SUPPLEMENTARY MATERIAL

A new sesquiterpenoid and further natural products from *Taraxacum portentosum* Kirschner & Štěpánek, an endangered species

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The chemical studies of roots and aerial parts of *Taraxacum portentosum* Kirschner & Štěpánek, a member of the section Palustria (H. Lindb.) Dahlst. (Asteraceae), led to the isolation of one new eudesmanolide and 13 known compounds, including five sesquiterpenoids: taraxinic acid, 11β,13-dihydrotaraxinic acid, taraxinic acid β-glucopyranosyl ester and its 11β,13-dihydroderivative, ixerin D, one apocarotenoid – loliolide and seven phenolics: scopoletin, 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone, methyl *p*-hydroxyphenylacetate, 5-methoxy-eugenyl-4-*O*-β-glucopyranoside, syringin, dihydroconiferin, and dihydrosyringin. Their structures were established by ^{1}H NMR. The new compound was characterized as 3-oxo-4β*H*-11,13-eudesmen-12,6-olide-8-*O*-β-glucopyranoside based on spectroscopic data (1D and 2D NMR) and HRESI mass spectrometry.

Keywords: Asteraceae; Palustria; Phenolic compounds; Sesquiterpenoids; *Taraxacum portentosum*

3. Experimental

3.1. General experimental procedures

HRESI mass spectra were obtained in the positive ion mode using Maldi-Synapt G2-S HDMS mass spectrometer equipped with an electrospray ion source and q-TOF type mass analyzer. All NMR spectra were recorded in $CDCl_3$ and in pyridine-d_5 on a Bruker AVANCE III 400 (400.17 MHz for ^1H and 100.63 MHz for ^{13}C) and a Bruker AVANCE III 600 (600.20 MHz for ^1H). The lock signal of solvent was used as an internal standard. Optical rotation was determined in MeOH on a PolAAr31 polarimeter with a sodium lamp operating at 589.44 nm.

Column chromatography was carried out using Merck silica gel 60 (0.063 - 0.2 mm). TLC was performed on Merck silica gel 60 (0.25 mm) plates and compounds were detected by viewing under UV light as well as by spraying with 20% sulfuric acid and heating. Analytical HPLC was carried out on a LiChroCART RP-18e column (particle size 5 μm, 3 x 125 mm) (Merck) eluted with H_2O-MeOH mixtures at a flow rate of 0.5 ml min^{-1}. Semipreparative HPLC was performed on a Waters instrument coupled to a dual wavelength UV/VIS detector operating at 210 and 260 nm, using Delta-Pak C-18 column (particle size 15 μm, 25 x 100 mm) (Waters Corp.) eluted with H_2O-MeOH mixtures at a flow rate of 3.0 ml min^{-1}. All solvents of analytical grade ($CHCl_3$, EtOAc, MeOH) were supplied by POCh S.A, and methanol for liquid chromatography and n-hexane were obtained from Merck. Water was purified by Milli-Q Compact Range system (Millipore Corp.).

3.2. Plant material

The roots and aerial parts of *T. portentosum* were collected in their natural habitat: Krześlin (E Polska, geographical coordinates: 52˚13′19″N, 22˚21′28″E) – wet meadows and lowmoors in the Liwiec River valley on 10 May 2014, and identified by Jolanta Marciniuk. A voucher specimen (no 47531) was deposited at the herbarium of the Department of Botany, University of Podlasie in Siedlce (WSRP).

Morphologically, the species is marked with quite big (7,5-10,0 mm long and 2,0-3,5 mm wide) as well as wide bordered outer bracts. The leaf shape is also very distinctive with long and narrow interlobes and side lobes turned up on one side and turned down on the other side. *T. portentosum* is an apomictic tetraploid 2n=4x=32 (Marciniuk et al. 2010).

The phytochemical analysis was performed in the year of collection of the plant material.

3.3. Extraction and isolation

The dried roots (92 g) and aerial parts (122 g) of the plant were separately powdered and extracted with EtOH at room temp. and the solvent was evaporated under reduced

pressure to give 13.0 g and 16.0 g of crude extracts, respectively. The crude extracts were chromatographed on a silica gel (Merck, Art. 7754) columns eluted with hexane-EtOAc (up to 100% EtOAc), followed by EtOAc-MeOH (up to 10% MeOH) gradient solvent systems. Further separation was conducted by prep. TLC and semiprep. HPLC.

Less polar fractions from the roots eluted with hexane-EtOAc (1:1, v/v) afforded **9** (1.2 mg), **2** and **3** in a mixture (*ca*. 1:1.5, 10.6 mg), after purification by semiprep. HPLC (H_2O-MeOH, 1:1, v/v). Fractions from the EtOAc elution were separated by semiprep. HPLC (H_2O-MeOH 3:2, v/v) to give **1** (1.5 mg), **10** (2.9 mg), **4** (13.0 mg), **5** (2.8 mg), and **11** (1.0 mg). More polar fractions eluted with EtOAc-MeOH (49:1, v/v) were processed by prep. TLC ($CHCl_3$-MeOH, 17:3, v/v) and semiprep. HPLC (H_2O-MeOH, 7:3, v/v) to yield **12** and **13** in a mixture (*ca*. 1:1.5, 2.5 mg), **12** (12.1 mg), **12** and **14** in a mixture (*ca*. 1:2, 1.9 mg) and **6** (3.4 mg).

The crude extract from the aerial parts was chromatographed, as described above, to give **1** (7.6 mg), **4** (12.1 mg), **5** (3.2 mg), **10** (11.5 mg), and **12** (11.8 mg). The elution of the silica gel column with hexane-EtOAc (3:2, v/v), followed by separation by semiprep. HPLC (H_2O-MeOH, 3:2, v/v) afforded **7** (4.4 mg) and **8** (1.2 mg).

All compounds were characterized by direct comparison of their spectroscopic data (^{1}H NMR) with those of the reference compounds previously isolated in our laboratory from the different *Taraxacum* species (**2**–**6**, **8**–**14**) (Kisiel and Michalska 2005, 2006; Michalska and Kisiel 2004; Michalska et al. 2010, 2019) and from *Lactuca tenerrima* (**7**) (Michalska et al. 2012).

3.3.1. 3-Oxo-4βH-11,13-eudesmen-12,6-olide-8-O-β-glucopyranoside (1)

Colourless solid: $[\alpha]_D^{26}$ - 17.8^o (c = 0.7, MeOH); ^{1}H- and ^{13}C NMR, HMBC: Table S1; ESIMS (pos. mode) m/z: 449.17 $[M + Na]^+$; HRESIMS (pos. mode) *m/z*: 449.1761 $[C_{21}H_{30}O_9Na]^+$; calc. 449.1782.

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List of supporting information

Table S1. ^{1}H NMR (400.17 MHz) and ^{13}C NMR (100.63 MHz) data of compound **1** in pyridine-d_5.

Position	δ_H (ppm), *J* (Hz)	δ_C (ppm)	HMBC (H→C)
Aglycone moiety			
1α	1.64 br dd (12.4, 2.8)	20.97	-
1β	1.32 ddd (12.4, 10.0, 3.2)		C-10
2α	1.44[a] m	36.55	C-10, C-14, C-15
2β	2.33[b] m		-
3	-	207.71	-
4	2.52 dq (12.0, 6.4)	44.27	C-3, C-5, C-6, C-15
5	1.46[a] dd (11.6, 11.2)	50.21	C-4, C-6, C-7, C-8, C-10, C-14, C-15
6	3.87 dd (10.8, 10.8)	82.79	C-4, C-11
7	2.34[b] br d (13.6)	50.37	C-5
8	3.96 m	81.66	C-2, C-10, C-14, C-1’
9α	3.19 dd (15.2, 5.6)	43.85	C-3, C-4, C-8, C-10
9β	2.80 dd (14.8, 11.2)		C-3, C-8
10	-	41.49	-
11	-	139.45	-
12	-	170.04	-
13a	5.27 d (2.8)	116.51	C-7, C-12
13b	6.09 d (3.2)		C-7, C-11, C-12
14	1.09 s	12.51	C-2, C-5, C-8, C-10
15	1.38 d (6.4)	13.84	C-3, C-4, C-5
Glucosyl moiety			
1’	4.80 d (7.6)	102.20	C-8, C-3’
2’	3.99 dd (8.4, 7.6)	74.83	C-1’, C-3’
3’	4.23[c] m	78.36	C-2’, C-4’
4’	4.20[c] m	71.60	C-3’, C-5’, C-6’a
5’	3.95 m	78.39	-
6’a	4.39 dd (11.6, 6.0)	62.86	C-4’, C-5’
6’b	4.61 dd (11.6, 2.4)		C-4’

[a,b,c] Signals overlapped.

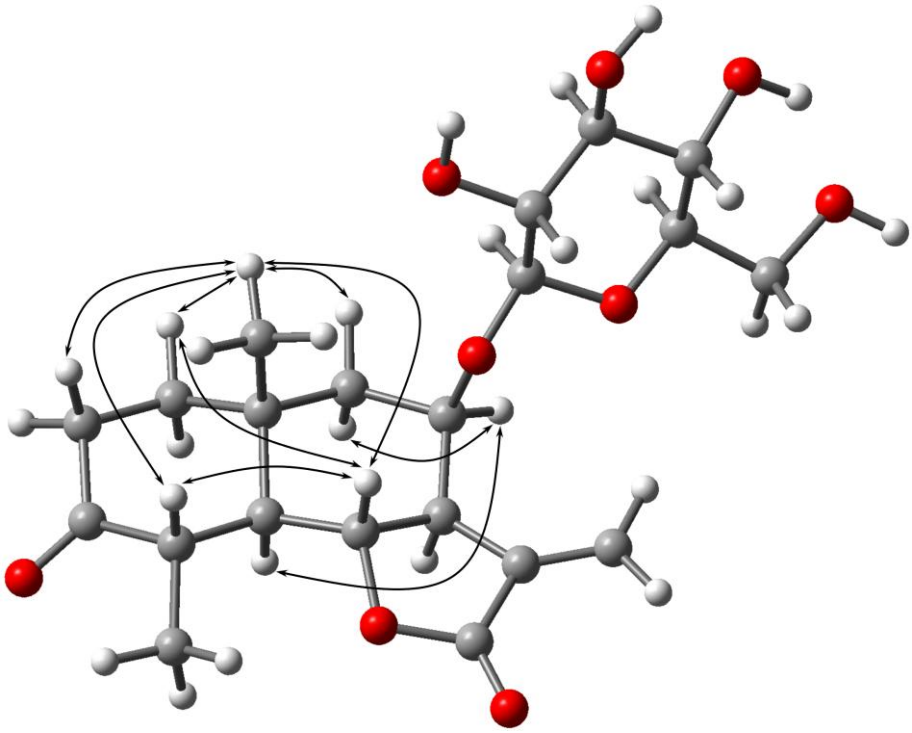

Figure S2. Key NOESY correlations for **1**.

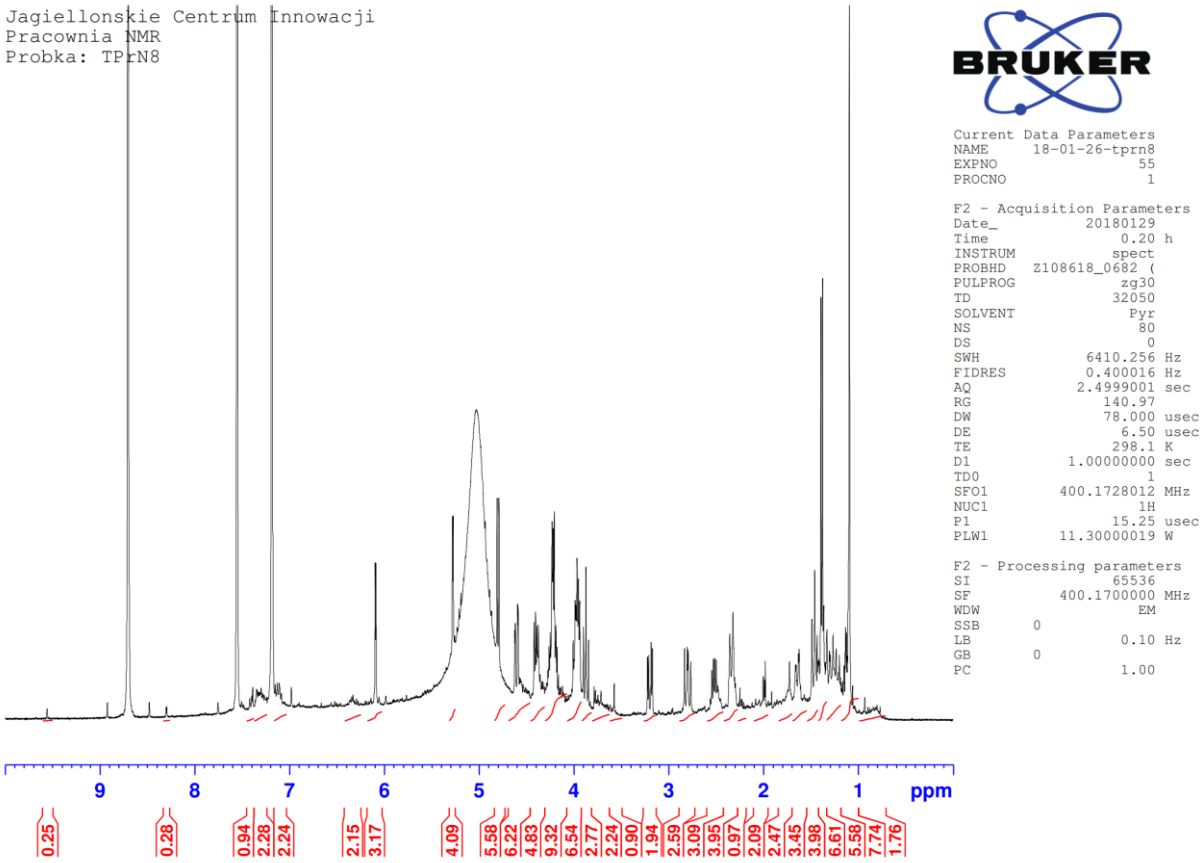

Figure S3. ^{1}H NMR spectrum of compound **1**.

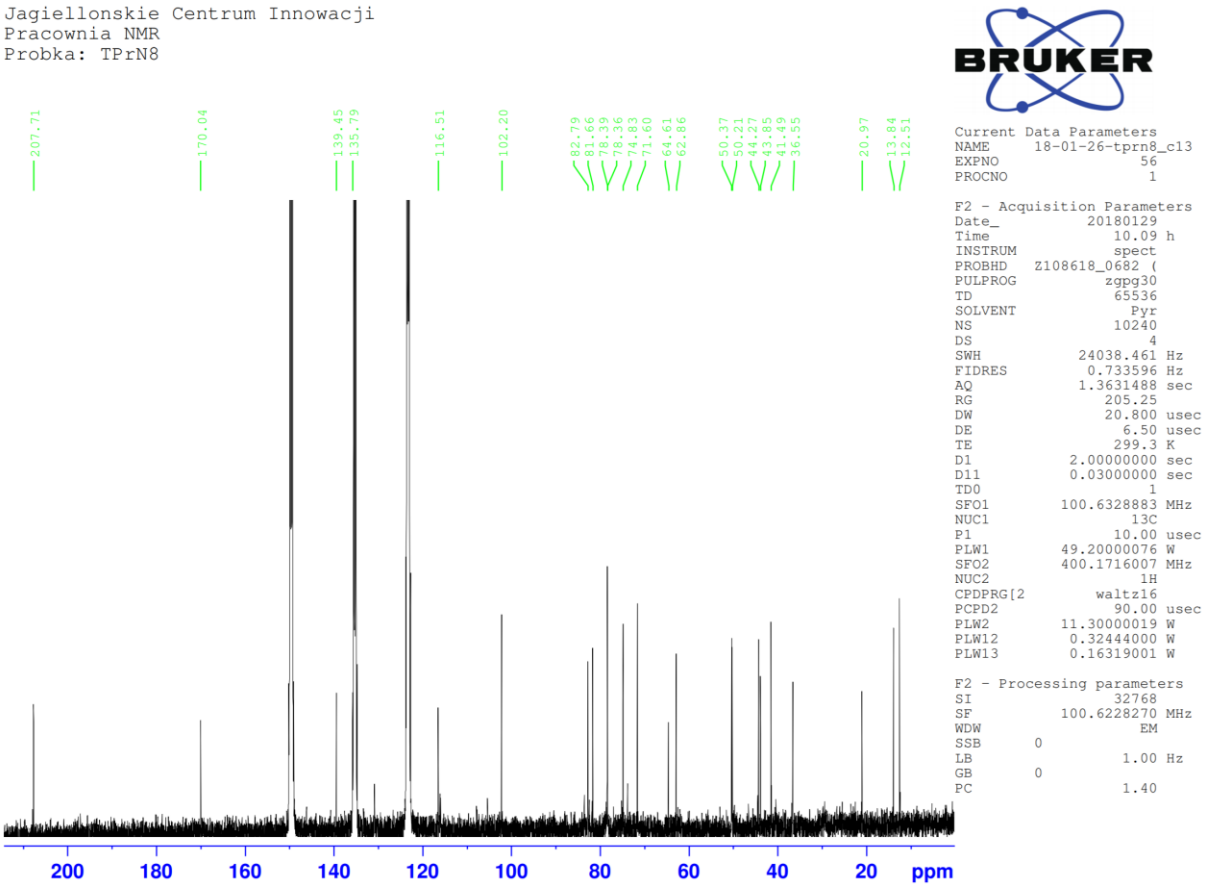

Figure S4. ^{13}C NMR spectrum of compound **1**.

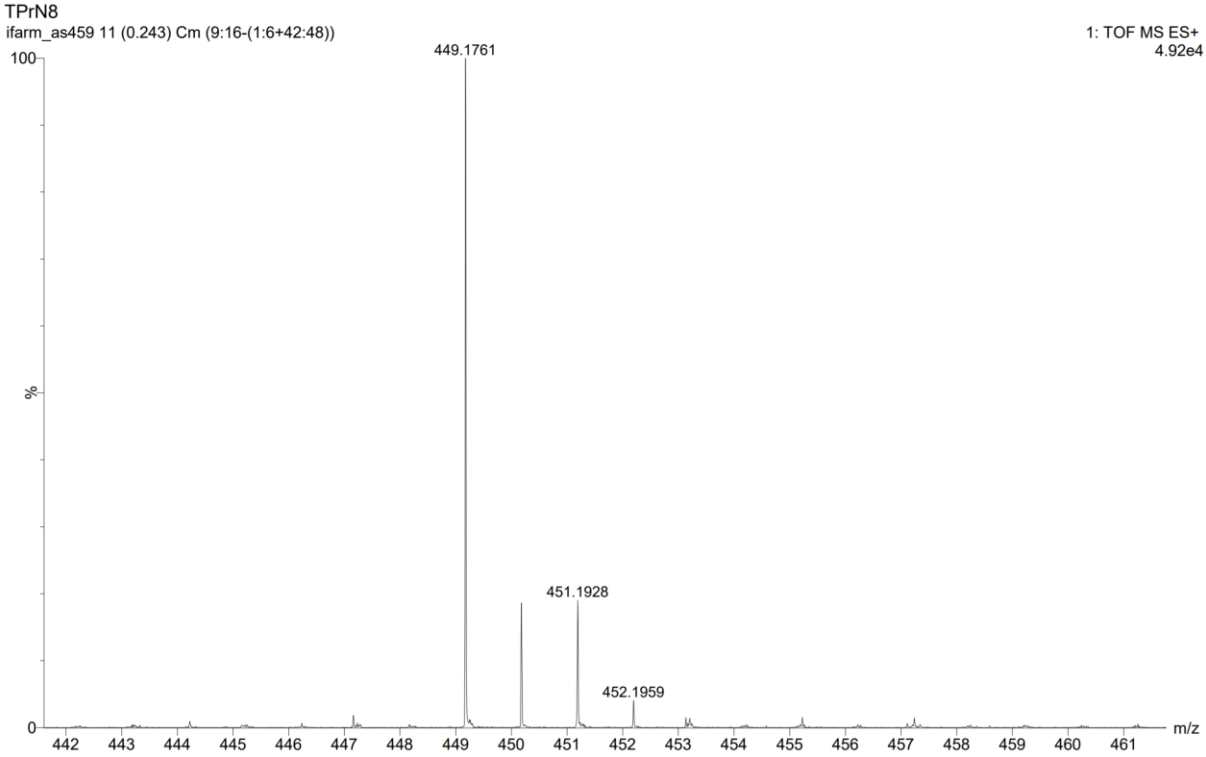

Figure S5. HR ESIMS spectrum of compound **1**.

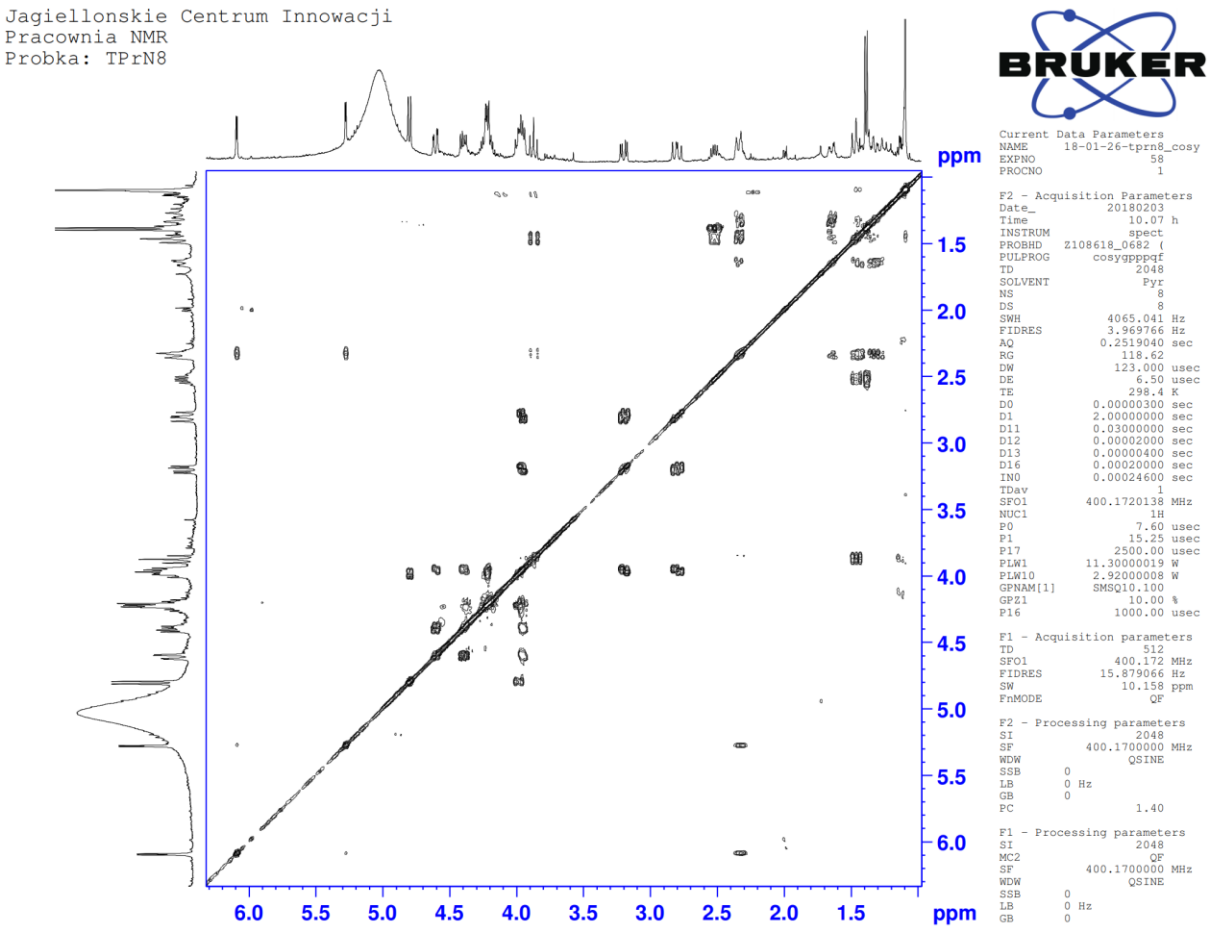

Figure S6. ^{1}H-^{1}H COSY spectrum of compound **1**.

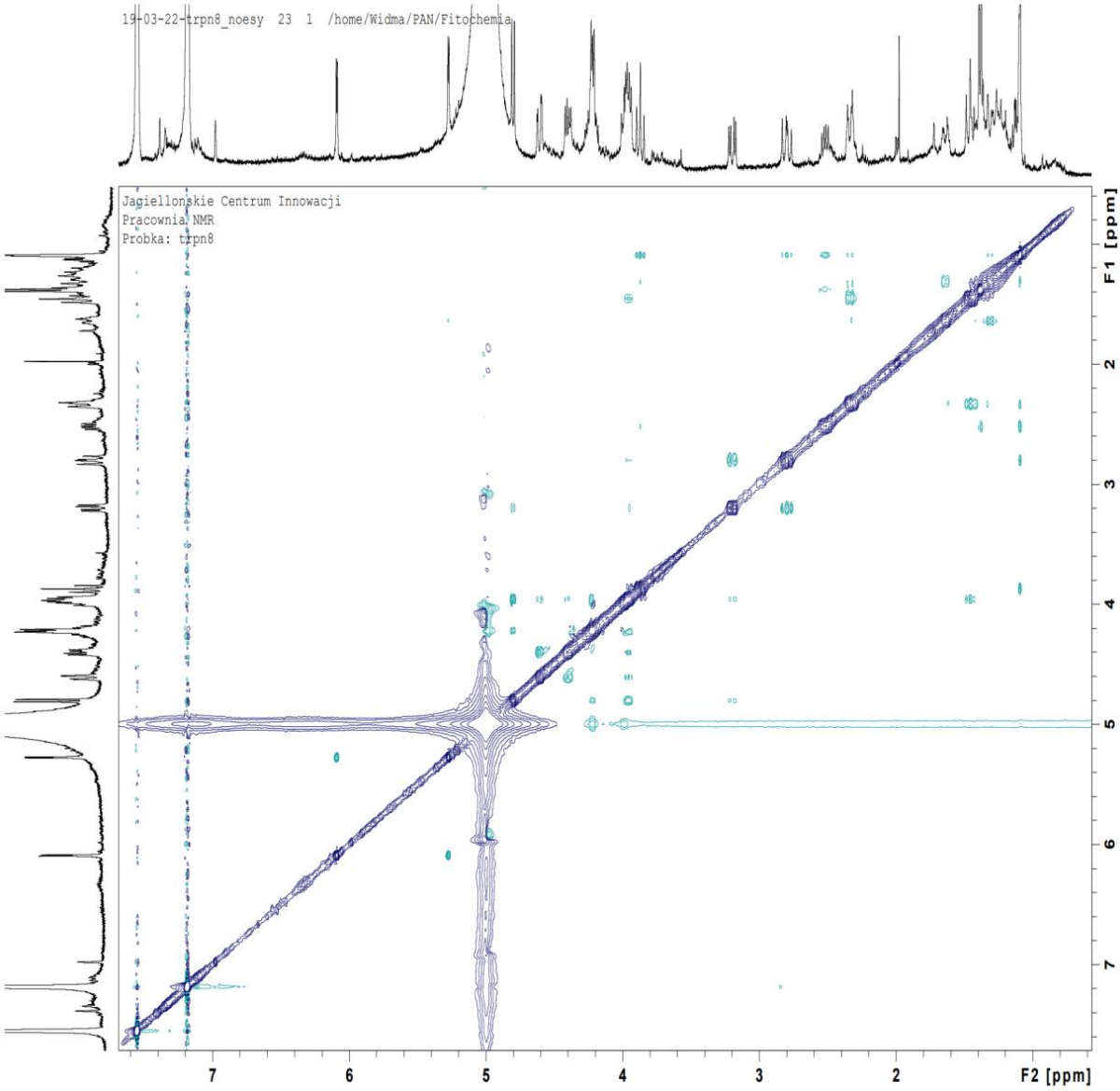

Figure S7. NOESY spectrum of compound **1**.

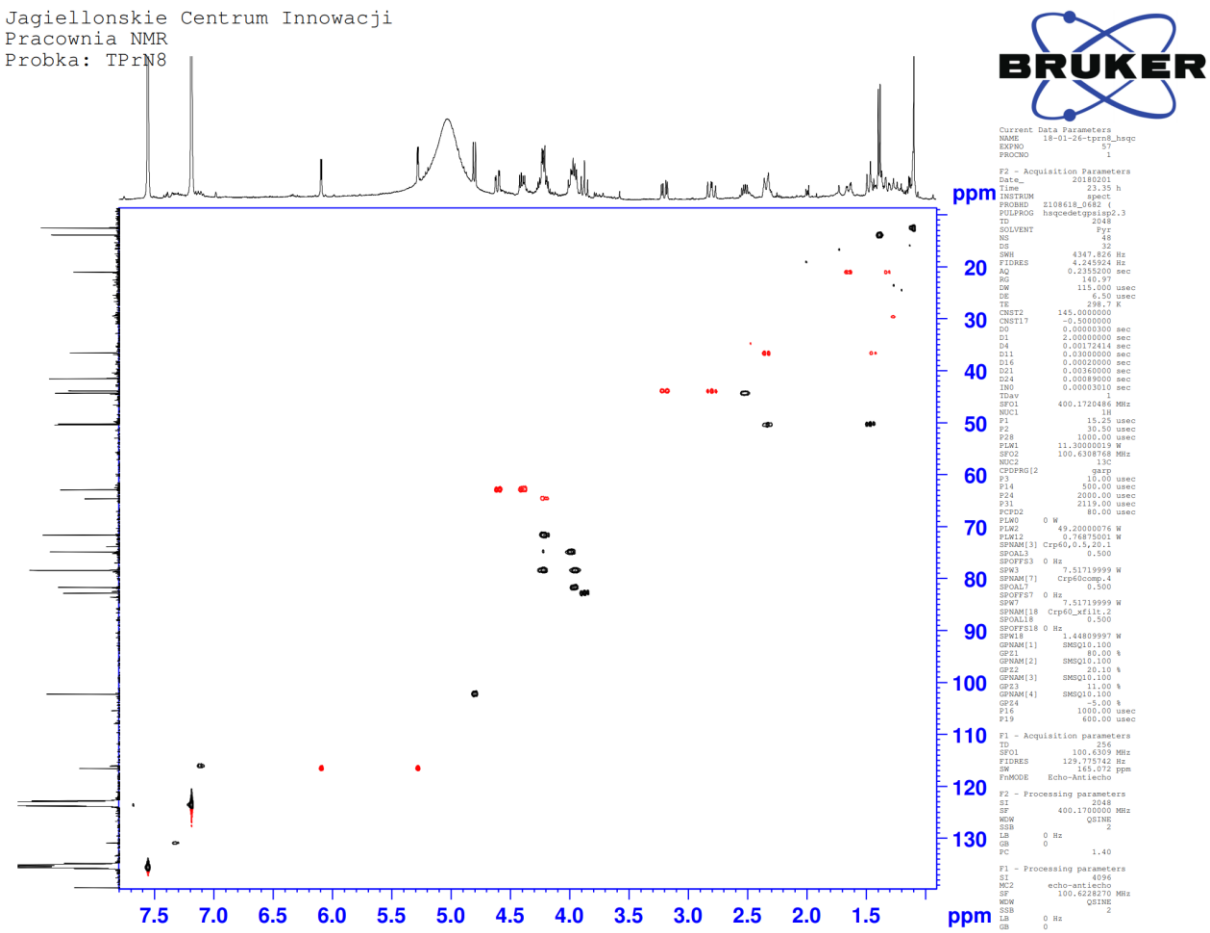

Figure S8. HSQC spectrum of compound **1**.

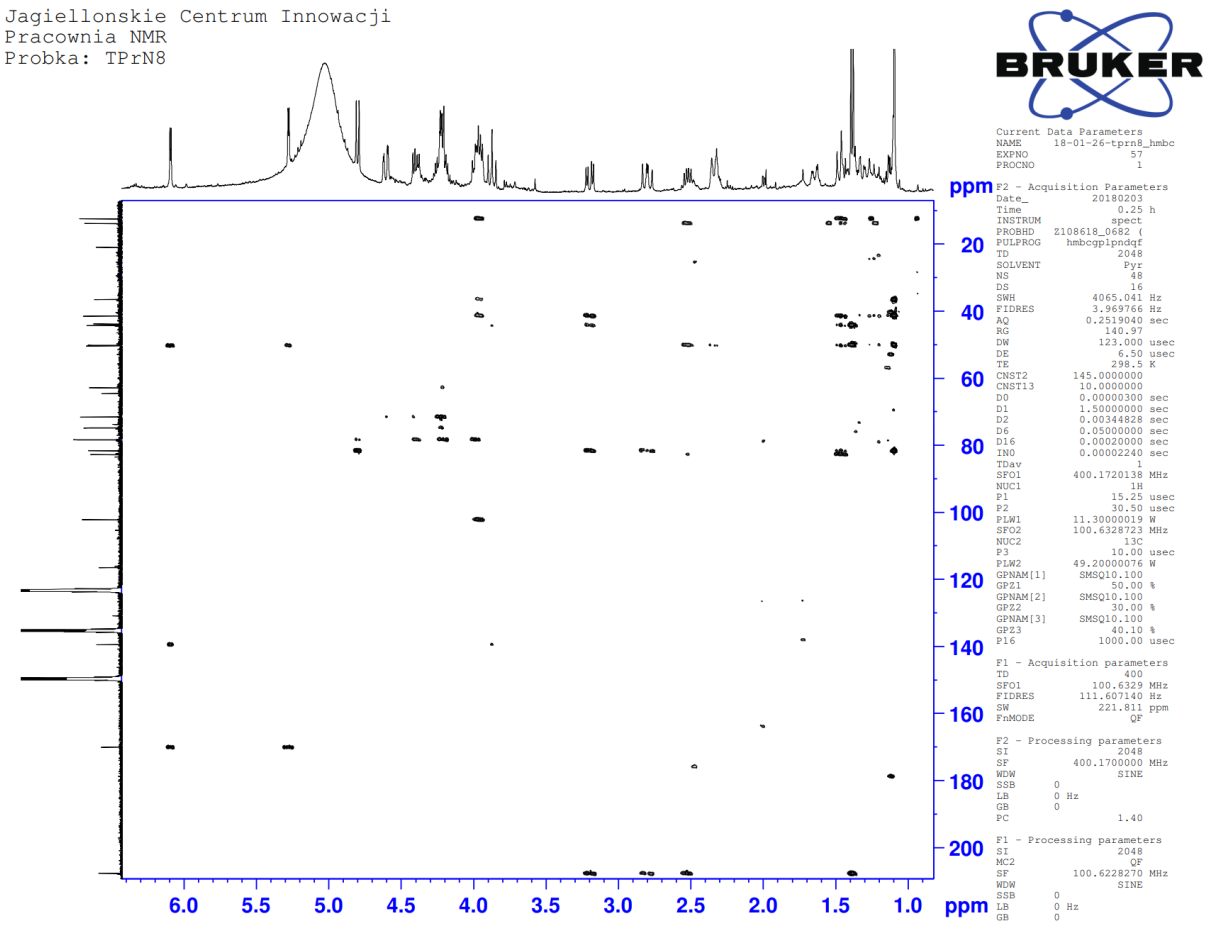

Figure S9. HMBC spectrum of compound **1**.