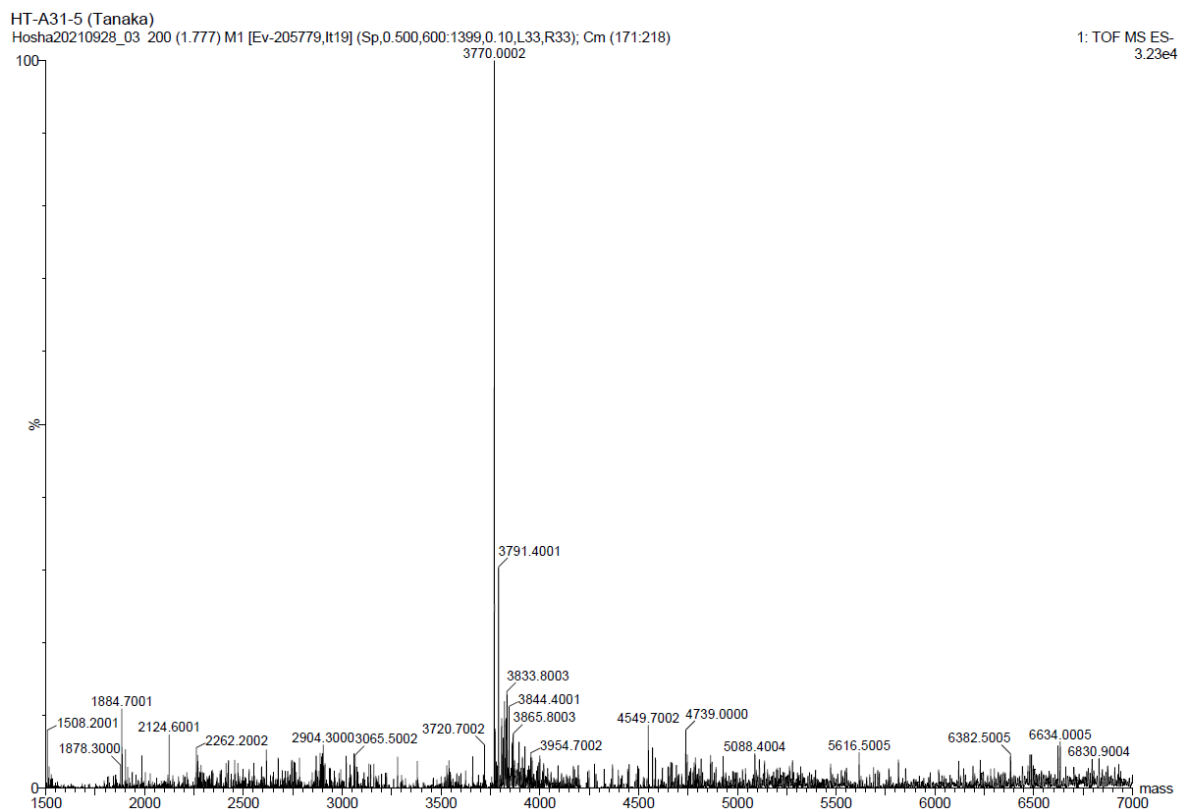
For **ON6a**

HPLC chromatogram of purified **ON6a**



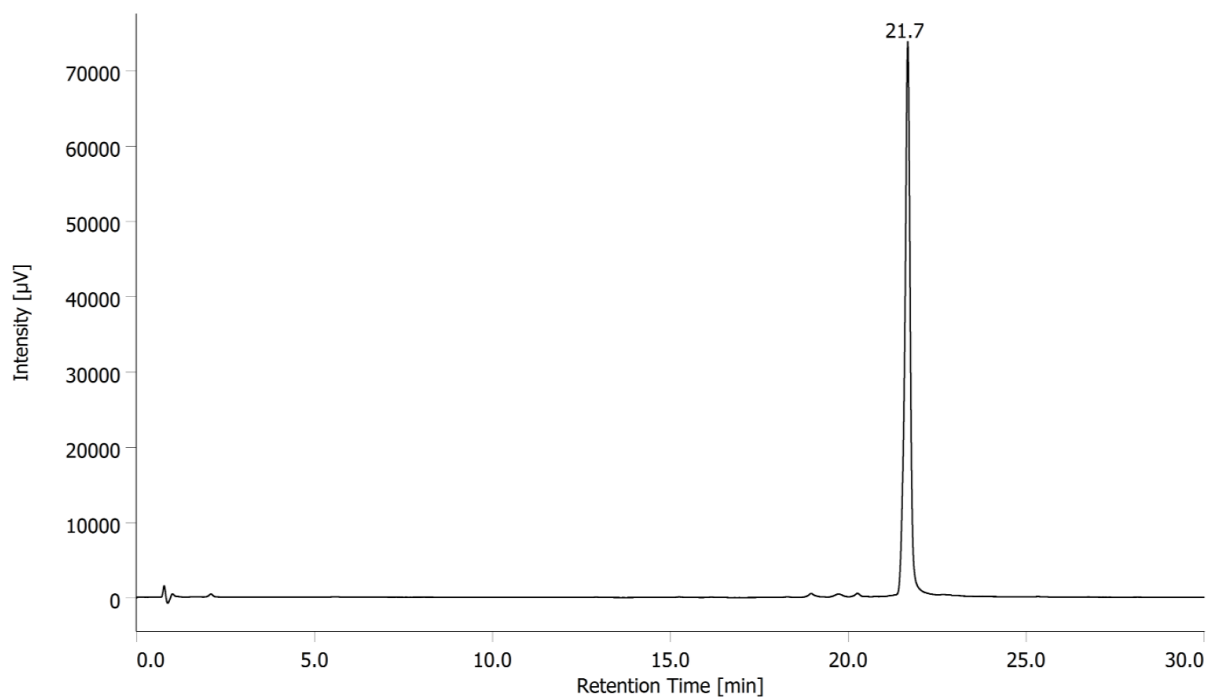
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 $^{\circ}\text{C}$.

ESI-MS spectrum of **ON6a**



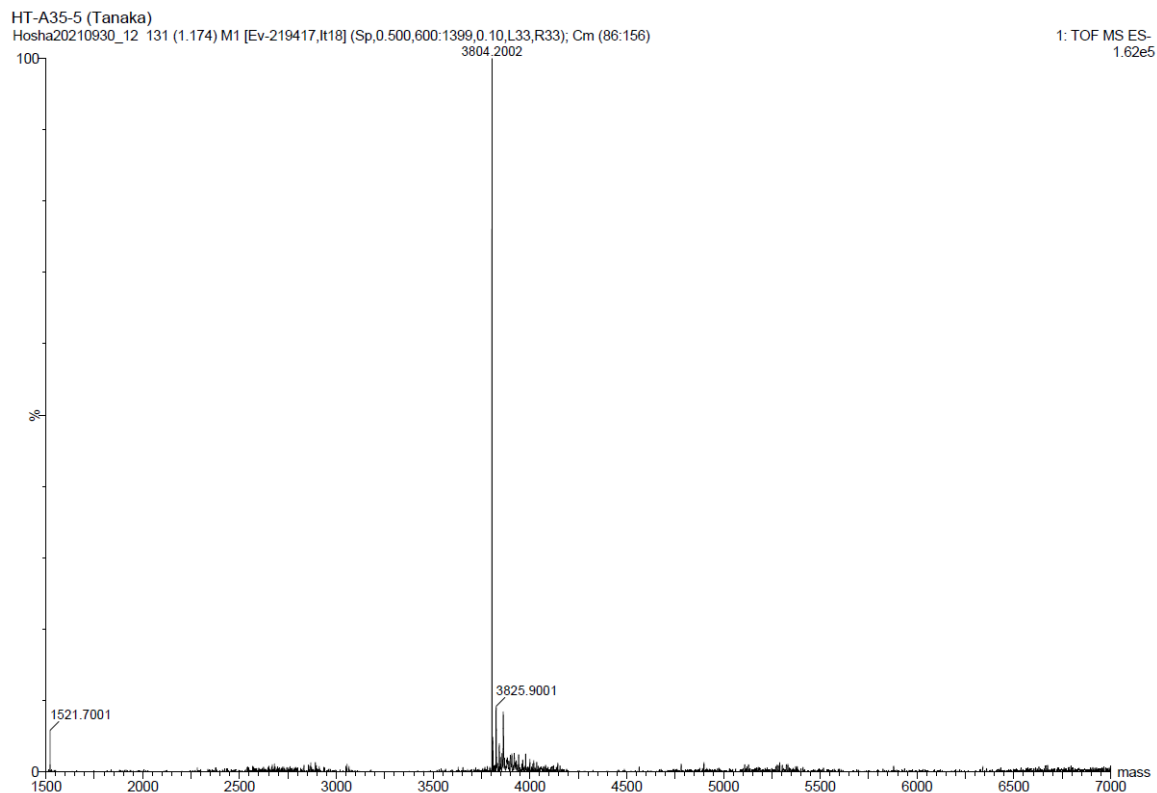
For **ON6b**

HPLC chromatogram of purified **ON6b**



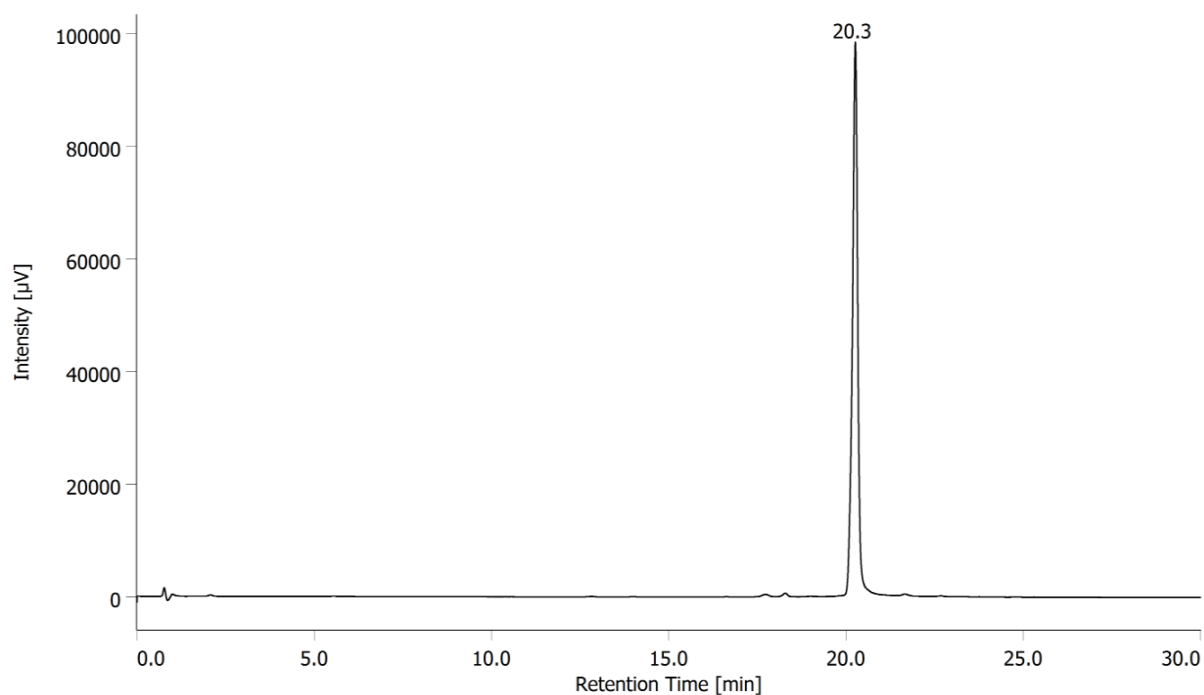
Column: Waters XBridgeTM Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 $^{\circ}\text{C}$.

ESI-MS spectrum of **ON6b**



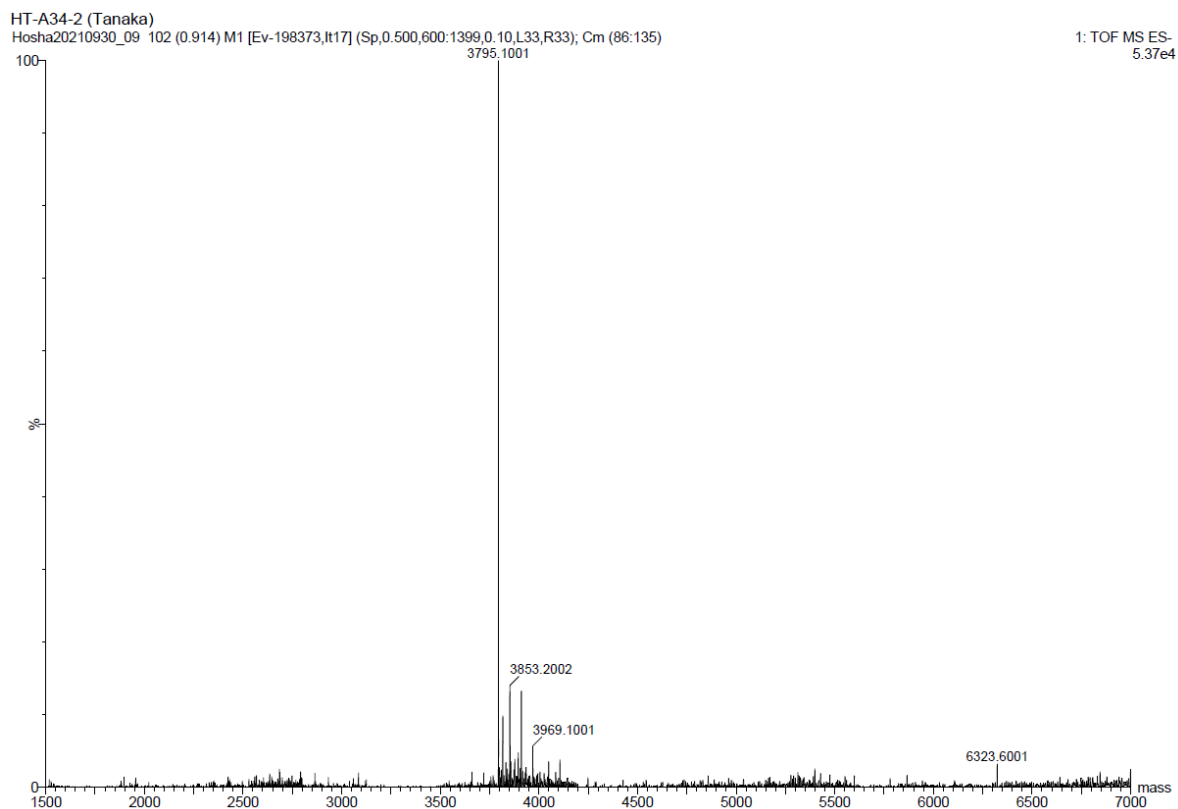
For **ON6c**

HPLC chromatogram of purified **ON6c**



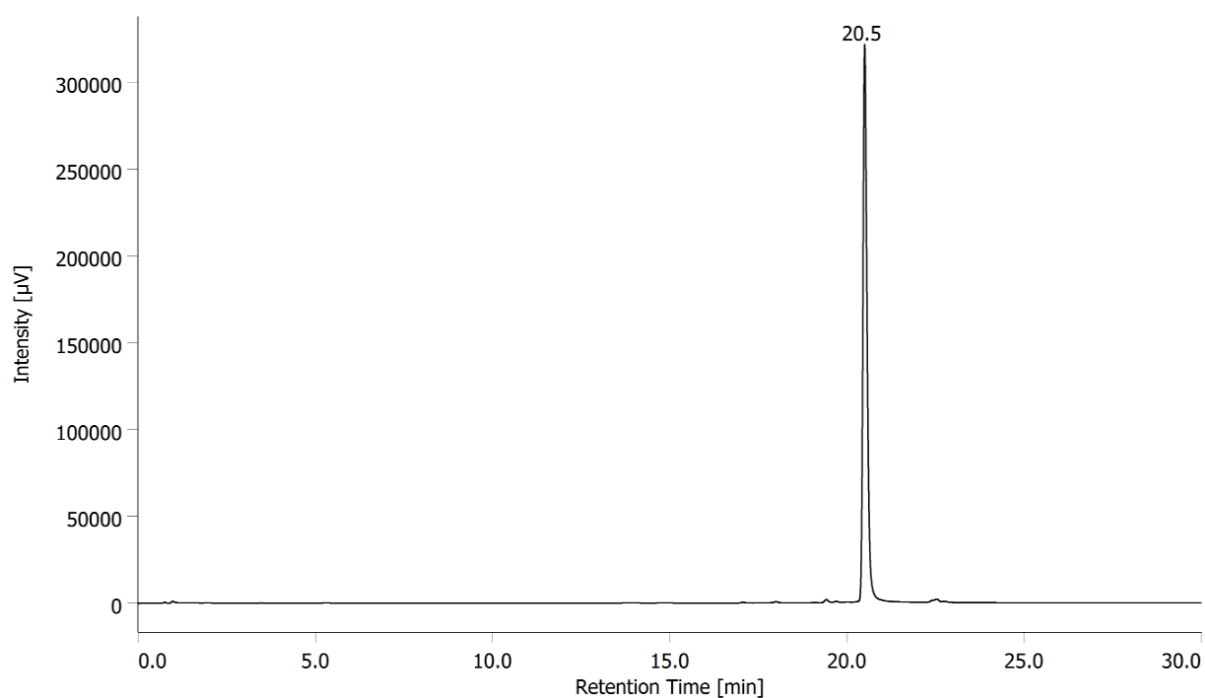
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON6c**



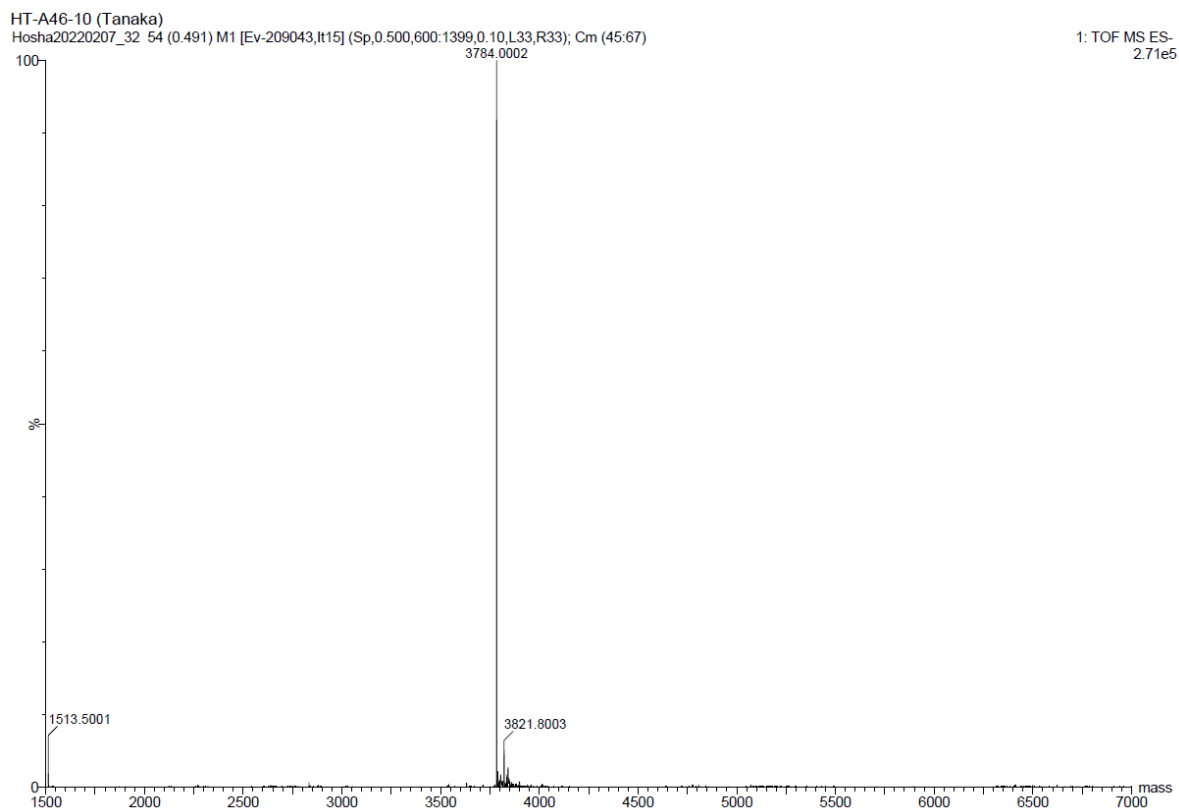
For **ON6d**

HPLC chromatogram of purified **ON6d**



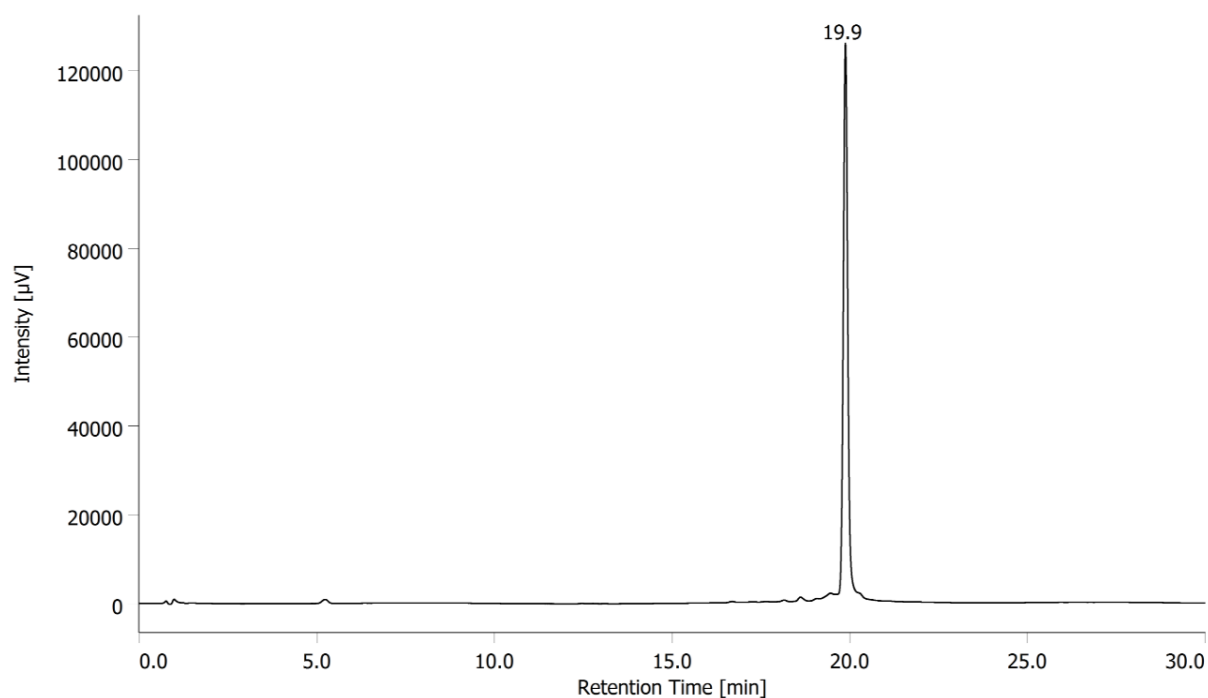
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON6d**



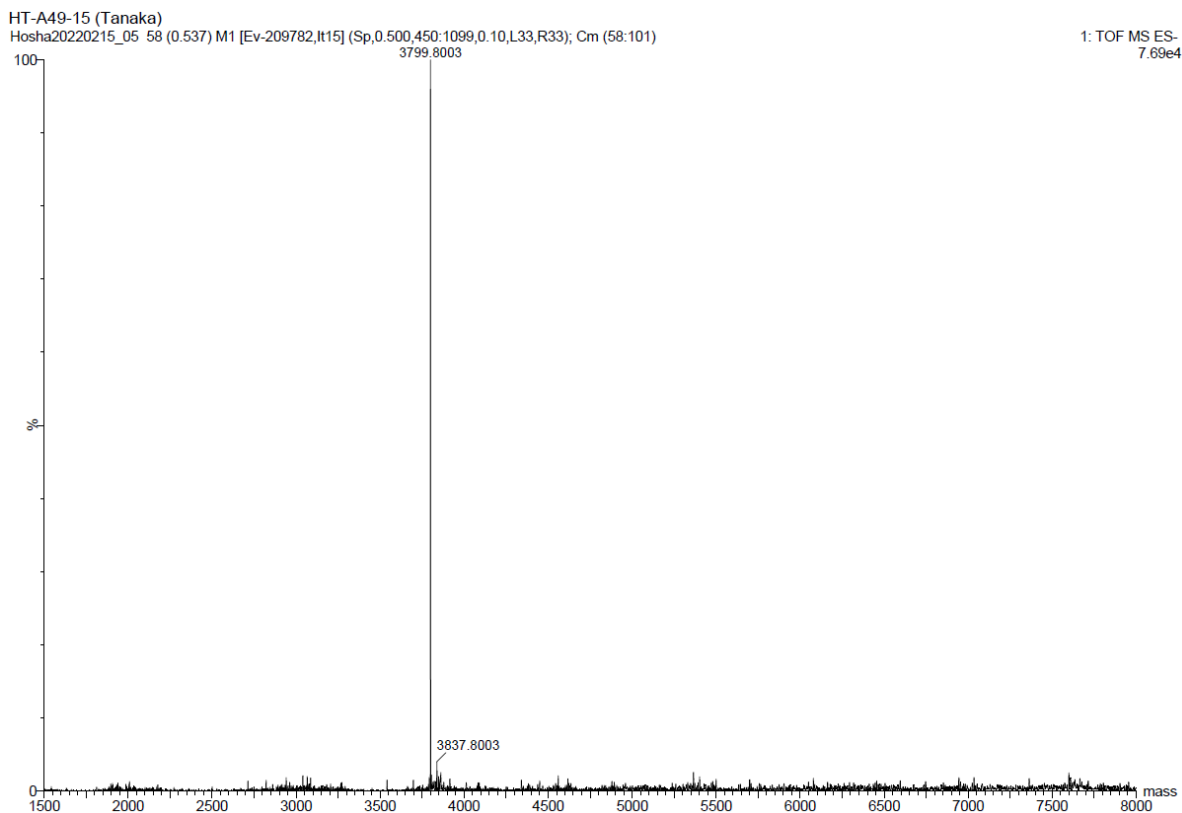
For **ON6e**

HPLC chromatogram of purified **ON6e**



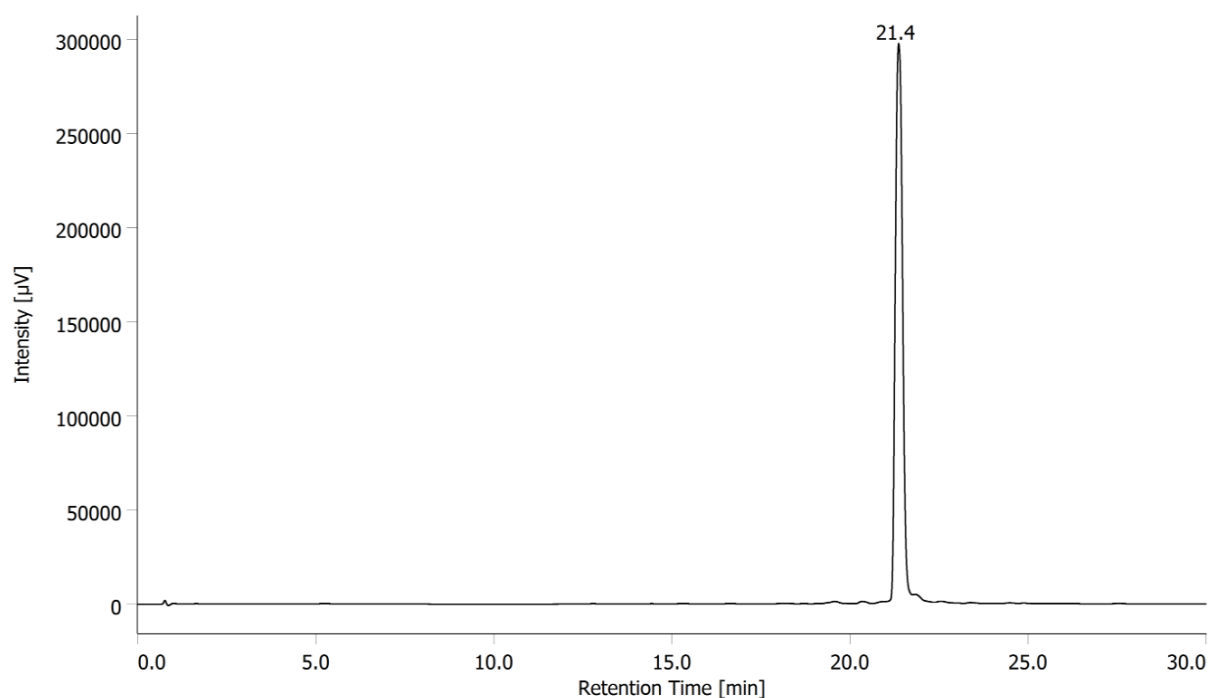
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON6e**



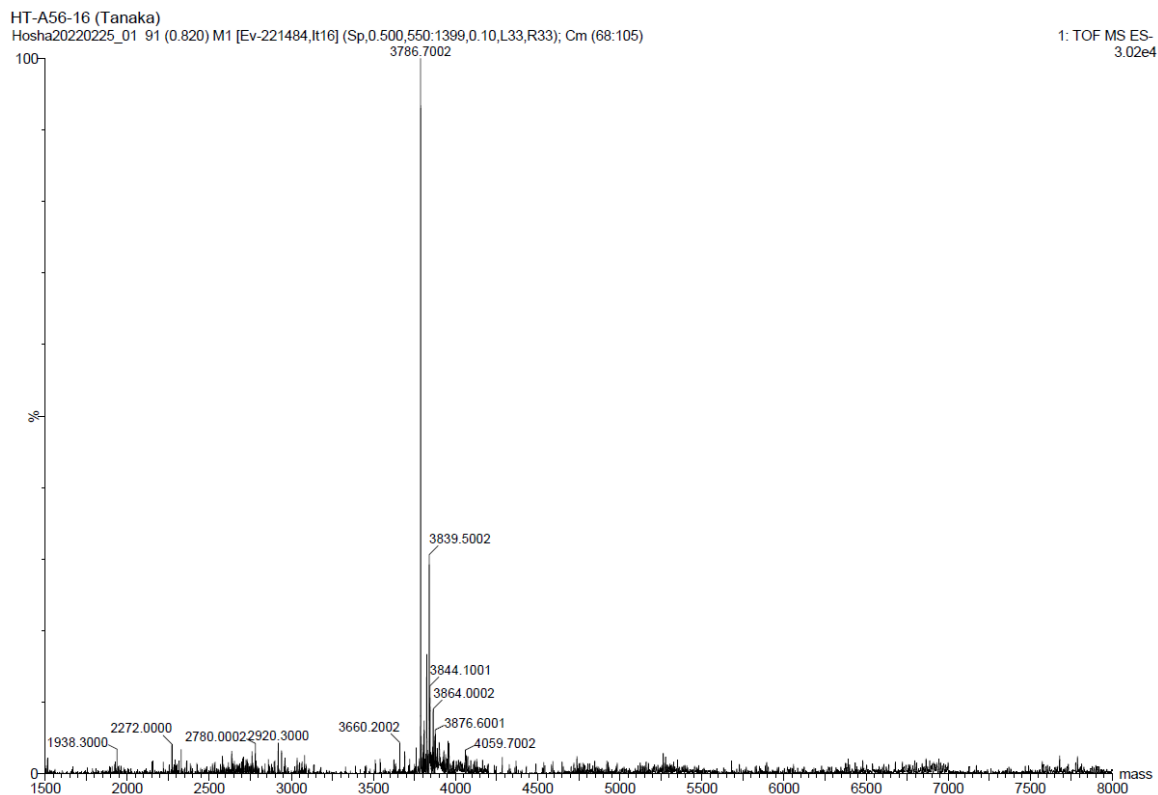
For **ON9a**

HPLC chromatogram of purified **ON9a**



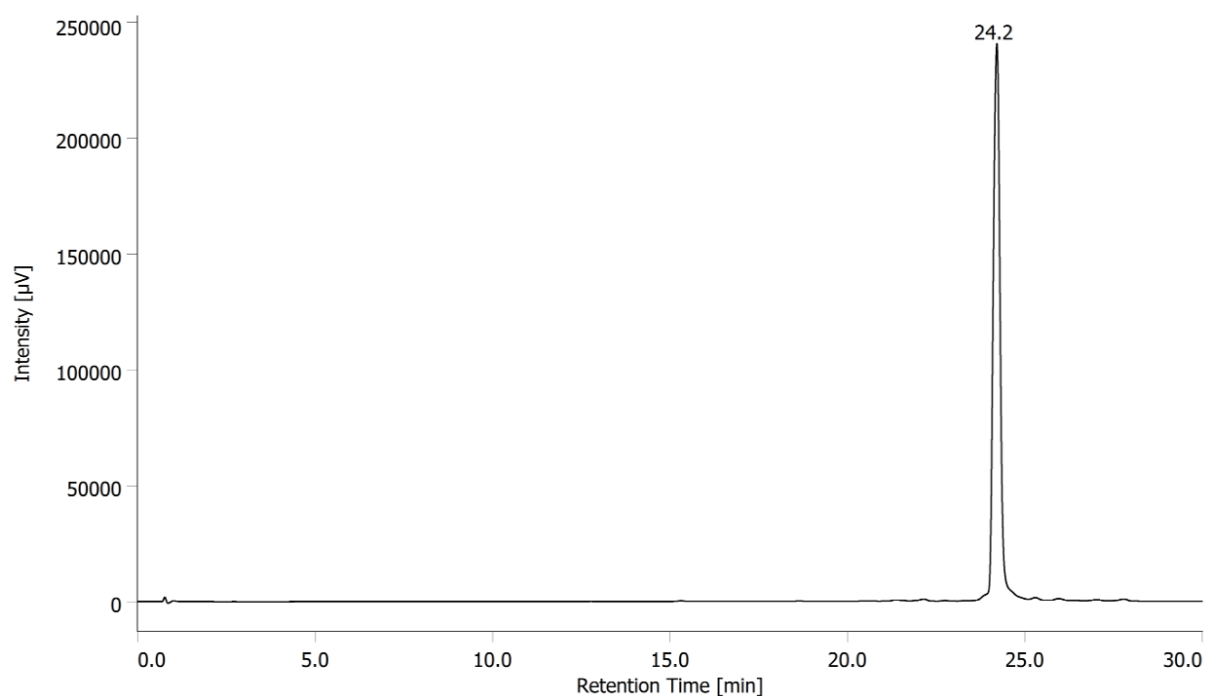
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON9a**



ON9b

HPLC chromatogram of purified **ON9b**

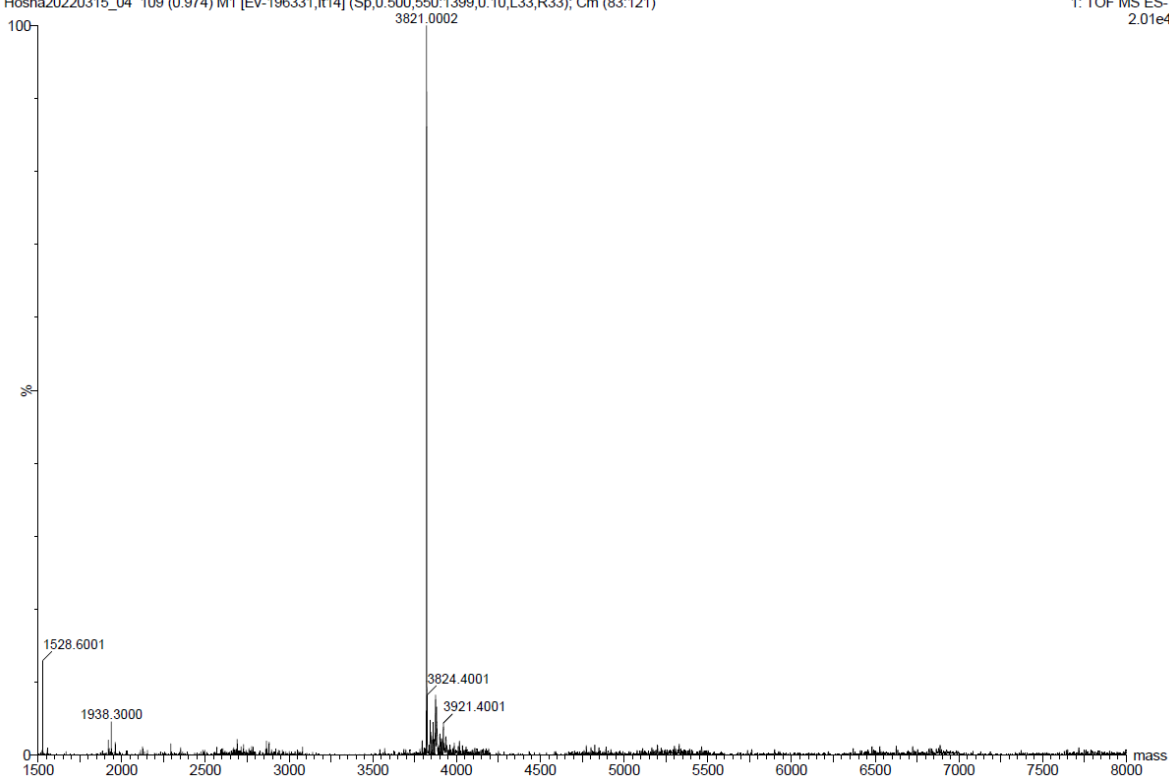


Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON9b**

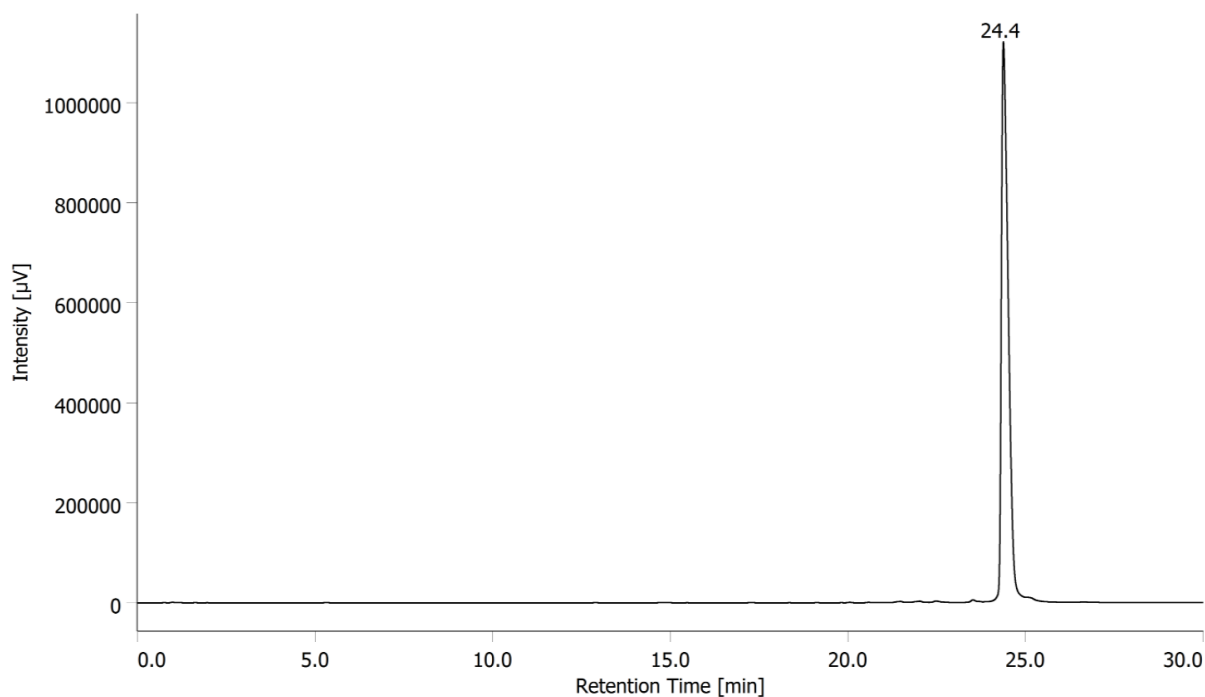
HT-A61-8 (Tanaka)
Hosha20220315_04 109 (0.974) M1 [Ev-196331,It14] (Sp,0.500,550:1399,0.10,L33,R33); Cm (83:121)

1: TOF MS ES-
2.01e4



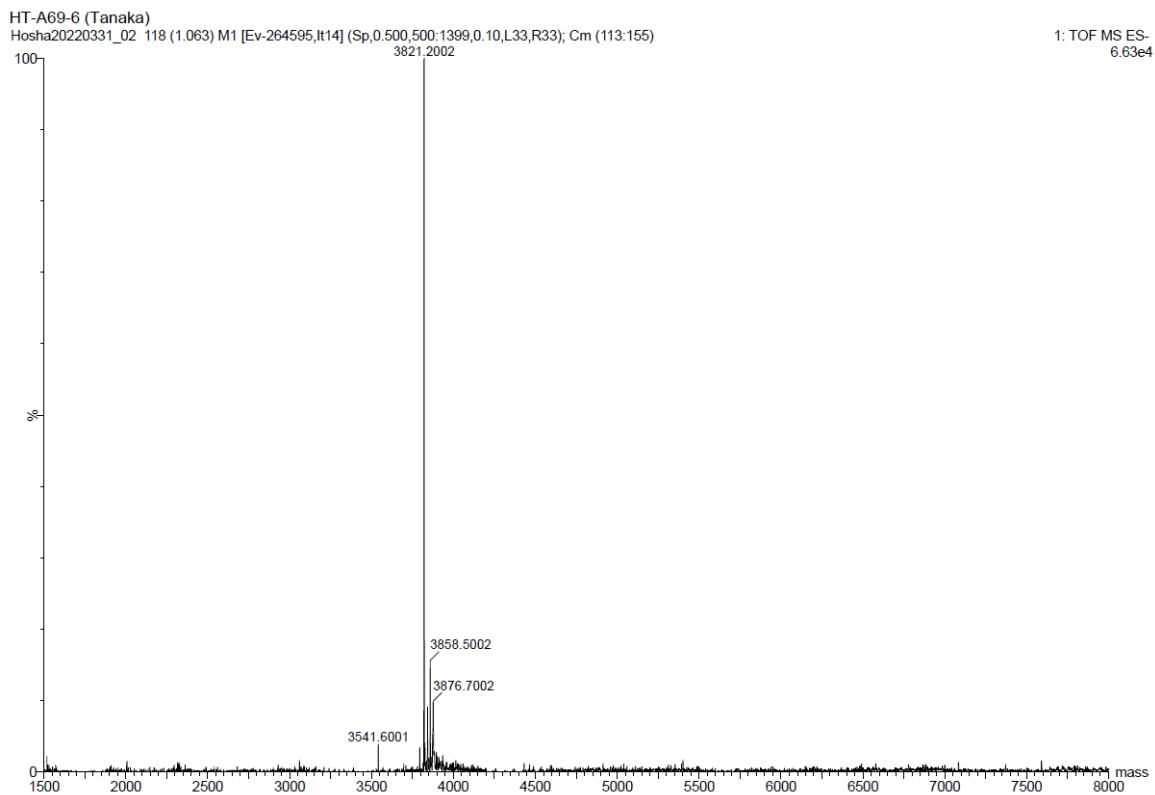
For **ON9c**

HPLC chromatogram of purified **ON9c**



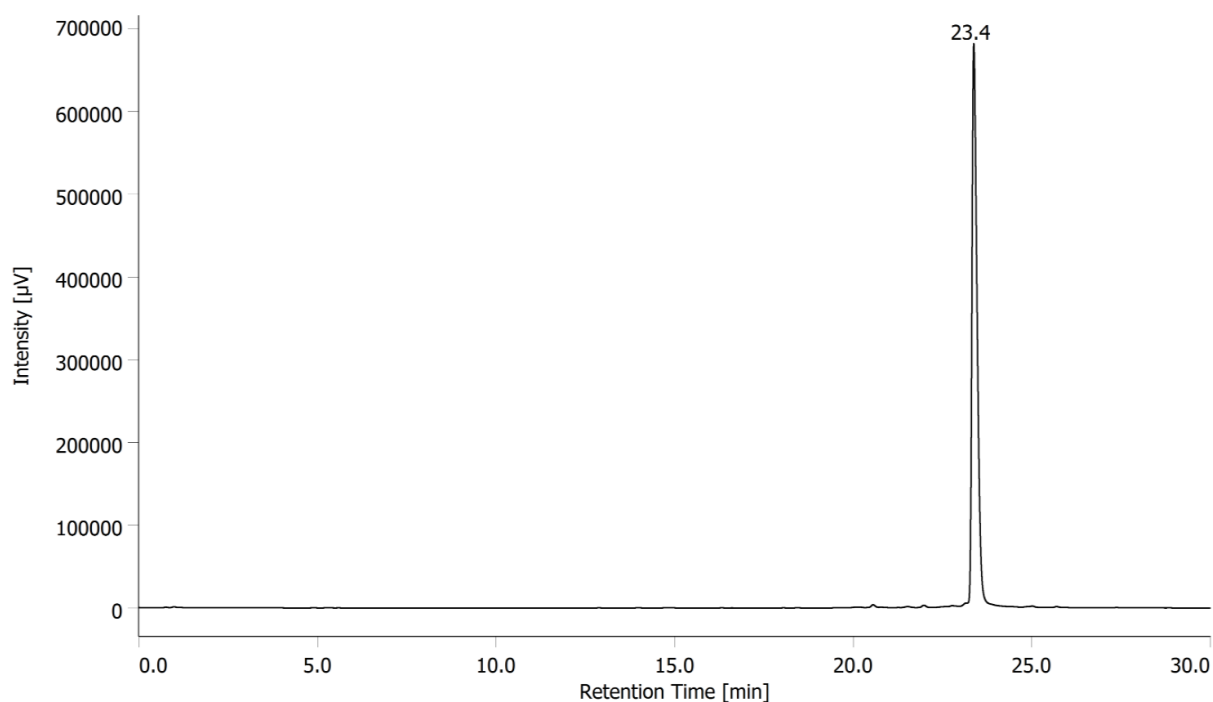
Column: Waters XBridgeTM Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 $^{\circ}\text{C}$.

ESI-MS spectrum of **ON9c**



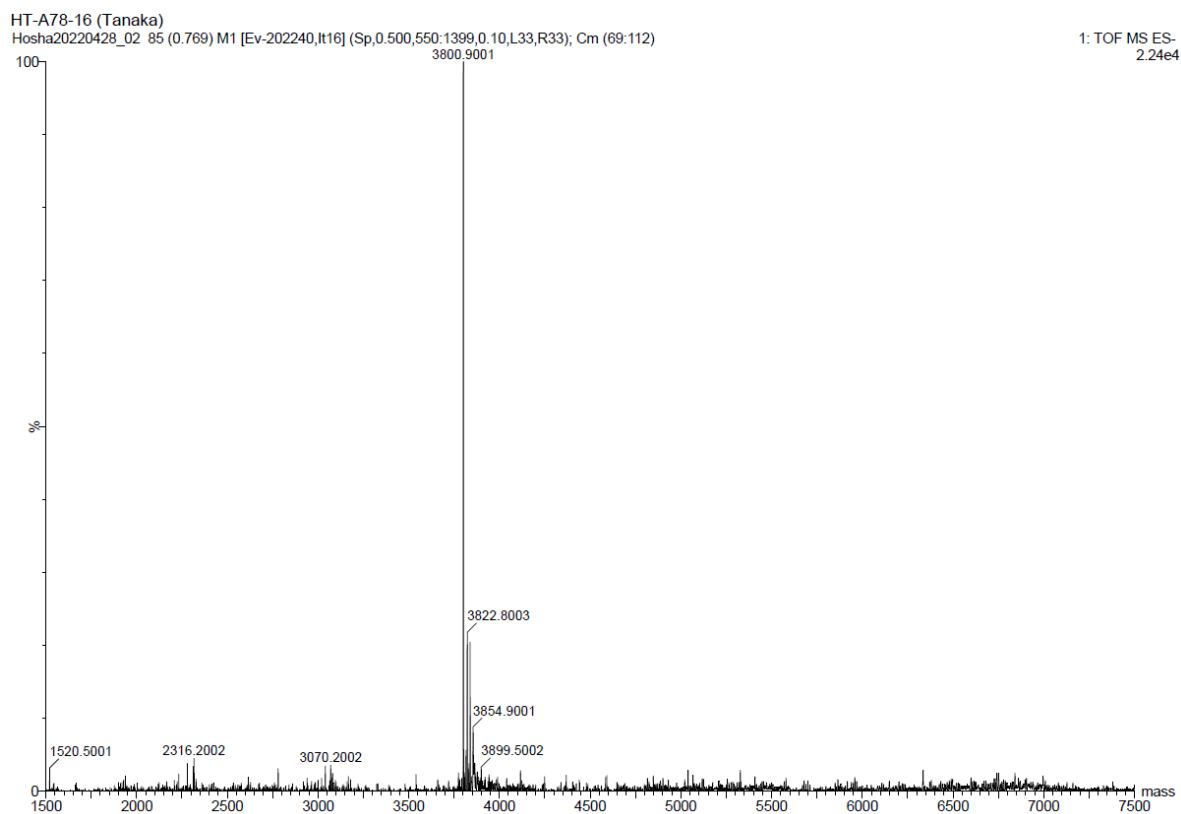
For **ON9d**

HPLC chromatogram of purified **ON9d**



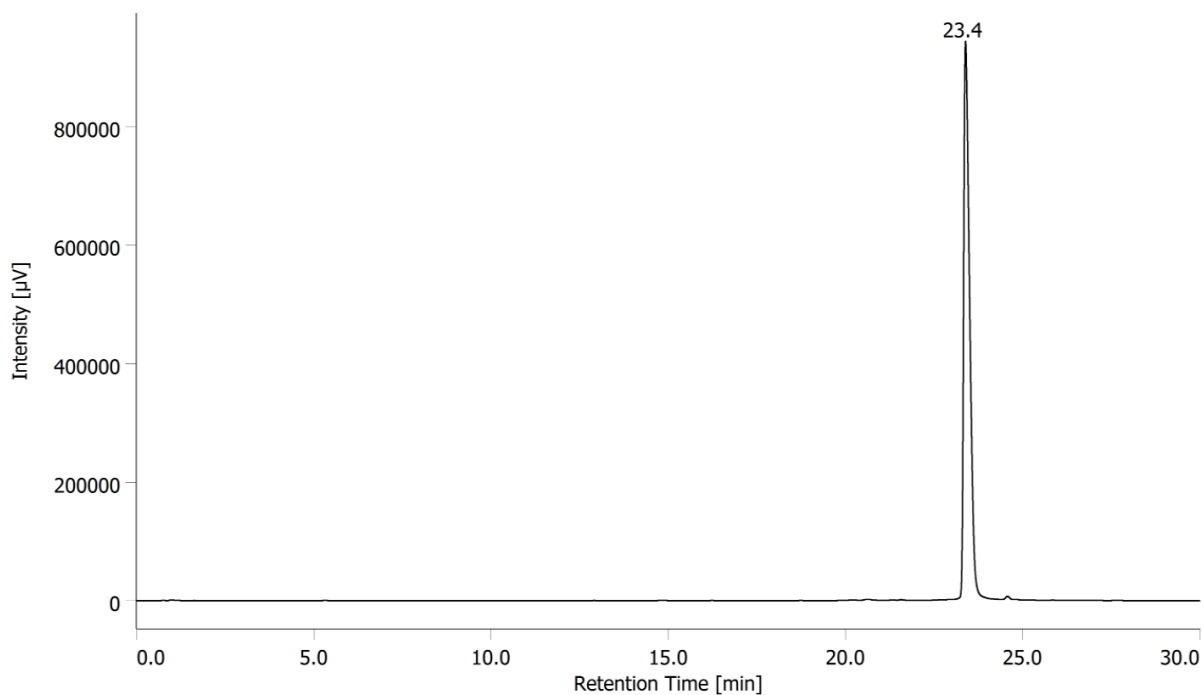
Column: Waters XBridge™ Shield RP18 2.5 μm, 4.6 × 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON9d**



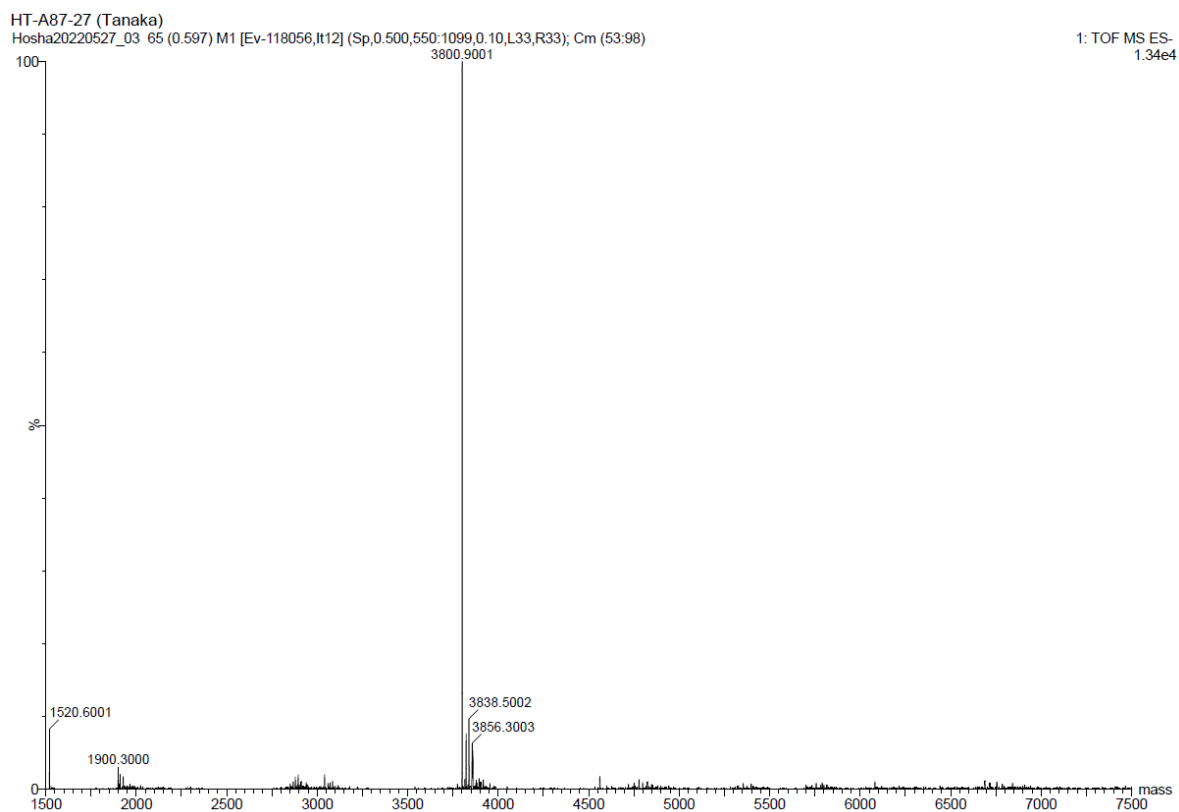
For **ON9e**

HPLC chromatogram of purified **ON9e**



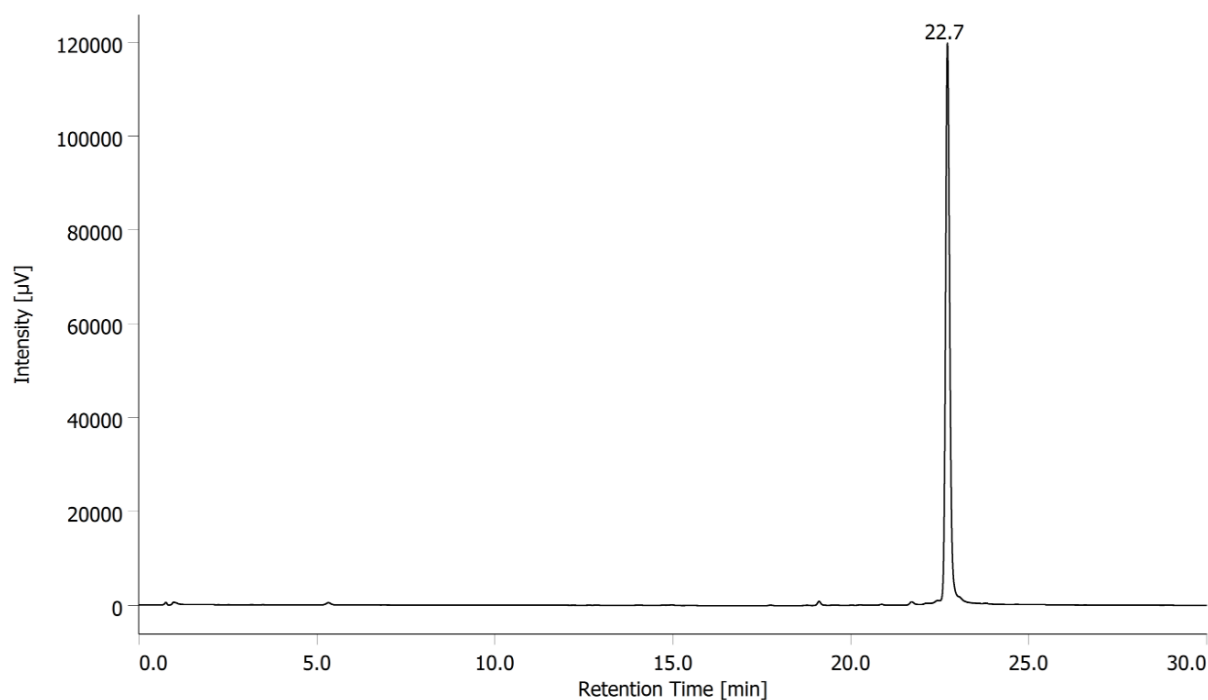
Column: Waters XBridgeTM Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 $^{\circ}\text{C}$.

ESI-MS spectrum of **ON9e**



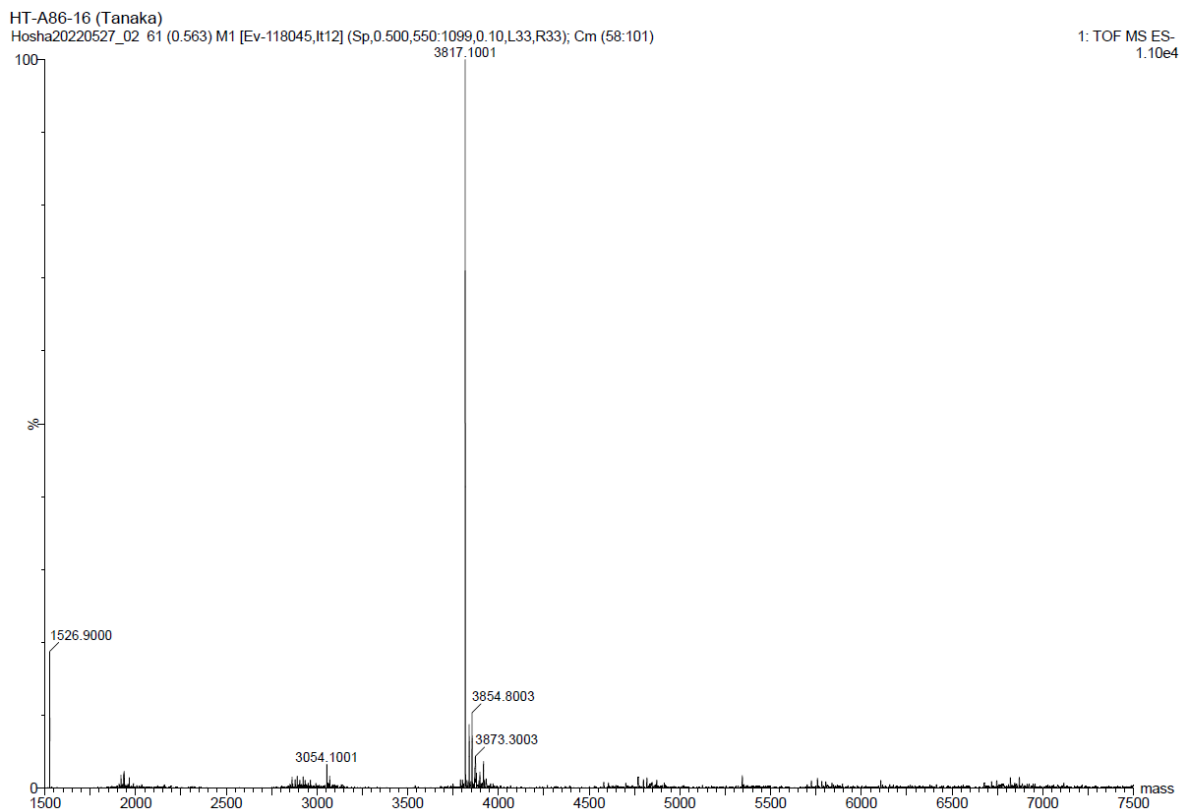
For **ON9f**

HPLC chromatogram of purified **ON9f**



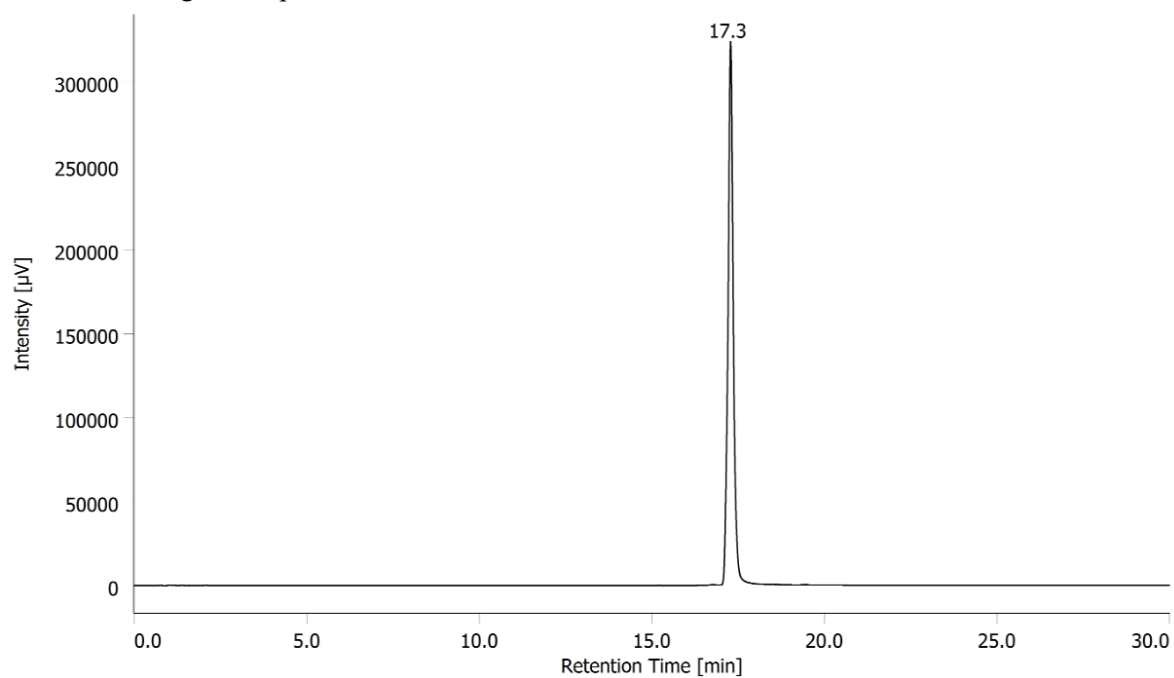
Column: Waters XBridge™ Shield RP18 2.5 μm, 4.6 × 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON9f**



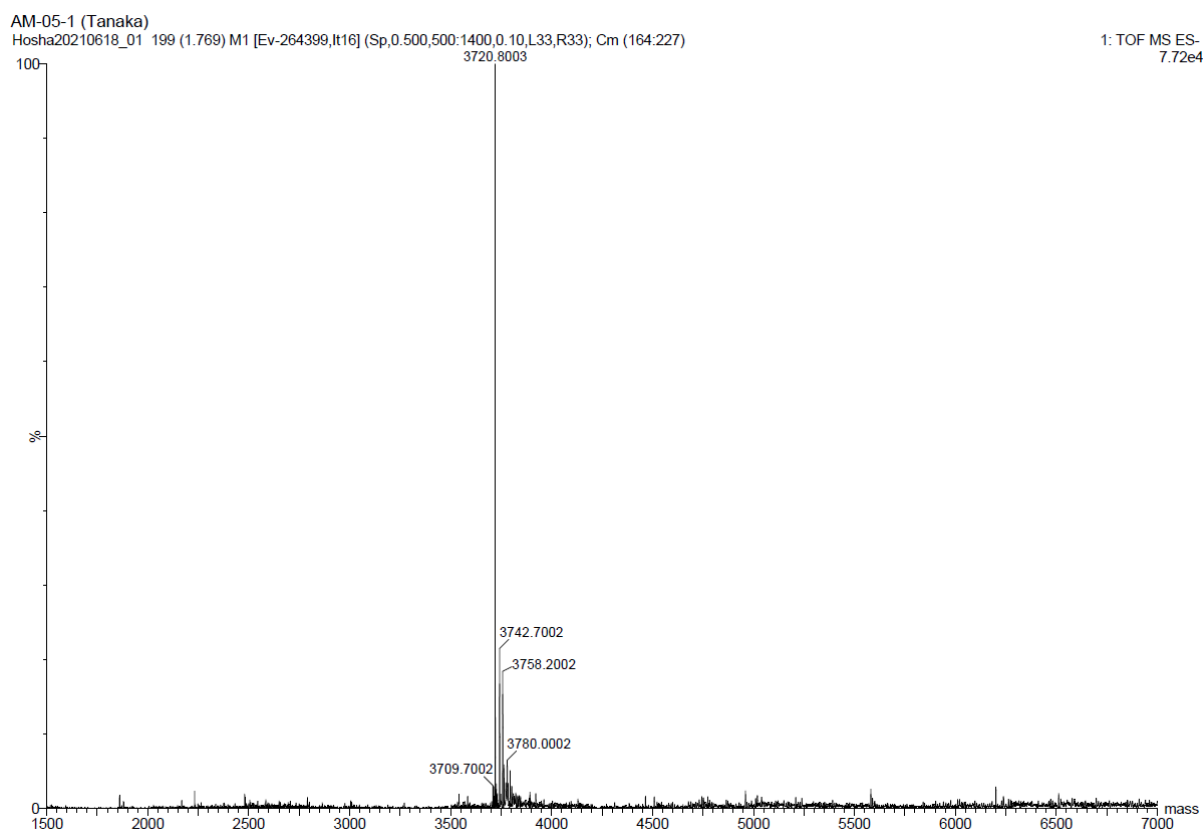
For **ON10**

HPLC chromatogram of purified **ON10**



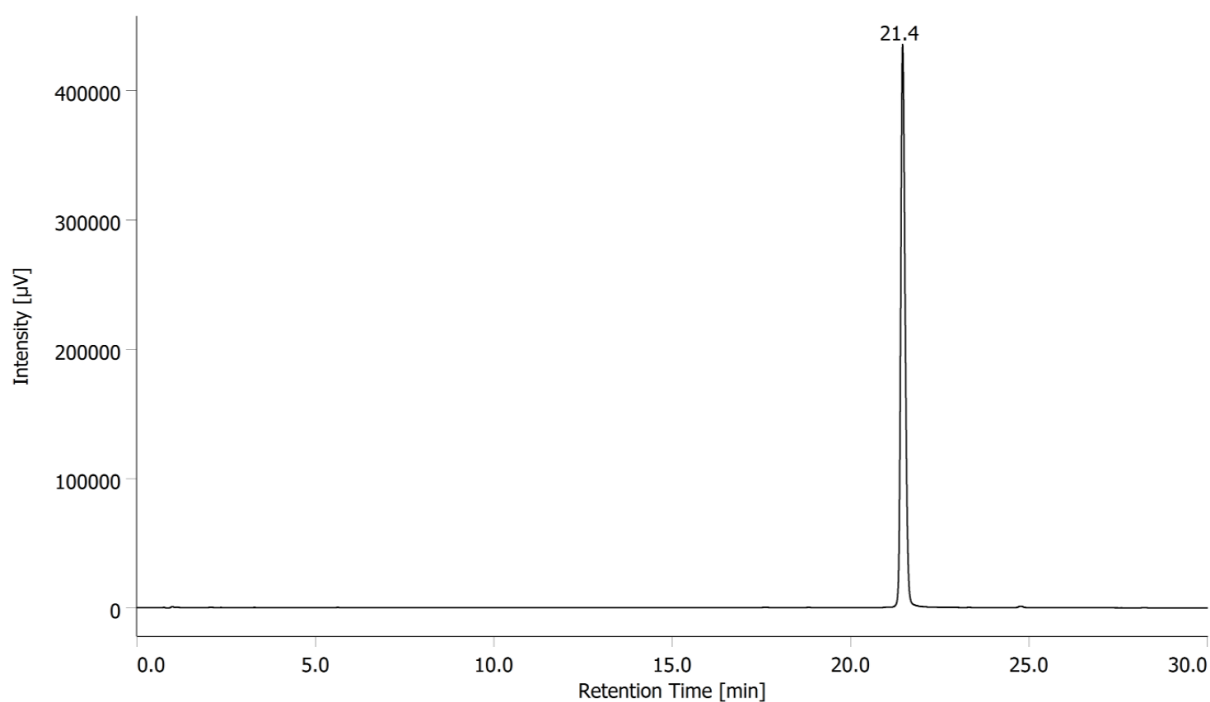
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON10**



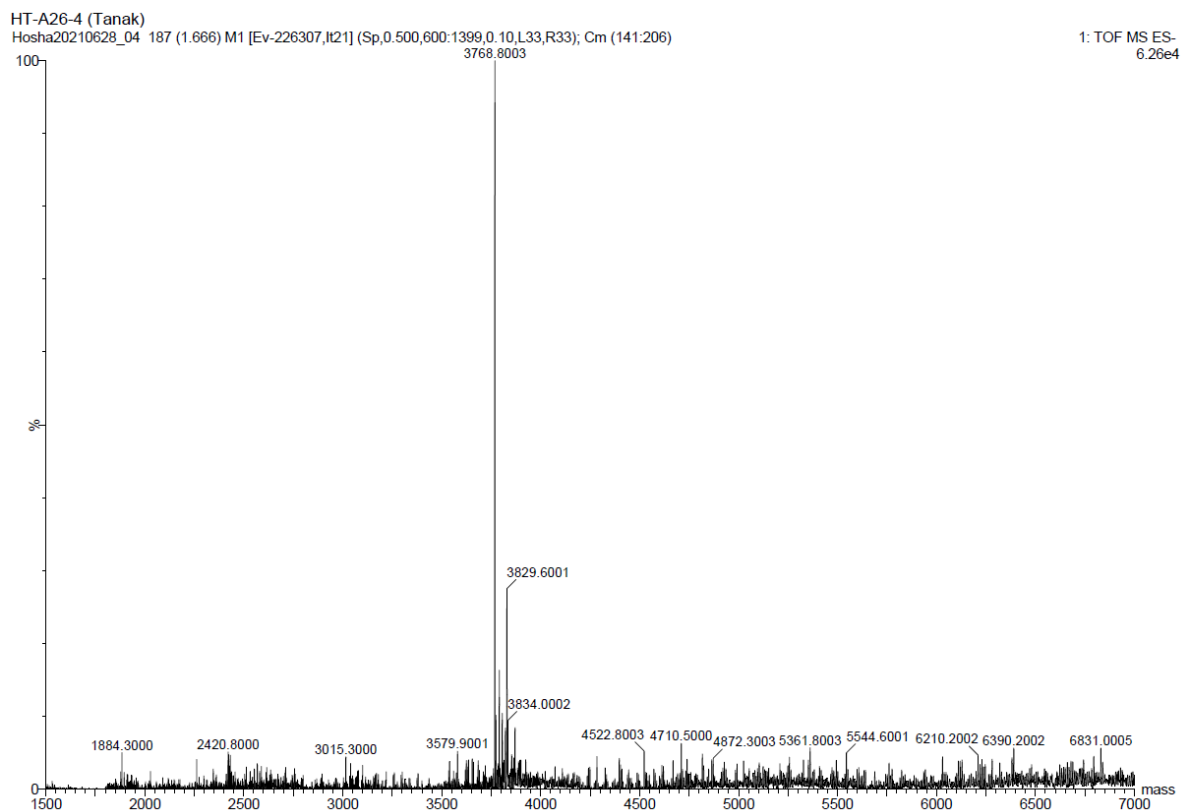
For **ON11a**

HPLC chromatogram of purified **ON11a**



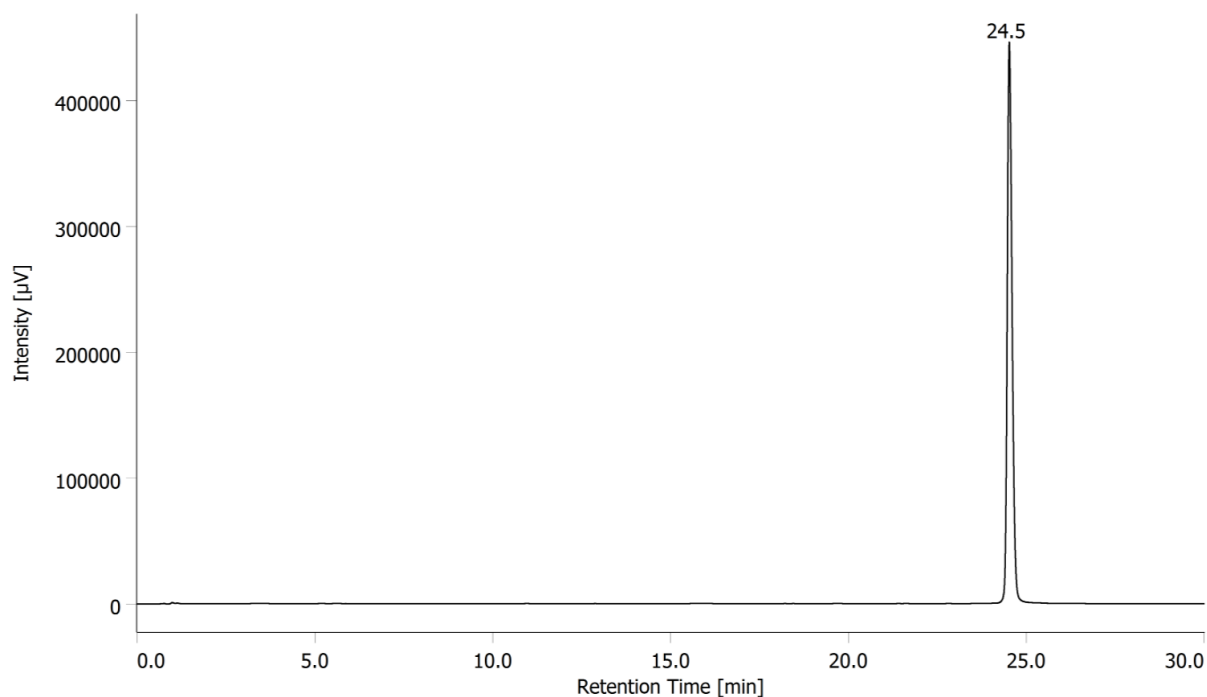
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON11a**



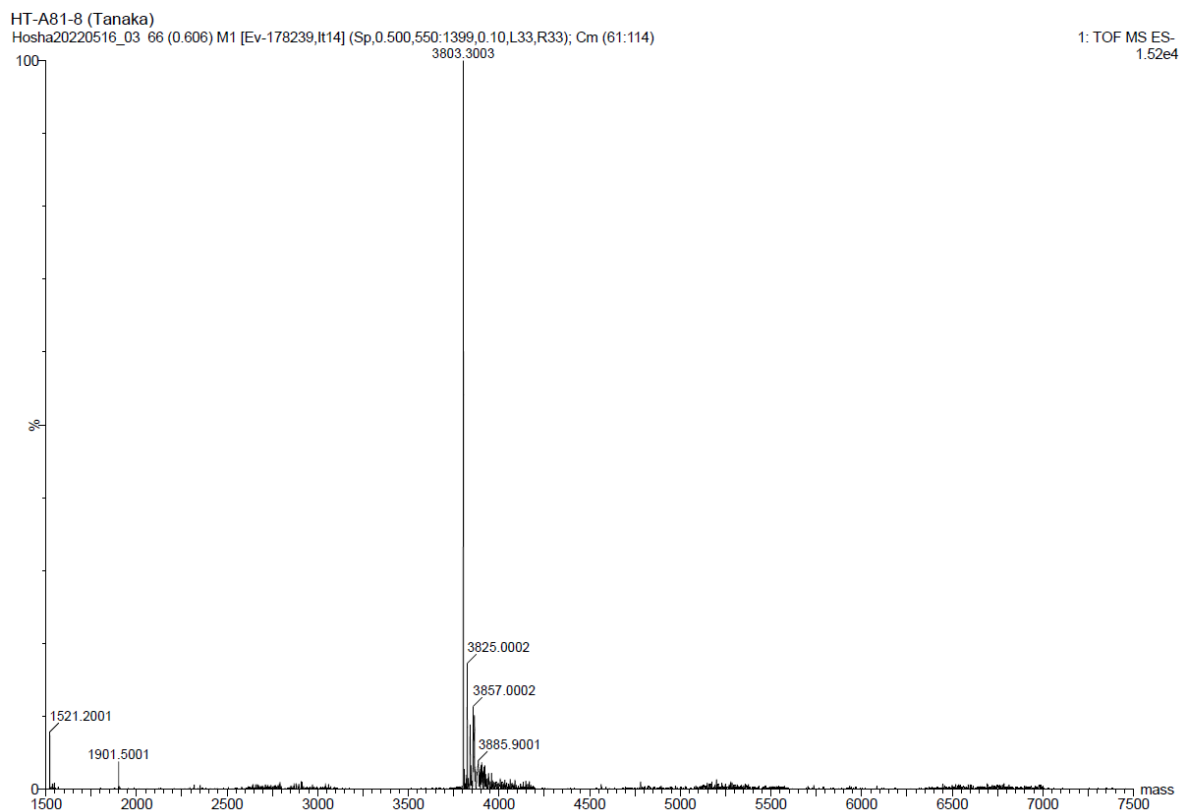
For **ON11b**

HPLC chromatogram of purified **ON11b**



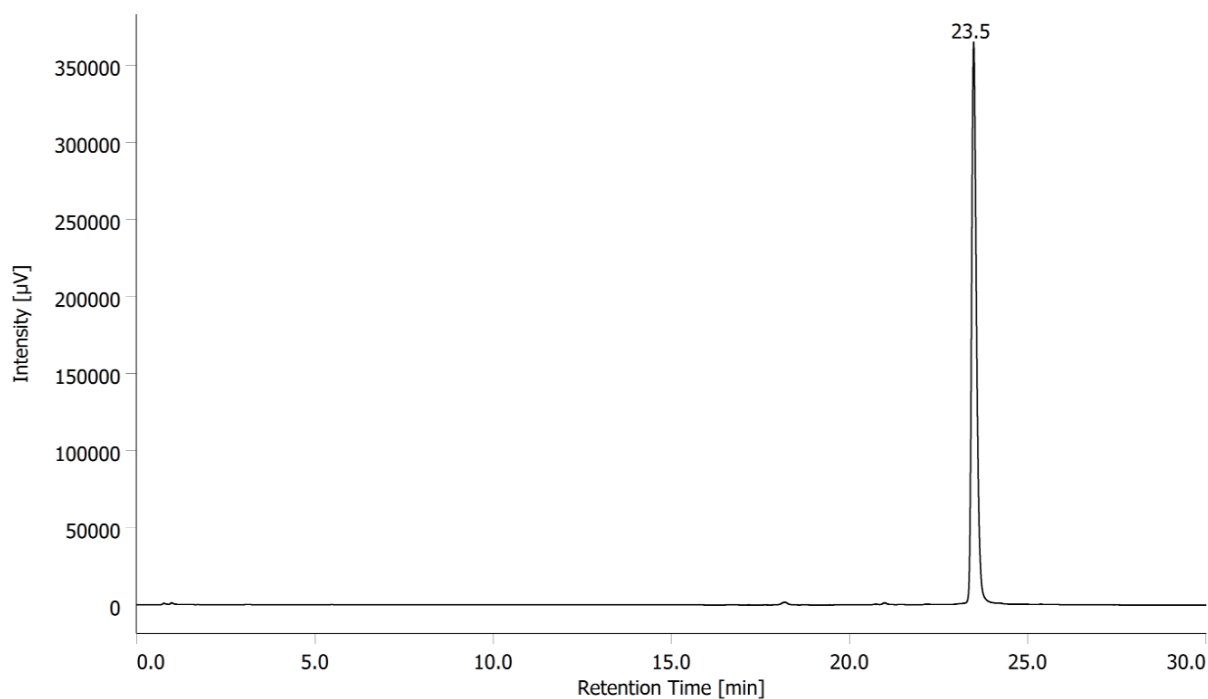
Column: Waters XBridgeTM Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 $^{\circ}\text{C}$.

ESI-MS spectrum of **ON11b**



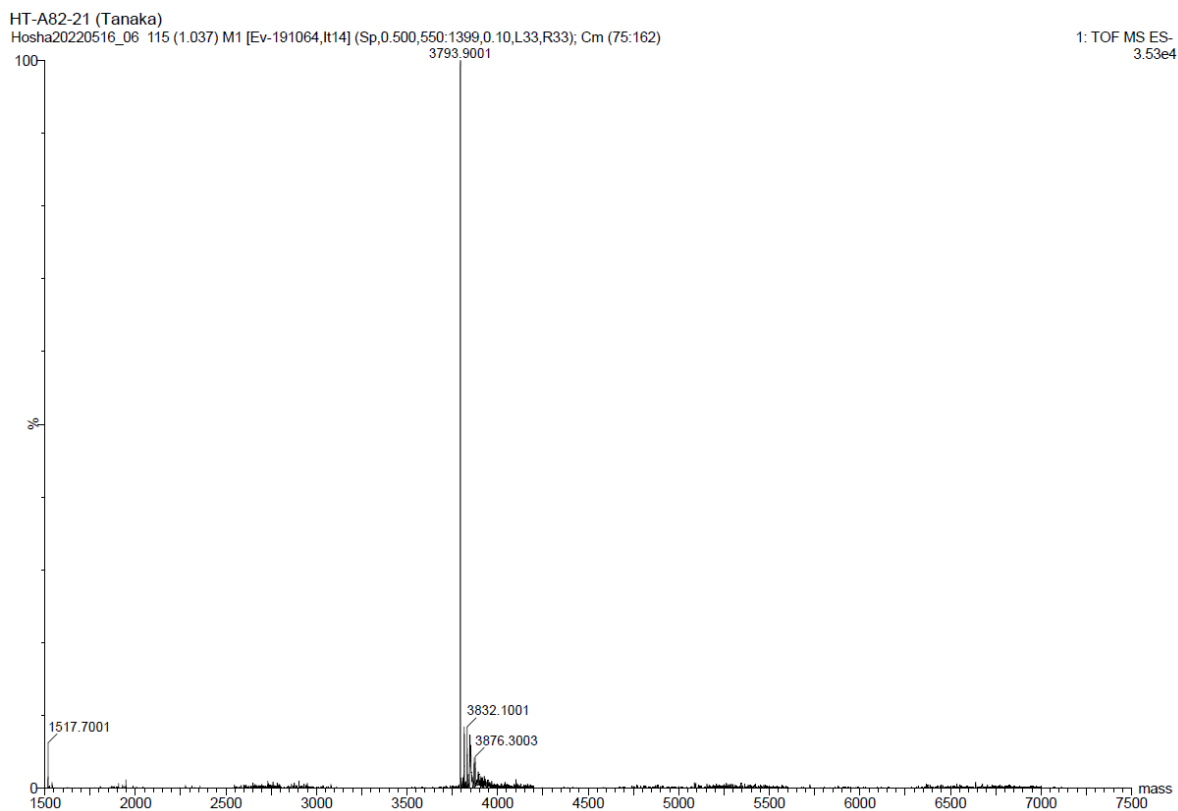
For **ON11c**

HPLC chromatogram of purified **ON11c**



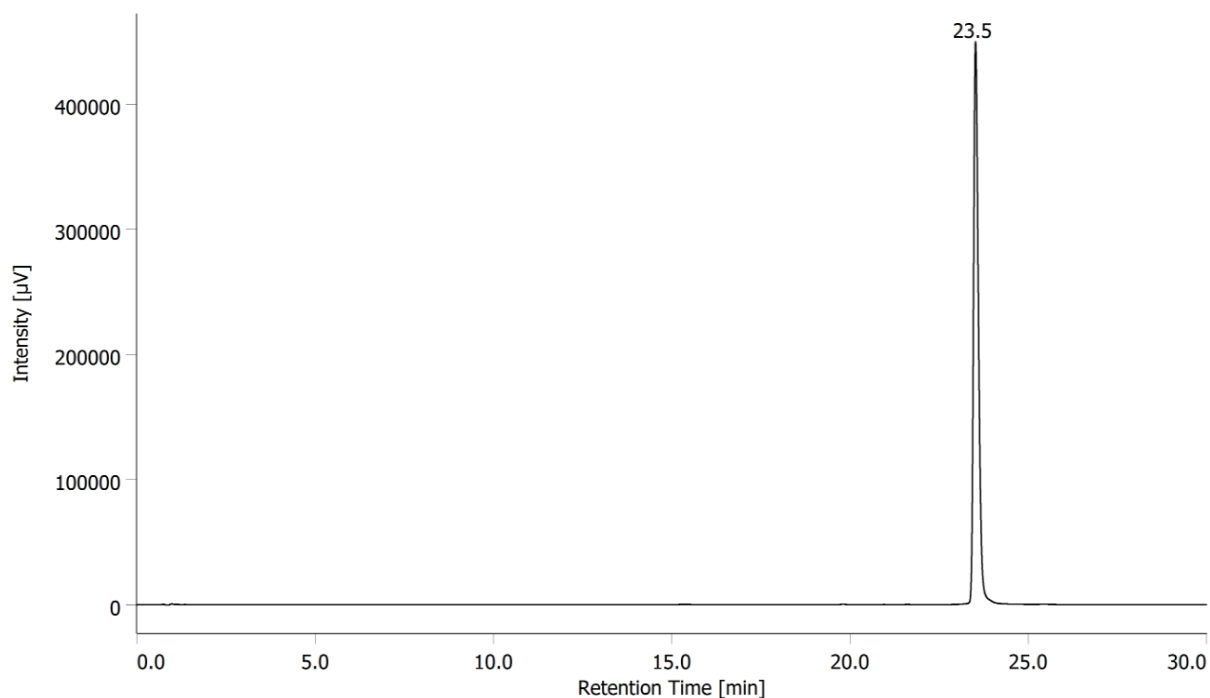
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON11c**



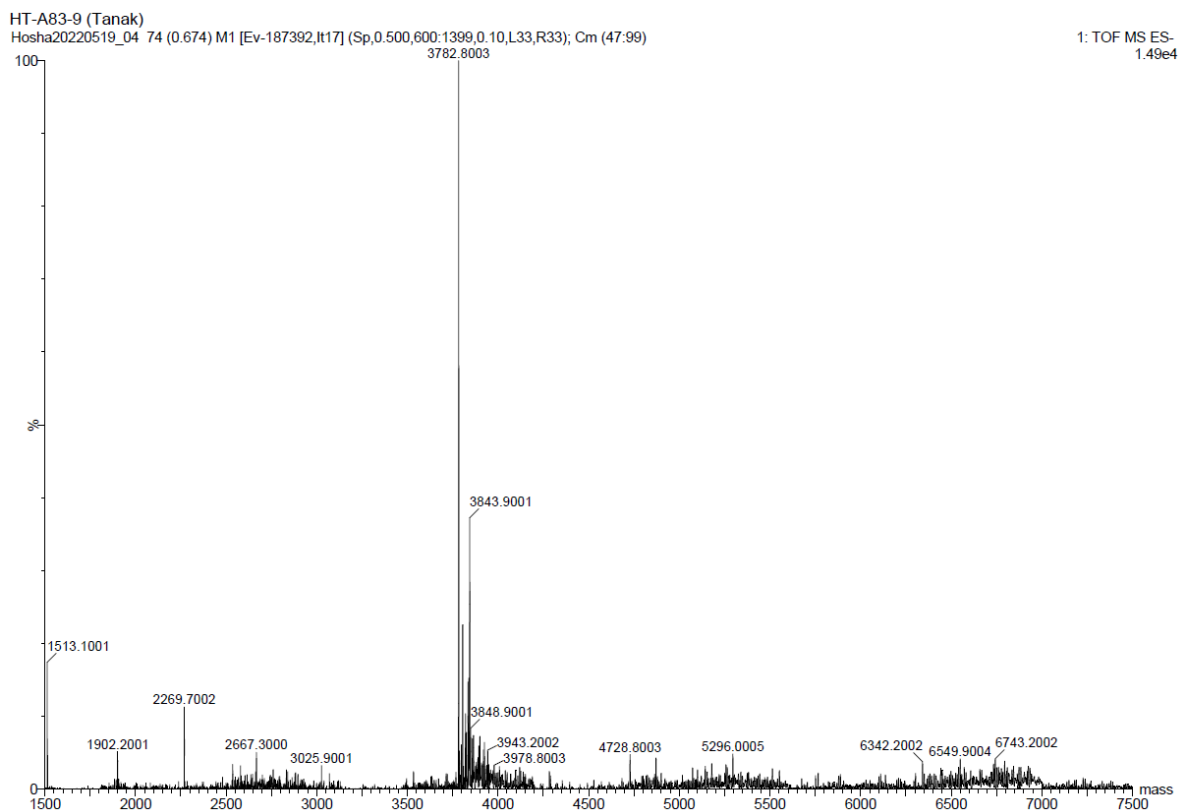
For **ON11d**

HPLC chromatogram of purified **ON11d**



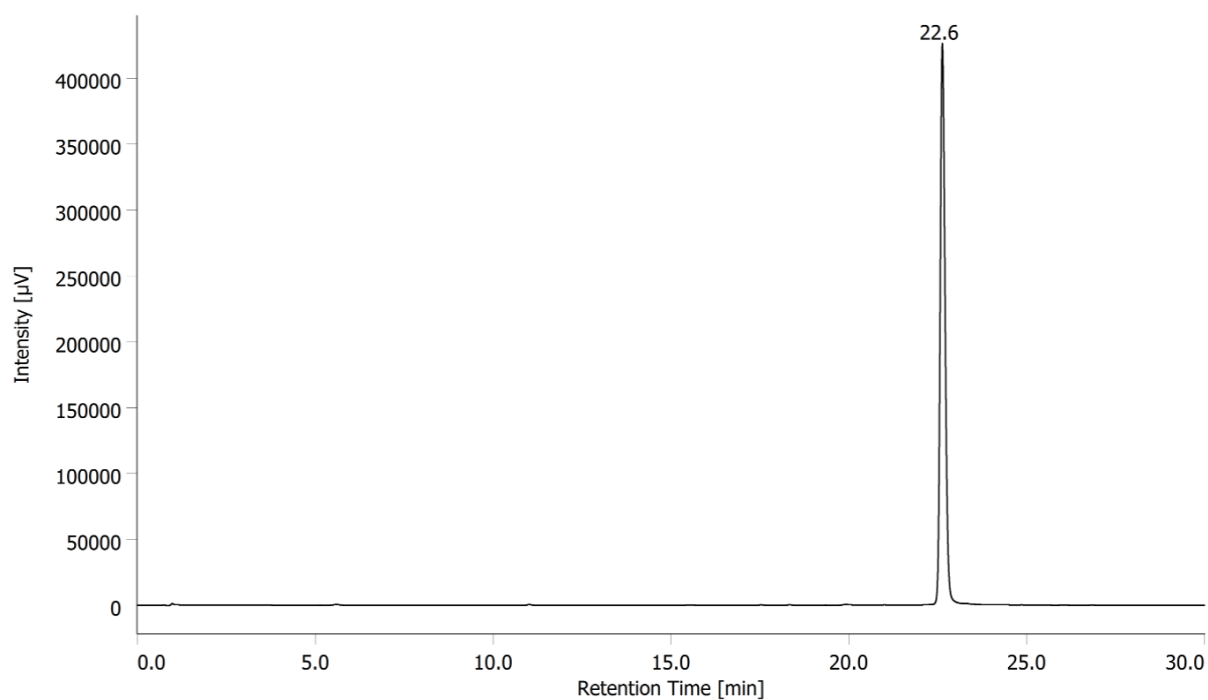
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON11d**



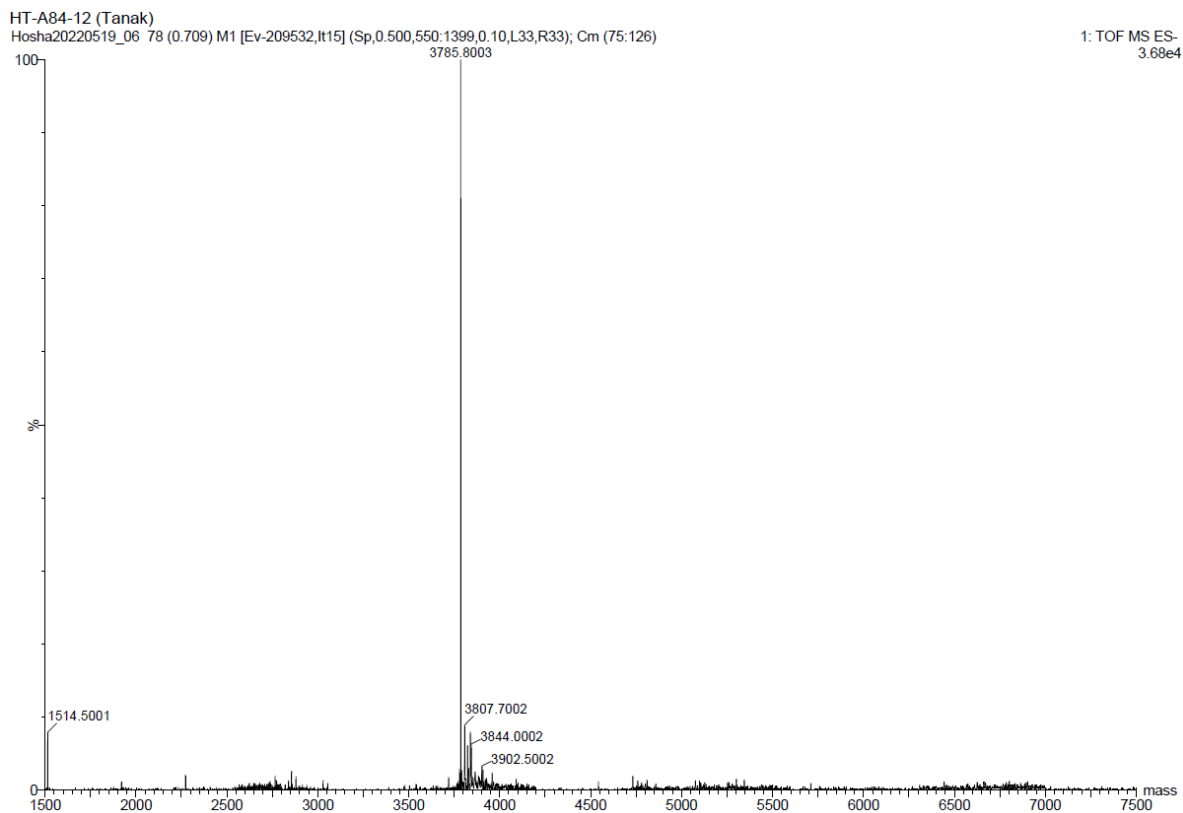
For **ON12a**

HPLC chromatogram of purified **ON12a**



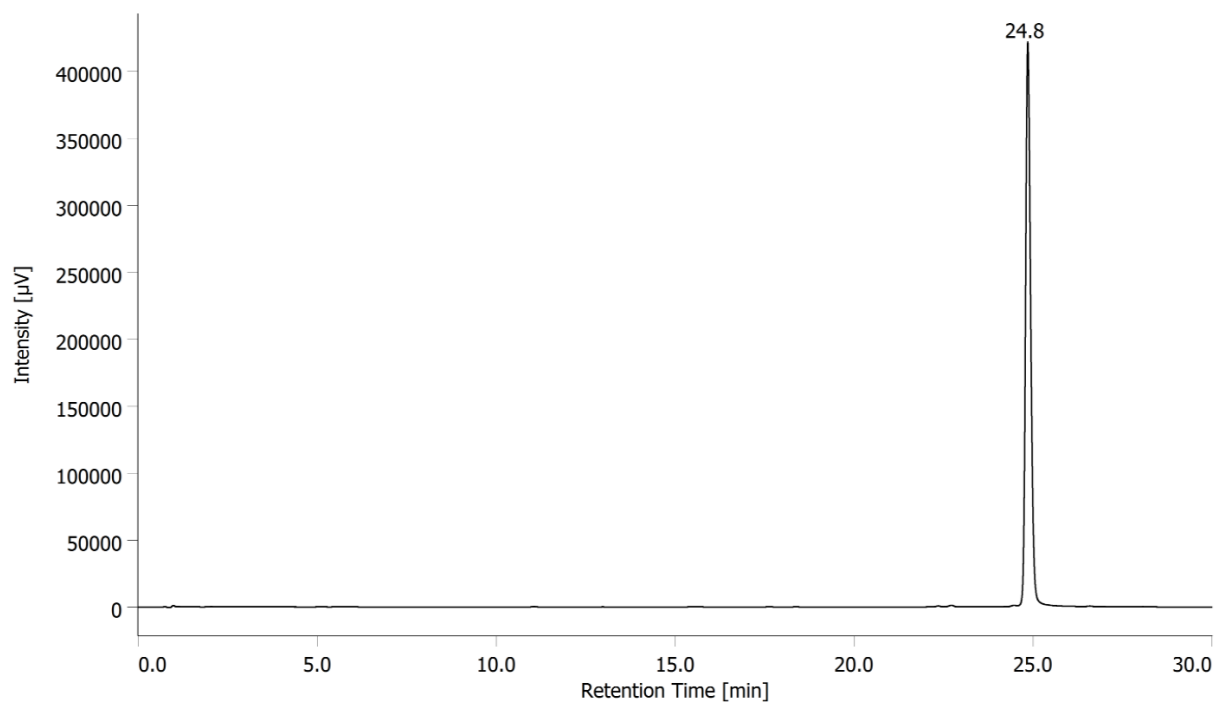
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON12a**



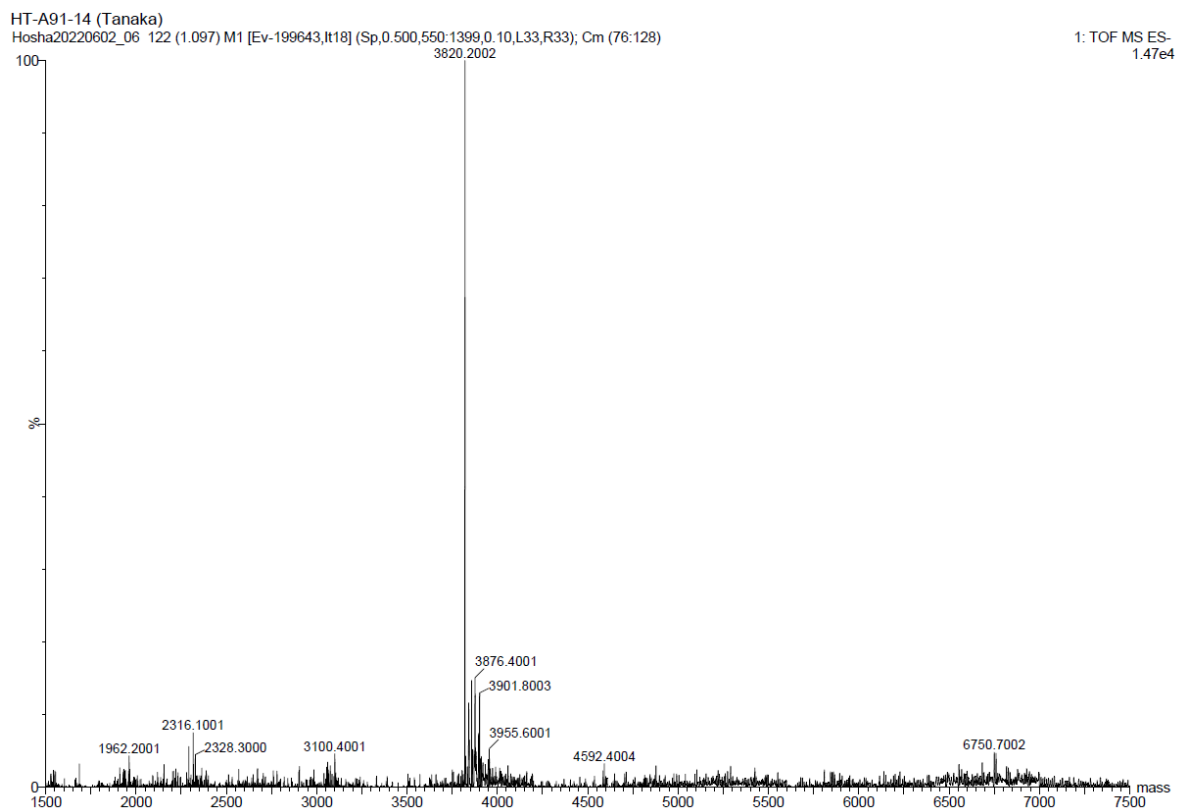
For **ON12b**

HPLC chromatogram of purified **ON12b**



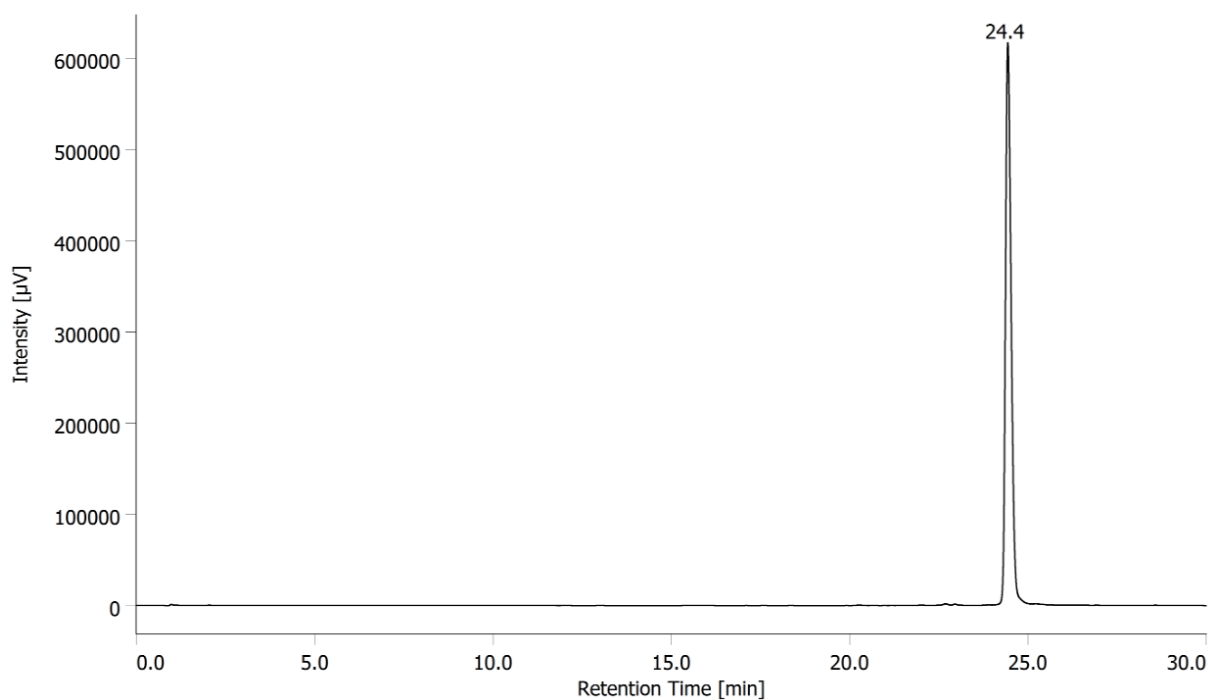
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON12b**



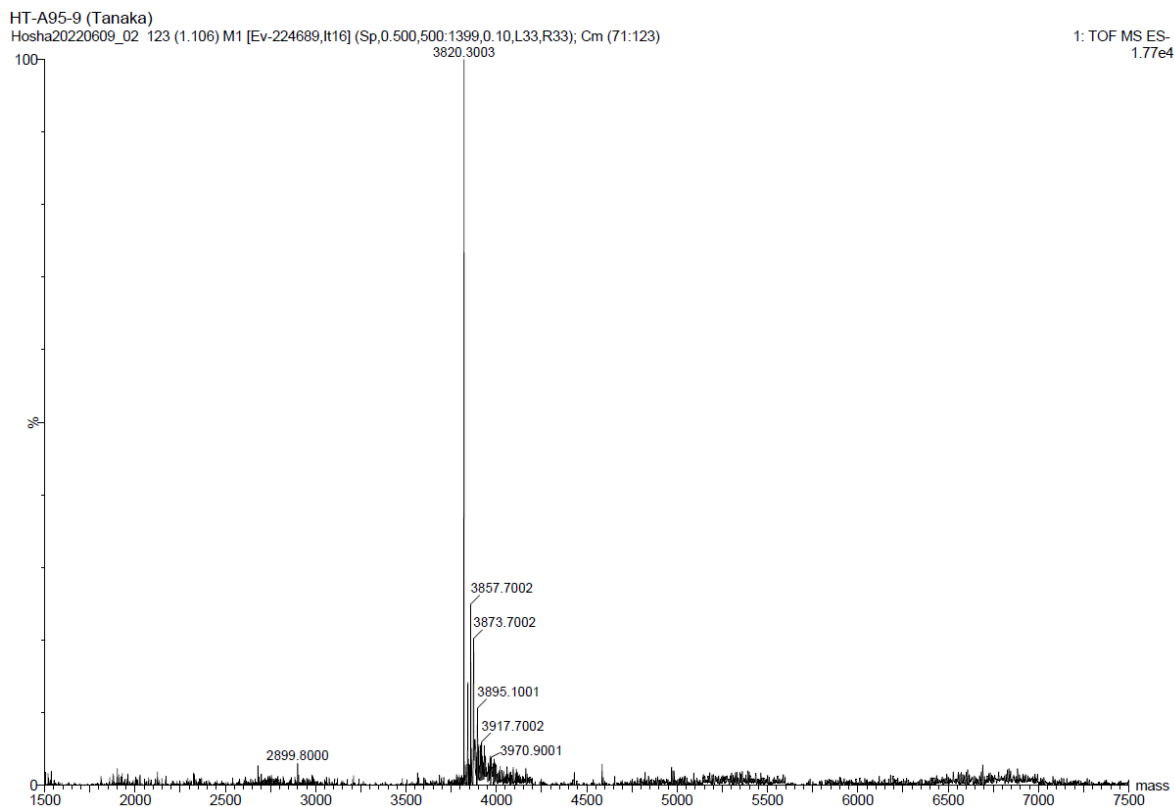
For **ON12c**

HPLC chromatogram of purified **ON12c**



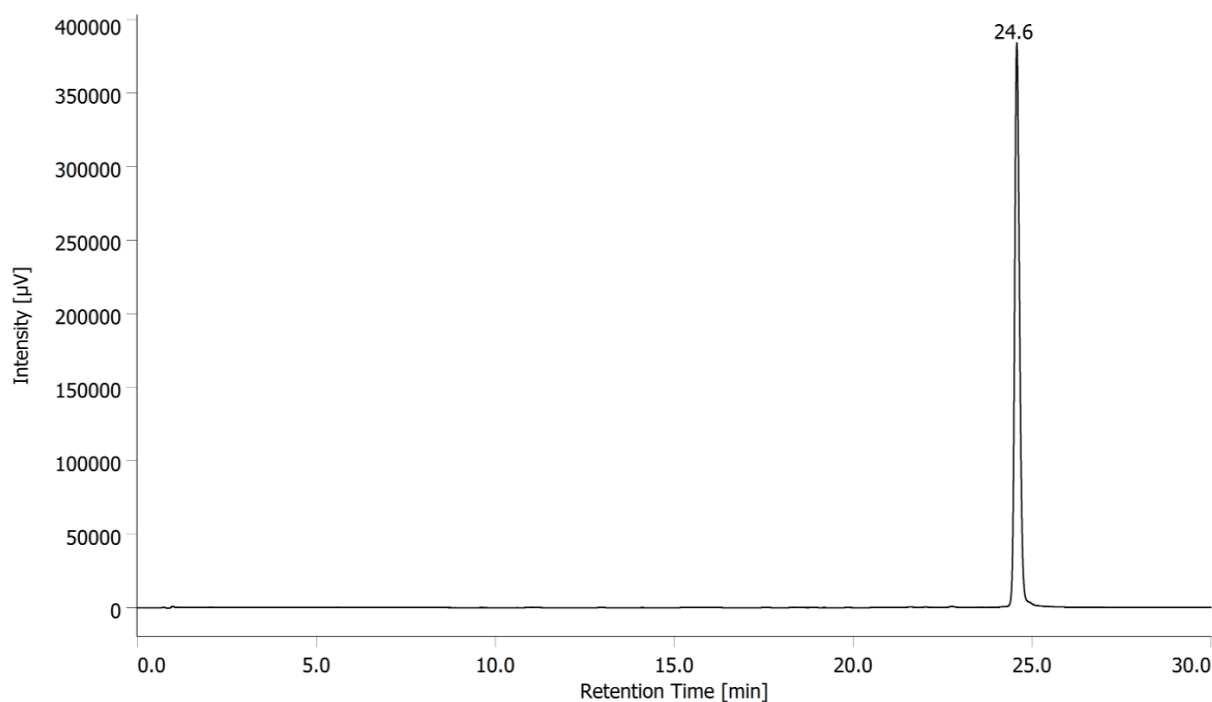
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON12c**



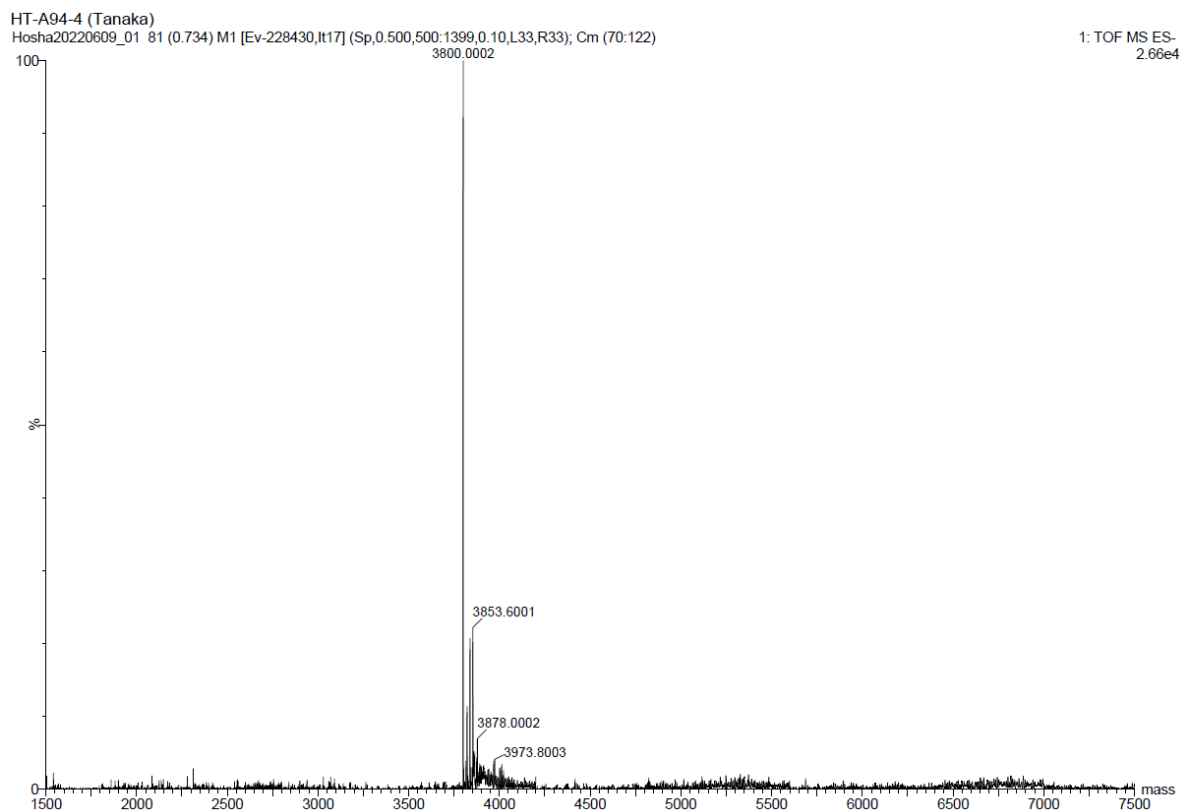
For **ON12d**

HPLC chromatogram of purified **ON12d**



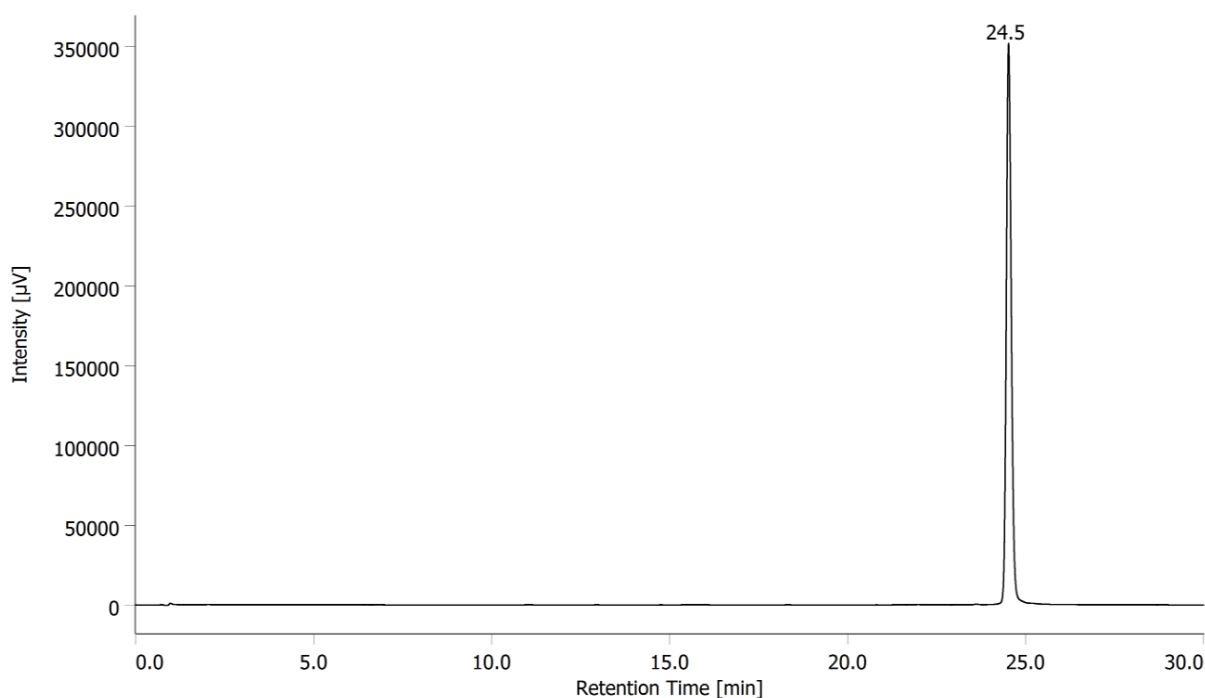
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON12d**



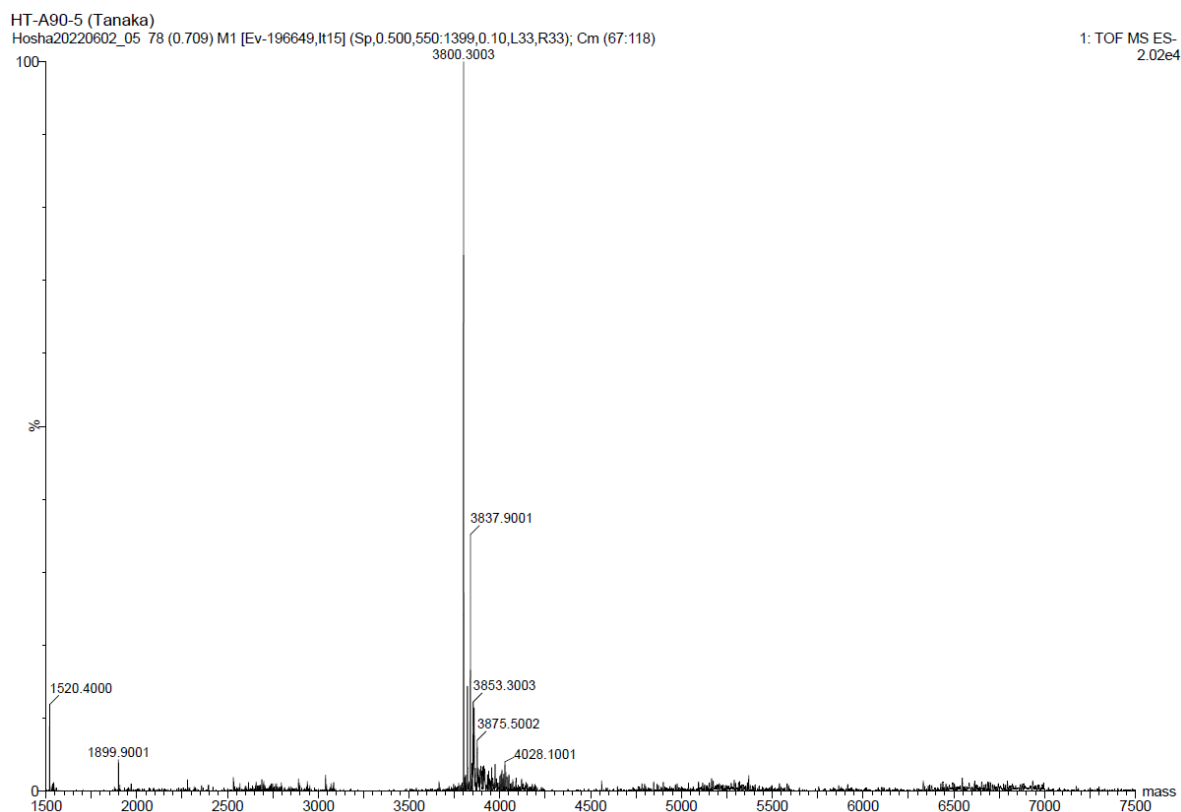
For **ON12e**

HPLC chromatogram of purified **ON12e**



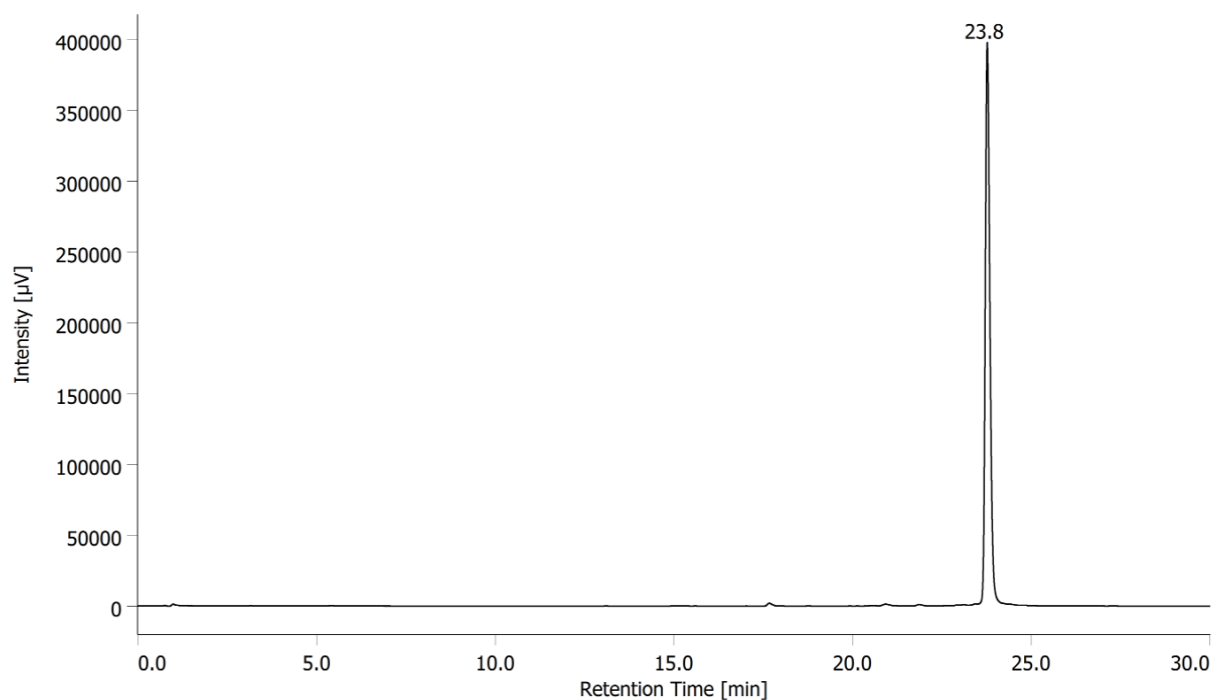
Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON12e**



For **ON12f**

HPLC chromatogram of purified **ON12f**



Column: Waters XBridge™ Shield RP18 2.5 μm , 4.6 $\times$ 50 mm.
Gradient: 5-15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.
Flow rate: 1.0 mL/min.
Column temp.: 40 °C.

ESI-MS spectrum of **ON12f**

