Supporting Information

Facile synthesis of a polyether-tethered dimeric berberine as a highly effective DNA-cleaving agent in the presence of Cu(II) ion

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Generals. ^{1}H NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 using a Varian Mercury 400 spectrometer and TMS as an internal reference. ESI-MS and HR-ESI-MS spectra were measured on Waters UPLC/Quattro Premier XE and Agilent 6460 Triple Quadrupole mass spectrometers, respectively. Agarose GE was conducted on DYY-8C electrophoresis apparatus and DYCP-31DN electrophoresis chamber, and detected on Alpha Hp 3400 fluorescence and visible light digital image analyzer. UV-Vis and fluorescence spectra were measured on a TU-1901 spectrophotometer and a HITACHI F-2500 spectrofluorimeter, respectively.

CT DNA and plasmid pBR322 DNA were obtained from Sigma-Aldrich and Takara Chemical Co., respectively. Their solutions were prepared in 5 mM Tris-HCl buffer (5 mM NaCl, pH 7.0).

The concentration of CT DNA was determined spectrophotometrically using the molar extinction coefficient of 13,200 $M^{-1}cm^{-1}$ per base pair (bp) at 260 nm. Compound **I** and berberrubine **3** were prepared according to reported protocols. [1, 2] All the other chemicals and reagents were obtained from commercial sources and used without further purification. Buffer solutions were prepared in triply distilled deionized water.

Synthesis of compounds 1 and 2

*1,11-Ditosyl-3,6,9-trioxaundecane **4***. The synthesis was conducted according to reported protocols. [2] Specifically, tetraethylene glycol (1.37 g, 7.0 mmol) was dissolved in dichloromethane (15 mL). *p*-Toluenesulfonyl chloride (2.70 g, 14 mmol) was added and the mixture was cooled to 0 °C with an ice bath. Powdered KOH (3.19 g, 56 mmol) was carefully added in small portions so that the mixture was kept below 5 °C. After stirring for 4.5 h at 0 °C, dichloromethane (15 mL) and ice-water (30 mL) were added. The organic layer was separated and the water layer was extracted with dichloromethane (15 mL×2). The combined organic layers were washed with saturated $NaHCO_3$ aqueous solution (30 mL×2), dried over anhydrous $MgSO_4$. After removal of solvent under reduced pressure, the obtained residue was purified by chromatography on a silica gel column, eluting with petroleum ether (60~90 °C)/ethyl acetate (2/1, v/v), to give compound **4** (2.72 g, 77%) having ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.4 Hz, 4H), 7.36 (d, *J* = 8.0 Hz, 4H), 4.17 (t, *J* = 4.8 Hz, 4H), 3.70 (t, *J* = 4.8 Hz, 4H), 3.58 (br, 8H), 2.46 (s, 6H). The data of ^{1}H NMR were in accordance with those reported by Bazan *et al*. [3]

*Compound **1***. To a solution of berberrubine **3** (320 mg, 1.0 mmol) in MeCN (20 mL) was added

1,11-ditosyl-3,6,9-trioxaundecane **4** (243 mg, 0.48 mmol). The resulting mixture was refluxed for 6 days, and then concentrated under reduced pressure. The obtained residue was subject to anion exchange into chloride form, and subsequently purified by chromatography on a reverse-phase column, eluting with a gradient of methanol in water (0-10%), to give compound **1** (360 mg, 86%) as a yellow powder having ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.66 (s, 2H), 8.85 (s, 2H), 8.05 (d, J = 9.6 Hz, 2H), 7.88 (d, J = 9.2 Hz, 2H), 7.69 (s, 2H), 7.05 (s, 2H), 6.15 (s, 4H), 4.95-4.92 (m, 4H), 4.28-4.26 (m, 4H), 3.89 (s, 6H), 3.78-3.76 (m, 4H), 3.65 (s, 8H), 3.20 (m, 4H); ESI MS *m/z*: 401.9 ($[M-2Cl^-]^{2+}$) and HRMS for $C_{46}H_{46}N_2O_{11}$ ($[M-2Cl^-]^{2+}$) Calc: 401.1545, Found: 401.1538.

*Tetraethylene glycol monomethyl ether tosylate **5**.* The synthesis was conducted according to reported protocols. [3] Specifically, a solution of tetraethylene glycol monomethyl ether (1.54 g, 7.42 mmol) and *p*-toluenesulfonyl chloride (1.49 g, 7.83 mmol) in anhydrous dichloromethane (15 mL) was cooled to 0 °C with an ice bath. Powdered KOH (1.37 g, 30 mmol) was carefully added in small portions. The resulting mixture was stirred at 0 °C for 4 h, and dichloromethane (15 mL) and ice-water (30 mL) were added. The organic layer was separated and the water layer was extracted with dichloromethane (15 mL×2). The combined organic layers were washed with water (10 mL), dried over anhydrous $MgSO_4$. After removal of solvent under reduced pressure, the obtained residue was purified by chromatography on a silica gel column, eluting with ligroin (60~90 °C)/ethyl acetate (2/1, v/v), to give compound **4** (2.08 g, 78%) having ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.16 (t, J = 4.8 Hz, 2H), 3.69 (t, J = 4.8 Hz, 2H), 3.65~3.62 (m, 6H), 3.58 (s, 4H), 3.55~3.53 (m, 2H), 3.37 (s, 3H), 2.45 (s, 3H).

The data of ^{1}H NMR were in accordance with those reported by Bazan *et al.* [3]

Compound ***2***. To a solution of berberrubine **3** (134 mg, 0.42 mmol) in MeCN (15 mL) was added compound **5** (148 mg, 0.41 mmol). The resulting mixture was refluxed for 7 days, and then concentrated under reduced pressure. The obtained residue was subject to anion exchange into chloride form, and subsequently purified by chromatography on a reverse-phase column, eluting with a gradient of methanol in water (0-15%), to give compound **2** (173 mg, 79%) as a yellow powder having ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.78 (s, 1H), 8.96 (s, 1H), 8.20 (d, J = 9.2 Hz, 1H), 8.02 (d, J = 9.2 Hz, 1H), 7.81 (s, 1H), 7.10 (s, 1H), 6.18 (s, 2H), 4.94 (t, J = 6.2 Hz, 2H), 4.43-4.41 (m, 2H), 4.07 (s, 3H), 3.82-3.80 (m, 2H), 3.61-3.58 (m, 2H), 3.54-3.51 (m, 2H), 3.47-3.42 (m, 6H), 3.36-3.35 (m, 2H), 3.22 (t, J = 6.2 Hz, 2H), 3.16 (s, 3H); ESI MS *m/z*: 512.9 ($[M\text{-}Cl^{-}]^{+}$) and HRMS for $C_{28}H_{34}NO_8$ ($[M\text{-}Cl^{-}]^{+}$) Calc: 512.2284, Found: 512.2279.

Experimental procedures for Agarose GE

The cleavage experiments were conducted by using the methods similar to those described previously. [4-6] Specifically, a mixture of pBR322 DNA (0.5 g/L, 0.7 μL) and a metal complex of each ligand was diluted with 5 mM Tris-HCl buffer (5 mM NaCl, 5% DMF, pH 7.0) to 16 μL and incubated at 37 °C for 5 h. The reaction was quenched by adding loading buffer containing 0.035% bromophenol blue, 36% glycerol, 30 mM EDTA and 0.05% xylene cyanol FF. The solution was then loaded on 1% agarose gel containing EB (1.0 mg/L), and analyzed with electrophoresis in Tris-acetate-EDTA (TAE) buffer (pH 8.0). Bands were visualized by UV light

and photographed.

The kinetics for the DNA cleavage was investigated at 37 °C for different intervals of time, by varying the concentrations of **1**@Cu(II) from 0 to 200 μM in 5 mM Tris-HCl buffer (5 mM NaCl, 5% DMF, pH 7.0). The percentage of the supercoiled DNA form was determined, and plotted against time for each concentration of **1**@Cu(II). The data were fitted with a single-exponential curve (*pseudo* first-order kinetics) to give the k_{obs} values. The k_{obs} values were then plotted versus the concentrations of **1**@Cu(II) (Eq. (1)), allowing the determination of the corresponding maximal first-order rate constant k_{max} and Michaelis constant K_M.

$$k_{obs} = k_{max}[\mathbf{1}@Cu(II)] / (K_M + [\mathbf{1}@Cu(II)]) \qquad (1)$$

For mechanistic investigations, inhibition reaction was carried out in the presence of NaN_3 (10 mM), TMP (0.2 mM), KI (10 mM), DMSO (1.0 mM) and *t*-BuOH (1.0 mM), followed by addition of **1**@Cu^{2+}.

X-ray crystallography

All the measurements were made on a Rigaku Mercury CCD X-ray diffractometer by using graphite monochromated Mo K (λ = 0.71070 Å). The crystal of compound **1** with $CuCl_2$ was mounted at the top of a glass fiber with grease. Cell parameters were refined by using the program CrystalClear (Rigaku and MSC, Ver. 1.3, 2001). The collected data were reduced by using the program CrystalStructure (Rigaku and MSC, Ver. 3.60, 2004) while an absorption correction (multiscan) was applied. A summary of the key crystallographic information was

tabulated in Table S1. Crystallographic data (without structure factors) for the structures reported in this article have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publication numbers CCDC-928678.

Table S1. Crystallographic data for the crystal of compound **1** with $CuCl_2$.

Compound	Compound **1** with $CuCl_2$
Molecular formula	$C_{46}H_{46}N_2O_{11}Cl_6Cu_2$
Formula weight	1142.65
Crystal system	monoclinic
Space group	*P21/c*
a (Å)	13.515(3)
b (Å)	13.241(3)
c (Å)	29.681(9)
α (°)	90.00
β (°)	109.70(3)
γ (°)	90.00
V (Å^3)	5001(2)
Z	42
T/K	293(2)
D_{calc} (g cm^{-3})	1.518
λ (Mo-Kα) (Å)	0.71073
μ (cm^{-1})	1.230
Total reflections	40398
Unique reflections	11397
No. observations	4298
No. parameters	606

R^a	0.0908
wR^b	0.1852
GOF[c]	1.024
$\Delta\rho_{max}$ (e Å^{-3})	1.286
$\Delta\rho_{min}$ (e Å^{-3})	-0.725

[a] $R = \Sigma||F_o|-|F_c||/\Sigma|F_o|$;

[b] $wR = \{\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$;

[c] GOF (Goodness-Of-Fit) = $\{\Sigma[w(F_o^2-F_c^2)^2]/(n-p)\}^{1/2}$, where n = number of reflections and p = total numbers of parameters refined.

References:

1. Y. Qin, J.-Y. Pang, W.-H. Chen, Z. Cai, Z.-H. Jiang, *Bioorg. Med. Chem.* **2006**, *14*, 25–32.
2. K. Iwasa, M. Kamigauchi, M. Ueki, T. Taniguchi, *Eur. J. Med. Chem.* **1996**, *31*, 469–478.
3. C. A. Traina, R. C. Bakus II, G. C. Bazan, *J. Am. Chem. Soc.* **2011**, *133*, 12600–12607.
4. C.-Q. Zhou, Y.-L. Lin, J.-X. Chen, L.-S. Wang, N.-N. Yang, W. Zeng, W.-H. Chen, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5853–5856.
5. C.-Q. Zhou, Y.-L. Lin, J.-X. Chen, W.-H. Chen, *Chem. Biodiv.* **2012**, *9*, 1125–1132.
6. J.-X. Chen, W.-E. Lin, C.-Q. Zhou, L. F. Yau, J.-R. Wang, B. Wang, W.-H. Chen, Z.-H. Jiang, *Inorg. Chim. Acta* **2011**, *376*, 389–395.

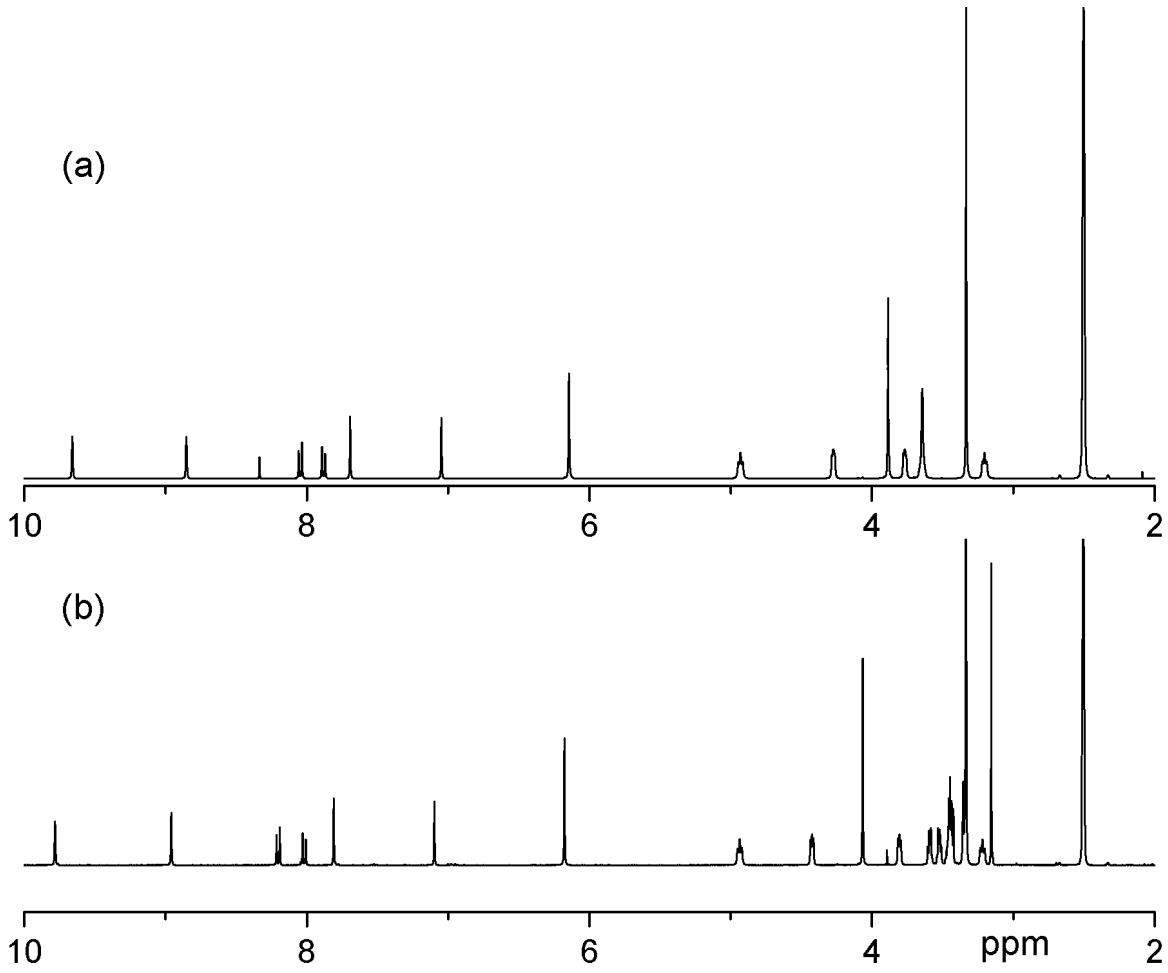

Figure S1. ^{1}H NMR (400 MHz, DMSO-d_6) spectra of compounds **1** (a) and **2** (b). Signal assignments are described in the synthetic procedures.

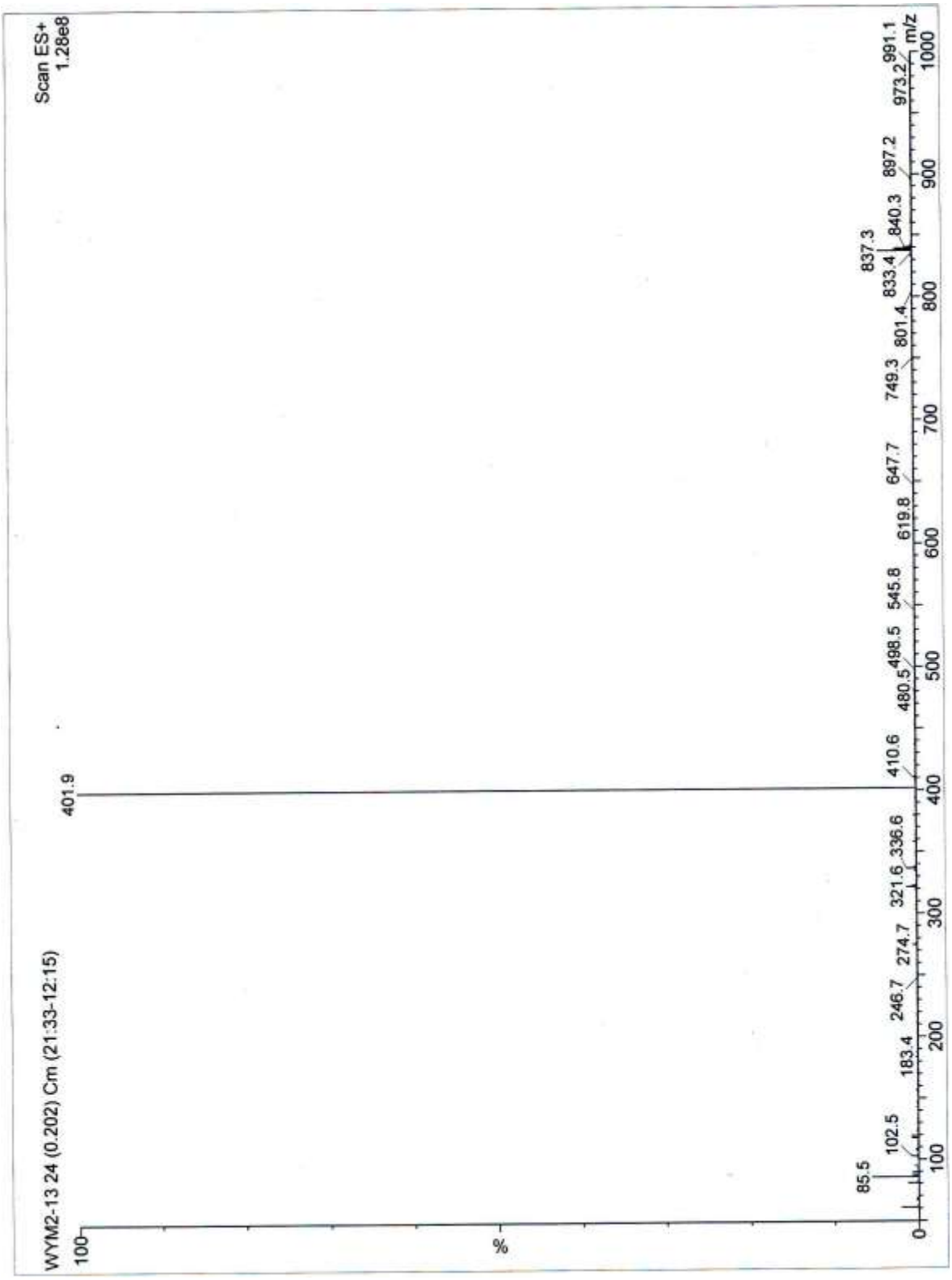

Figure S2. ESI-MS spectrum of compound **1**.

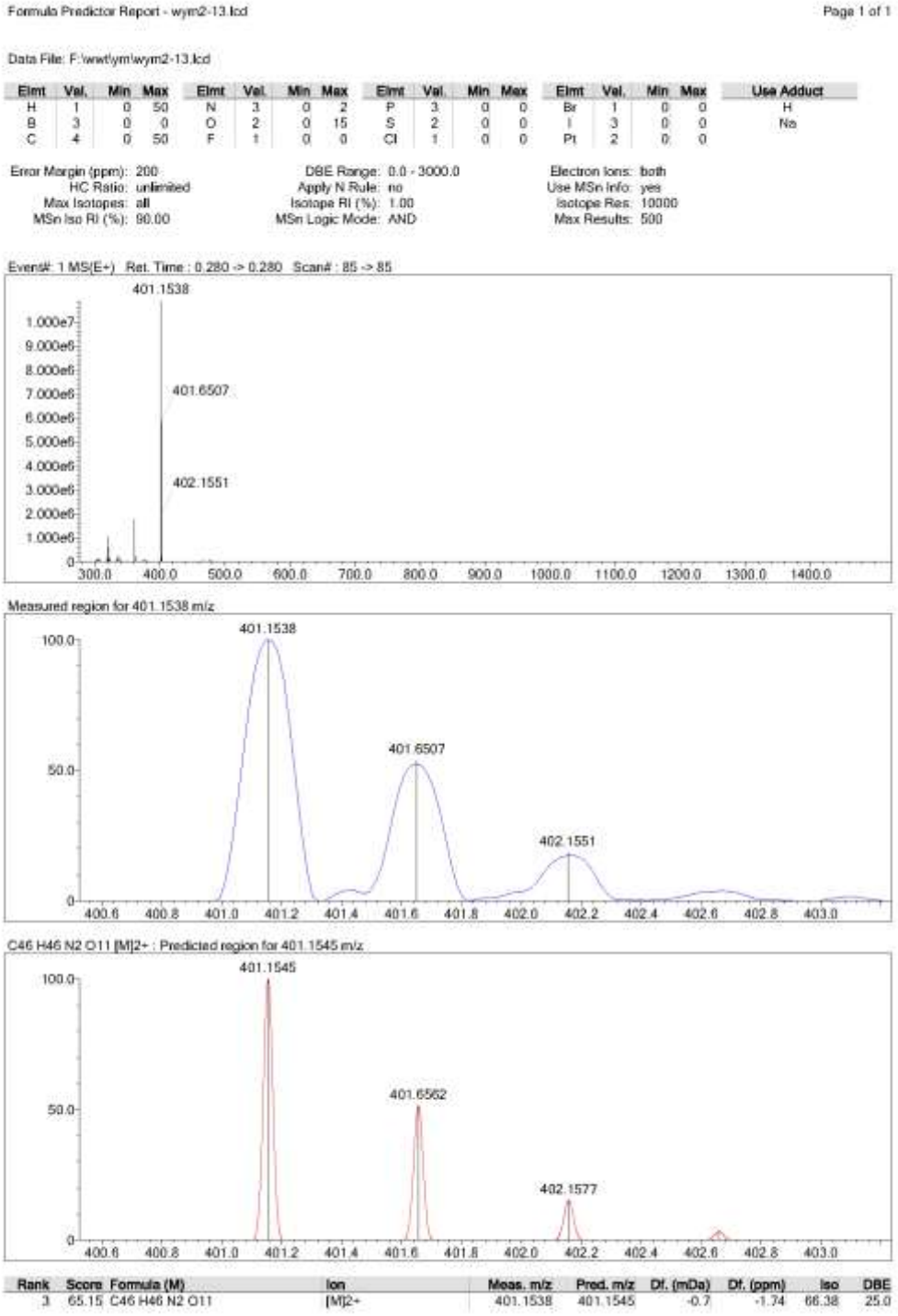

Figure S3. HR-ESI-MS spectrum of compound **1.**

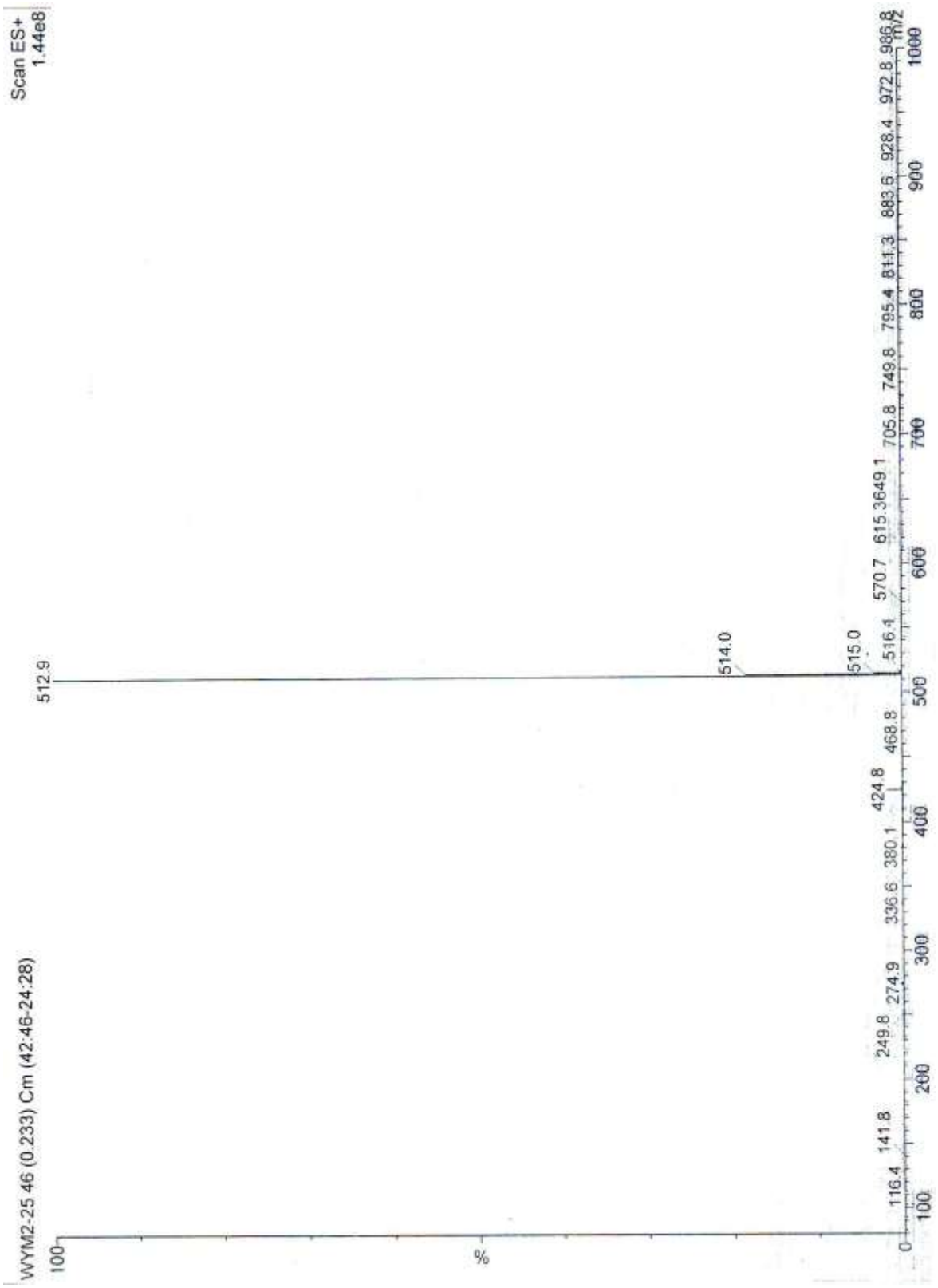

Figure S4. ESI-MS spectrum of compound **2**.

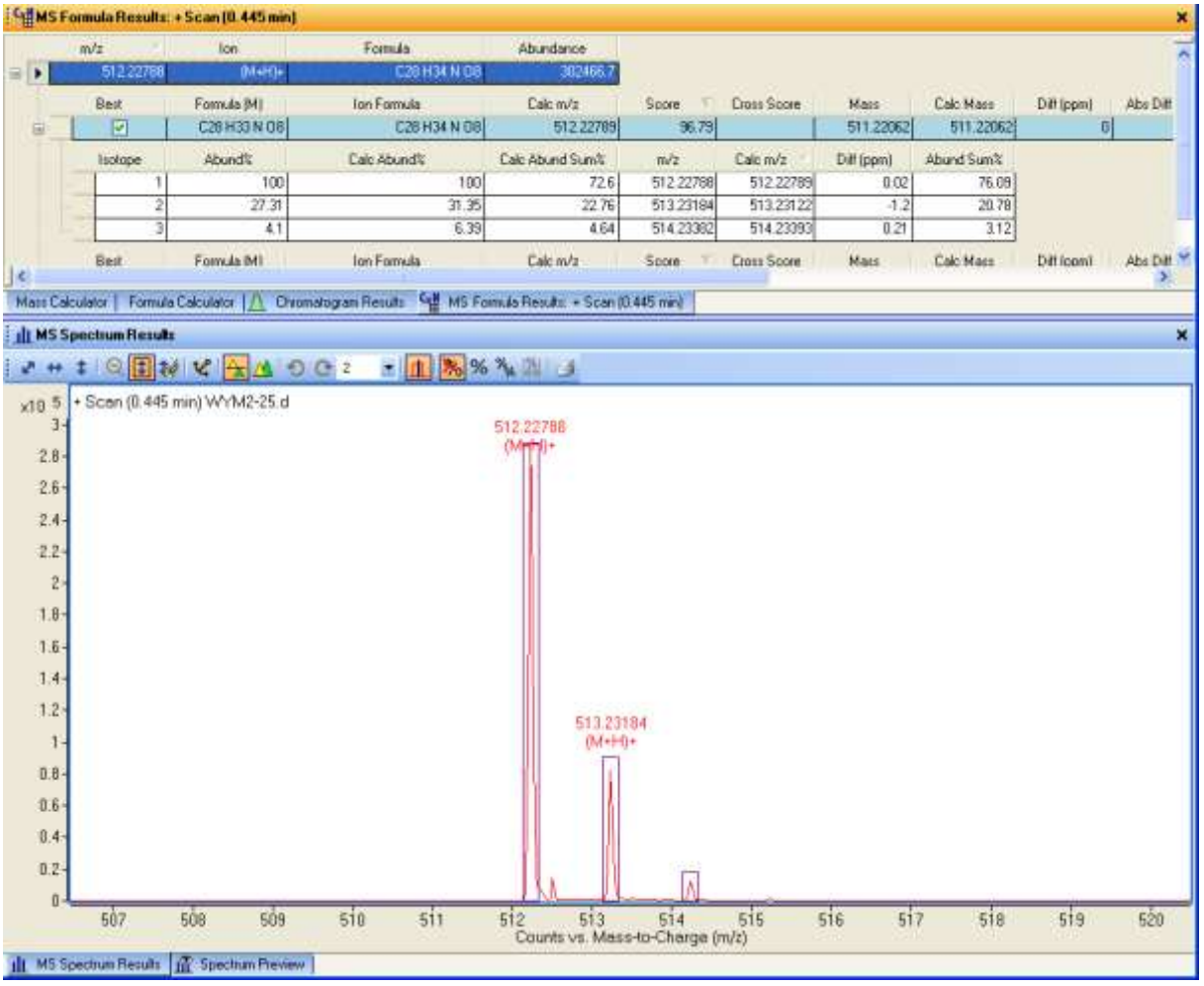

Figure S5. HR-ESI-MS spectrum of compound **2**.