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# Discovery of New Stilbene Antioxidants of the Bio-Elicited Peanut Sprout Powder (BPSP) and Longevity Extension of Mice Fed with BPSP-Supplemented Diets

**Robin Y.-Y. Chiou[1*], Po-Chang Chiu[2], Ju-Chun Chang[1], Yu-Jang Li[2], Chia-Wen Hsieh[3], Jin-Yi Wu[3], Shu-Mei Lin[1], Yun-Lian Lin[4], Brian B.-C. Weng[3]**

[1]Department of Food Science, National Chiayi University, Taiwan
[2]Department of Applied Chemistry, National Chiayi University, Taiwan
[3]Department of Microbiology, Immunology and Pharmaceutical Sciences, National Chiayi University, Taiwan
[4]Department of Chinese Pharmaceutical Science and Chinese Medicine Resources, China Medical University, Taiwan
Email: *rychiou@mail.ncyu.edu.tw

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## Abstract

Biosynthesis of peanut stilbenes, including resveratrol as the secondary metabolites, could be enhanced by subjecting the kernels to germination and wound-stress. Investigations of the bio-elicited peanut sprout powder (BPSP) addressed on characterization of the comprising stilbenes and effectiveness in longevity extension deserves intensive research. In this study, peanut kernels were subjected to germination and wound-stress in preparation of BPSP. The methanol extracts of BPSP were medium pressure liquid chromatographic (MPLC) fractionated and semi-preparative HPLC recovered and followed by instrumental identification and biological activity determinations of the isolated stilbenes. In longevity experiments, 16 female 11-mon-old BALB/c mice and both genders of 12-mon-old ICR mice were daily fed with BPSP-supplemented diets at doses of 0, 0.1 and 0.5 g BPSP/kg bw for 750 and 762 days, respectively. Based on chemical characterization, enriched quantity of stilbenes in the BPSP up to ca. 1% (w/w) was detected. Two new stilbene compounds, namely, 4, 5'-dihydroxy-6"-hydroxymethyl, 6"-methylpyrano [2", 3": 3', 4'] stilbene and 3, 4, 5'-trihydroxy-6", 6"-dimethylpyrano [2", 3": 3', 4']stilbene along with 5 known stilbenes were isolated. The 7 stilbenes exhibited potent antioxidative and antiglycative activities and varied with structure-activity nature. Based on the resultant survival curves and average lifespans of both mouse models, basal diets supplemented with BPSP are effective to extend mouse longevity by a dose dependent manner. It is of merit to demonstrate that peanut kernels as a potent producer could be bio-elicited to biosynthesize a broad spectrum of bioactive stilbenes to prepare BPSP which is effective to extend mouse longevity as science-evidenced by the two long-term animal experiments.

**Keywords**

Bio-Elicited Peanut Sprout Powder (BPSP), Wound-Stress, MPLC, Peanut Stilbenes, Longevity Extension

## 1. Introduction

In addition to one of worldwide-favored nuts, peanut plant and seeds are capable of biosynthesizing bioactive stilbenes and phytochemicals [1]-[6]. Peanut stilbenes are small molecular secondary metabolites and induced by biotic and/or abiotic elicitations to enhance biosynthesis [6]-[13]. Among those discovered stilbenes, resveratrol is one of the most noticed, due to its virtual biological activities and emerging high values in development of the health-enhancing products. Since the first discovery of peanut resveratrol in 1976 [14], many other stilbenes originated from peanut have been isolated and identified [8] [10] [15]-[22]. Accordingly, a broad spectrum addressed on enhancement of biosynthesis and organic synthesis along with biological and/or pharmaceutical activities characterization of the stilbenes has been extensively and intensively investigated [4] [8] [12] [13] [17] [23]-[28].

As conducted in our laboratory, subjection of peanut kernels to germination in preparation of peanut sprouts as a functional vegetable has been reported [29] [30]. Extracts of peanut sprouts resulting in anti-obesity effects have also been demonstrated [31]. In comparison, peanut seeds after germination bear higher resveratrol content and antioxidant activities than that of un-germinated seeds [29] [32] [33] [34]. Biosynthesis of peanut stilbenes could be remarkably enhanced by subjecting kernels to germination and wound-stress elicitation [8]. The isolated peanut stilbenes, such as arachidin-1, have exhibited potent anti-inflammatory and anti-cancer along with anti-oxidant activities [8] [23] [24] [26]. In a recent report, arachidin-1 and resveratrol exhibit phytoestrogenic activities and modulate regulatory T cell functions responsible for successful aging of the aged ICR mice [28]. These findings point out a perspective of potency to use the bio-elicited peanut sprout powder (BPSP) in development of value-added and health-beneficial products. In this study, in order to discover the unknown comprising stilbenes and isolate sufficient quantities of pure compounds for chemical and biological activities characterization, BPSP was subjected to medium pressure liquid chromatographic (MPLC) fractionation and followed by semi-preparative HPLC purification. Two new compounds along with 5 known stilbenes have been isolated, identified and followed by determination of antioxidative and antiglycative activities. Importantly, based on the findings that arachidin-1 and resveratrol exhibit phytoestrogenic activities and modulate regulatory T cell functions responsible for successful aging of the aged ICR mice [28] and arachidin-1 is one of the major bioactive stilbenes of BPSP [8], two long-term longevity experiments have been conducted, respectively. Both BALB/c mice as an in-bred mouse model and ICR mice as an out-bred mouse model were fed with basal diets supplemented with BPSP at various doses for 750 and 762 days.

## 2. Materials & Methods

### 2.1. BPSP Preparation and Nutrition Fact Analysis

Basically, the previously reported procedure [8] was followed with modification. In brief, sound kernels (*Arachis hypogaea* L., a Spanish cultivar) were soaked with water at ambient temperature (25˚C - 28˚C) for 16 h, drained and incubated under dark and humidified condition 3 days for germination. Cotyledons removed from each of the germinated kernels were sliced to create a wound stress and spread onto perforated plates and heated in a forced-air-oven at 120˚C for 45 min to dehydrate and inactivate enzymes. The dried peanut slices were ground into paste with a peanut butter grinder and defatted with *n*-hexane. BPSP was prepared after solid-solvent separation through filtration and vacuum evaporation in a closed chamber for complete solvent removal. Then, the obtained BPSP was packed in heavy-duty polyethylene bags and frozen-stored (−20˚C) for later use. BPSP was subjected to nutrition fact analysis by Food Inspection and Analysis Center, College of Life Sciences, National Chiayi University.

### 2.2. MPLC and HPLC Fractionation and Recovery of the BPSP Stilbenes

BPSP 500 g was refluxed with methanol (5 liters) for 2 h and filtered to obtain methanol extract and followed by rotary vacuum evaporation to get a final concentrated volume ca. 150 ml. The concentrate was partitioned twice with 300 ml ethyl acetate (EA). The pooled EA fractions were evaporated to dryness and dissolved in 20 ml of 80% methanol. The dissolved solution was membrane-filtered (0.45 μm) and loaded onto a MPLC column (ODS, 3.6 × 23 cm, Buchi Glass Column) for MPLC (Buchi Pump Manager C-615 with two Pump Module C-601, Buchi Labortechnik, Flawil, Switzerland). MPLC was run with dual solvents of methanol and water from initial 65% methanol and linearly increased to final 75% methanol in 60 min with 25 ml/min of flow rate. The eluted solution was collected every 3 min. Then, the column was eluted for clean-up with 90% methanol for 15 min. The MPLC loading solution and each of the eluted and collected fractions was subjected to analytical HPLC analysis (Hitachi L-7100 pump, L-7455 PDA detector) (Hitachi Co., Ltd., Tokyo, Japan) equipped with an ODS column (Hypersil ODS, 250 × 4.6 mm, 5 μm, Thermo Electron Corp. Hypersil Ltd., Cheshire, UK) run with a dual solvent system of methanol (A solvent) and water (B solvent) with 1 ml/min, 20 μl and 254 nm of flow rate, injection volume and monitoring wavelength, respectively. The mobile phase was programmed and run at 0 min with initial 50% A and linearly increased to 75% A at 5 min; increased to 100% A at 18 min; back to 50% A at 21 min and held for an additional 3 min prior to next sample injection.

Based on the obtained HPLC chromatograms of the loading solution for MPLC [**Figure 1(a)**] and MPLC separate fractions [**Figures 1(f1)-(f6)**], the fractions containing the selected peanut stilbenes were concentrated appropriately by vacuum rotary evaporation and membrane-filtered to prepare samples for semi-preparative HPLC fractionation (L-7100 pump, L-7420 UV/Vis detector, L-2200 auto-sampler, Hitachi Co.; and a fraction collector, CHF 121SA, Advantec Co.). Semi-HPLC equipped with an ODS-column (Hyperprep HS C18, 10 mm diameter × 25 cm length, Thermo Electron Corp. Hypersil Ltd., Cheshire, UK) was run at 35˚C (controlled by a column oven) with a dual solvent system containing methanol (A solvent) and water (B solvent) with 100

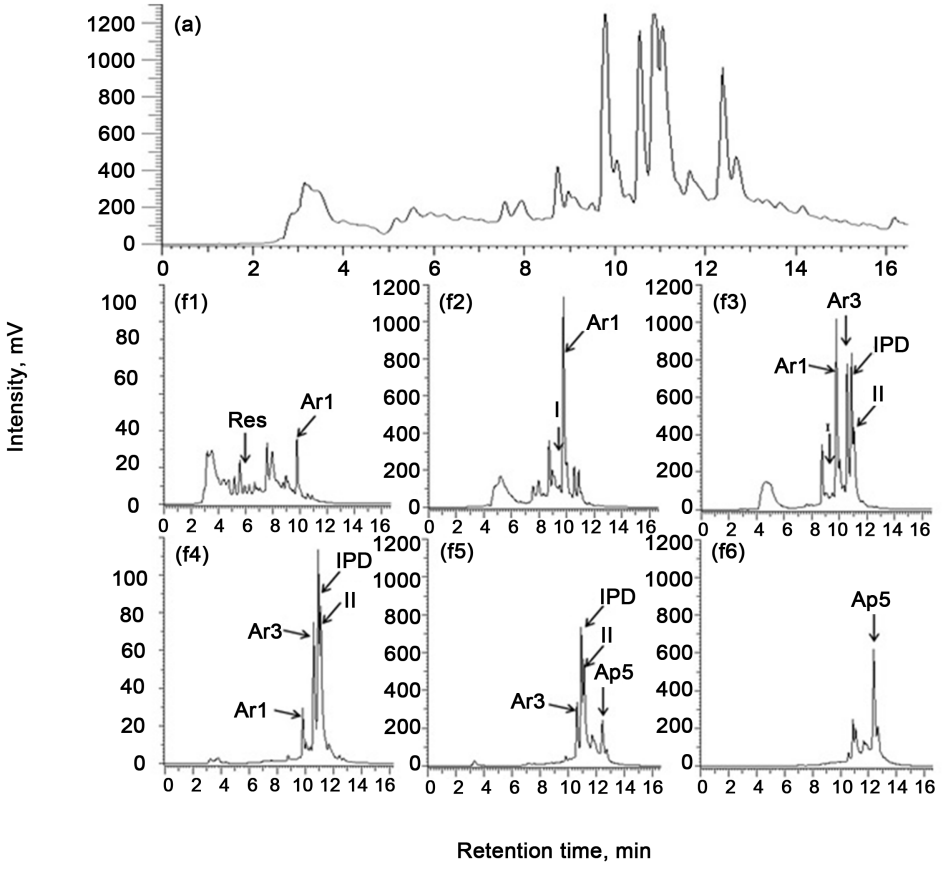



**Figure 1.** HPLC chromatograms of the loading solution extracted from bio-elicited peanut sprout powder (a) and the collected fractions (f1 to f6) after subjection of the loading solution to medium pressure liquid chromatographic (MPLC) fractionation.

μl, 3 ml/min and 254 nm of injection volume, flow rate and monitoring wavelength, respectively. The optimal mobile phase was programmed and adjusted according to the fractions destined for further purification. From each fraction, the repeatedly collected elutes corresponding to a targeted peak were pooled and vacuum evaporated to dryness. After further vacuum drying at 40˚C for 2 h, the substance was weighed, cap sealed in brown vial and stored under −20˚C for structural identification and bioactivity determinations. In addition to match of HPLC retention time and PDA spectrum, structures of the known stilbenes were confirmed by dissolving each in $CD_3OD$ or $CDCl_3$ for $^1H$ NMR analysis. As indicated in the HPLC chromatograms of the MPLC collected fractions by order of polarity, known stilbenoids of resveratrol (Res) was respectively isolated from MPLC fraction 1 [Figure 1(f1)], arachidin-1 (Ar 1), compound **I**, arachidin-3 (Ar 3), isopentadieneylresveratrol (IPD) and compound **II** were isolated from MPLC fraction 2 to 5 [Figures 1(f2)-(f5)] and arahypin-5 (Ap 5) was isolated from fraction 6 [Figure 1(f6)]. Among those, two new unknown compounds, *i.e.*, compound **I** [Figure 1(f2)] and compound **II** [Figure 1(f5)] were weighted and subjected to structural identification.

## 2.3. Structural Identification of the Unknown Compounds

The isolated and weighed dry solids of compounds **I** and **II** were dissolved in methanol

and $CH_2Cl_2$ for UV $\lambda$max determination with a spectrophotometer (Hitachi U-2100). ESI-HRMS (EI + VE + LMR) (*m/z*, $M^+$) analysis of the compounds was run on a LTQ Orbitrap XL Hybrid Ion Trap Mass Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). Optical rotation measurements employed an automatic polarimeter (Autopol IV, Rudolph Research Analytical, Hackettstown, NJ).

For NMR analyses, compound **I** (ca. 2.0 mg) and compound **II** (ca. 2.5 mg) were respectively dissolved in 0.6 ml $CD_3OD$ and $CDCl_3$ for sample preparation. All NMR experiments were performed by a Bruker Avance III 500 MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany), equipped with a 5-mm BBFO probe. The $^1H$ and $^{13}C$ NMR were measured at 298 K operating at 500.13 and 125.75 MHz, respectively. Chemical shift for each hydrogen was recorded with reference to the residual solvent signal of $CD_3OD$ (at $\delta_H$ 3.31 and 4.70, $\delta_C$49.2) and $CDCl_3$ (at $\delta_H$ 7.26, $\delta_C$ 77.2). Coupling constants (*J*) were reported in Hertz (Hz). All one dimensional ($^1H$ and $^{13}C$) and two dimensional NMR (COSY, HSQC, and HMBC) measurements were performed with standard Bruker pulse sequences.

### 2.4. Antioxidative Potency (AOP) and Antiglycative Activity Determination

For AOP determination by linoleic acid in an iron/ascorbate system, the previously reported procedure [34] was followed. For comparison on identical molecular basis, 100 μM resveratrol, compound **I**, arachidin-1, arachidin-3, IPD, compound **II**, arahypin-5 and butylated hydroxytoluene (BHT) as a reference were respectively prepared by dissolving in methanol and subjected to followed measurements. For antiglycative activity determination, the reported procedure [35] was followed. Briefly, the above 7 stilbenoid compounds were respectively prepared in 5% DMSO at 50 μM for determination. Aminoguanidine (AG) (Sigma Chem. Co., St. Louis, MO) at 3 mM was prepared as a reference and determined concurrently.

### 2.5. Longevity Experiments with BALB/c and ICR Mice

Two long-term animal experiments with BALB/c mice, an in-bred model, and ICR mice, an out-bred model, have been respectively conducted. Both experiment proposals have been approved by the Institutional Animal Care and Use Committee (IACUC) (Approval No. IACUC-2006005) of the National Chiayi University. For the first experiment with 11-mon-old female BALB/c mice, 48 mice provided by National Laboratory Animal Center, Taipei, Taiwan, after 2-week adaptation, were distributed randomly into three groups, namely, control group (BPSP 0 g/kg bw) (composition of the basal diet was listed in Supplementary Material Appendix Table S1), L-BPSP group (BPSP 0.1 g/kg bw) as a low dose treatment and H-BPSP group (BPSP 0.5 g/kg bw) as a high dose treatment. Four mice were raised and housed in a cage with a 12-h cycle of circadian rhythms and each was ear-tagged for identification. The temperature and relative humidity were controlled at 20˚C - 22˚C and 60% - 65%, respectively. The mice were daily fed with the estimated diets provided on 15 g diet/100 g·bw basis. The uneaten diets were weighed as a health monitoring purpose and average daily feed intake. Partial amount of soybean protein in the basal diet formula was substituted by BPSP to meet

the desired dose levels and adjusted to increase proportion according to the observed slight decrease of feed intake in the later periods. The BALB/c animal experiment started from November 3, 2006 and ended after the last death on November 14, 2008 for 750 days of entire period. Body weight for each survival mouse was determined every 2 weeks and each dead mouse was weighed and subjected to organ resection and combined with carcass for tissue fixation in a formalin jar for later use.

For the second longevity experiment, male and female ICR mice (12-mon-old, an out-bred animal model) obtained from the Laboratory Animal Center, National Taiwan University, Taipei, Taiwan were treated following the same protocol and treatment doses as that done with BALB/c mice. After 2-week adaptation, 10 male and 6 female mice were randomly distributed into a treatment and housed in a low density setting of 2 male/cage and 3 female/cage. Each mouse was weighed and identified with ear punched tagging. For diet preparation, BPSP was introduced in substitution of soybean protein of the basal diet (**Supplementary Material Appendix Table S1**). The treatment started from December 18, 2008 and ended after the last death on January 26, 2011 for 762 days of entire period.

### 2.6. Statistics

Means with standard deviation of the determinations ($n > 3$) are expressed. Data were subjected to one-way analysis of variance (ANOVA) for variance comparison among test groups. Significant differences between means were determined using Duncan's multiple-range test.

## 3. Results

### 3.1. BPSP Preparation and Nutrition Fact

In this study, BPSP preparation was initiated from dormant but of vitality peanut kernels transited to physiological and biochemical activation of germination-related metabolism and followed by wound-stress, heat treatment, grinding, defatting and powder packaging for storage. As subjected to compositional analysis to indicate nutrition fact of BPSP, its energy, protein, carbohydrate (sugars), fat (saturated fat and *trans* fat) and sodium per 100 g BPSP are 366 Kcal, 55 g, 30 g (1.1 g), 2.6 g (0.5 and 0 g) and 17 mg, respectively. BPSP is a high protein powder and used to substitute soy protein to formulate BPSP-supplemented diets (**Supplementary Material Appendix Table S1**) in the followed animal experiments.

### 3.2. Isolation and Identification of the Recovered BPSP Stilbenes

As shown in **Figure 1(a)** of HPLC chromatogram, presence of a complexity of comprising components in the BPSP methanol extracts after ethyl acetate partition is expected. The complex composition of the BPSP extract has been effectively separated by MPLC fractionation [**Figures 1(f1)-(f6)**]. From those collected fractions, series of isolation of the comprising stilbenes by semi-preparative HPLC were further accomplished. In reference of HPLC retention time, UV-spectrum shown by PDA detection and confirmation with $^1$H NMR analysis, 5 known peanut stilbenes, namely, *trans*-resveratrol (Res), *trans*-arachidin-1 (Ar 1), *trans*-arachdin-3 (Ar 3), *trans*-isopentadieneyl

resveratrol (IPD) and *trans*-arahypin-5 (Ap 5) were isolated (Figure 2). Two new compounds, i.e., compound **I** [Figure 1(f2)] and compound **II** [Figure 1(f4) and Figure 1(f5)] were subjected to spectroscopic analyses and structural elucidation. As estimated based on recovery yields, contents of resveratrol, arachidin-1, arachidin-3, IPD, arahypin-5, compound **I** and compound **II** are ca. 50, 2400, 1800, 3800, 980, 25 and 110 μg/g BPSP, respectively.

For the compound **I**, a light grey powder was obtained with UV ($CH_2Cl_2$) $\lambda$max (log $\varepsilon$): 340 nm (4.32); $[\alpha]_D^{22}$ +6.67 (c 0.1, $CH_2Cl_2$); ESI-HRMS [(EI+VE+LMR, $M^+$) m/z 310.1212, $C_{19}H_{18}O_4$ with theoretical mass 310.1205]. After subjection to 1D and 2D NMR spectroscopic analysis, $^1H$ and $^{13}C$ chemical shifts are shown in Table 1 and the related 1D ($^1H$ and $^{13}C$) and 2D (COSY, HSQC, HMBC) spectra are shown in **Supplementary Material**. $^1H$ NMR spectrum of **I** indicated the presence of a 1,4-disubstituted phenyl with protons at $\delta_H$ 6.78 (d, 2H, $J$ = 8.8 Hz), 7.35 (d, 2H, $J$ = 8.8 Hz), a 1,3,4,5-tetrasubstituted phenyl with protons at $\delta_H$6.48 (s) and 6.49 (s), a *trans*-olefin with protons at $\delta_H$ 6.75 and 6.96 (each d, $J$ = 15.5 Hz), and a *cis*-olefin with protons at $\delta_H$ 5.54 and 6.74 (each *d*, $J$ = 9.9 Hz) and a *gem*-hydroxyl methyl moiety with methylene protons at $\delta_H$ 3.52, 3.62 (each d, 2H, $J$ = 11.5 Hz) and methyl protons at $\delta_H$ 1.35 (3H, s). The proton correlations were confirmed by a COSY experiment. The $^{13}C$ NMR spectrum of compound **I** (Table 1) displayed 19 carbons, of which 14 were assigned to a stilbene moiety and 5 were assigned to a *gem*-hydroxymethyl-methylpyran unit.

The HMBC spectrum indicated correlations of H-7 to C-2 ($\delta_C$ 128.9) and C-6 ($\delta_C$ 128.9); H-8 to C-2' ($\delta_C$ 106.74) and C-6' ($\delta_C$ 106.9); H-5" to C-6" ($\delta_C$ 79.8) and C-4' ($\delta_C$ 110.1); H-7" to C-6" ($\delta_C$ 79.8); and H-8" to C-6" ($\delta_C$ 79.8) and C-7" ($\delta_C$ 68.49). Based on the above instrumentally supported evidence, compound **I** was identified as 4, 5'-dihydroxy-6"-hydroxymethyl, 6"-methylpyrano [2", 3": 3', 4']stilbene (Figure 2). It is a new assigned natural compound.

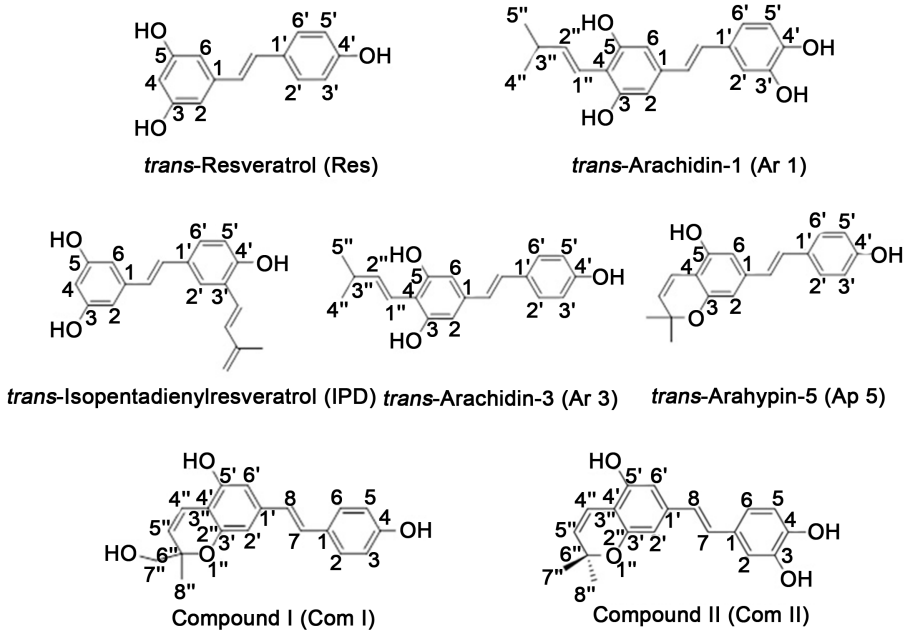
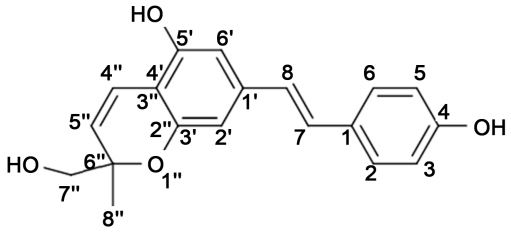



Figure 2. Chemical structures of stilbenes isolated from the bio-elicited peanut sprout powder.

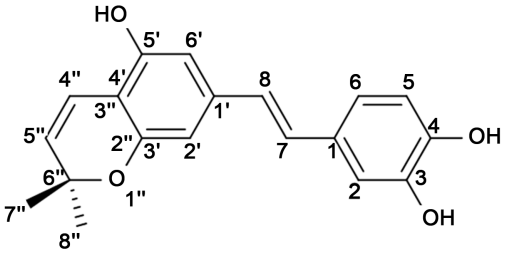
**Table 1.** $^{1}H$ and $^{13}C$ NMR chemical shifts respectively analyzed by 500 and 125 MHz of the compounds I and II isolated from the bio-elicited peanut sprout powder.

| Carbon position | Compound **I** (in $CD_3OD$) | | Compound **II** (in $CDCl_3$) | |
|---|---|---|---|---|
| | $\delta_H$[a] | $\delta_C$ | $\delta_H$[a] | $\delta_C$ |
| 1 | | 130.9 | | 130.8 |
| 2 | 7.35 (d, 1H, *J* = 8.8) | 128.9 | 7.03 (d, 1H, *J* = 1.6 Hz) | 112.9 |
| 3 | 6.78 (d, 1H, *J* = 8.8) | 116.6 | | 143.6 |
| 4 | | 140.5 | | 143.7 |
| 5 | 6.78 (d, 1H, *J* = 8.8) | 116.6 | 6.85 (d, 1H, *J* = 8.2) | 115.5 |
| 6 | 7.35 (d, 1H, *J* = 8.8) | 128.9 | 6.92 (dd, 1H, *J* = 1.6, 8.2) | 120.2 |
| 7 | 6.96 (d, 1H, *J* = 15.5) | 129.6 | 6.90 (d, 1H, *J* = 16.5) | 128.5 |
| 8 | 6.75 (d, 1H, *J* = 15.5) | 126.8 | 6.76 (d, 1H, *J* = 16.5) | 126.4 |
| 1’ | | 140.5 | | 138.6 |
| 2’ | 6.48 (s, 1H) | 106.74 | 6.43 (s, 1H) | 105.9 |
| 3’ | | 154.6 | | 151.4 |
| 4’ | | 110.1 | | 109.0 |
| 5’ | | 155.3 | | 154.1 |
| 6’ | 6.49 (s, 1H) | 106.9 | 6.58 (s, 1H) | 107.1 |
| 4” | 6.74 (d, 1H, *J* = 9.9) | 120.2 | 6.61 (d, 1H, *J* = 9.9) | 116.3 |
| 5” | 5.54 (d, 1H, *J* = 9.9) | 125.6 | 5.59 (d, 1H, *J* = 9.9) | 129.1 |
| 6” | | 79.8 | | 76.0 |
| 7” | 3.52,3.62 (d each, 2H, *J* = 11.5) | 68.49 | 1.44 (s, 3H) | 27.8 |
| 8” | 1.35 (s, 3H) | 30.98 | 1.44 (s, 3H) | 27.8 |
| -OH | | | 9.78, 9.80, 10.06 | |

[a]*J*: coupling constant in Hz; s: singlet; d: doublet.




Compound **I**




Compound **II**

For structural elucidation of the compound **II**, a light grey powder was obtained with UV (MeOH) $\lambda$max (log $\varepsilon$): 340 nm (4.74); ESI-HRMS [(EI+VE+LMR, M+) m/z 310.1200, $C_{19}H_{18}O_4$ with theoretical mass 310.1205]. $^1$H and $^{13}$C chemical shifts of compound **II** are shown in Table 1. The related 1D and 2D spectra are also shown in **Supplementary Material.** $^1$H NMR spectrum of **II** indicated signals of three hydroxyl protons ($\delta_H$ 9.78, 9.80 and 10.06), a 1,3,4-trisubstituted phenyl with protons at $\delta_H$6.85 (d, $J$ = 8.2 Hz), 6.92 (d, $J$ = 8.2 Hz), and 7.03 (s), a 1,3,4,5-tetrasubstituted phenyl with protons at $\delta_H$ 6.43 (s) and 6.58 (s), a *trans*-olefin with protons at $\delta_H$6.76 and 6.90 (each d, $J$ = 16.5 Hz), and a *cis*-olefin with protons at $\delta_H$ 5.59 and 6.61 (each d, $J$ = 9.9 Hz) and protons at 1.44 (6H, s) corresponding to a *gem*-dimethylpyran group. The proton correlations were confirmed by a COSY experiment. The $^{13}$C NMR spectrum of compound **II** (Table 1) displayed 19 carbons, of which 14 were assigned to a stilbene moiety and 5 to a *gem*-dimethylpyran unit.

The HMBC spectrum indicated correlations of H-2 and H-5 to C-3 ($\delta_C$ 143.6) and C-4 ($\delta_C$ 143.7), and H-6 to C-4, both H-7 and H-8 are correlated to C-1 ($\delta_C$ 130.8) and C-1' ($\delta_C$ 138.6). The *gem*-dimethylpyran moiety was determined to be attached to the 1,3,4,5-tetrasubstituted benzene ring by the long-range correlations of H-4" to C-3' ($\delta_C$ 151.4), C-4' ($\delta_C$ 109.0) and C-5' ($\delta_C$ 154.1) as well as H-5"to C-4'. Accordingly, based on the above instrument-supported data, compound **II** was identified as 3, 4, 5'-trihydroxy-6", 6"-dimethylpyrano [2", 3": 3', 4']stilbene (Figure 2). It is a new assigned natural compound.

### 3.3. AOP and Antiglycative Activities of the Recovered BPSP Stilbenes

As compared on the same molecular basis (100 μM), in comparison to BHT, all test stilbenes have exhibited potent AOP activities [Figure 3(a)]. Apparently, among the test stilbenes, AOP of each molecule varied with dependence on each own chemical structure. As a comparison was made on antiglycative activity, the test 7 peanut stilbenes [Figure 3(b)], archidin-1, arachidin-3 and compound **II** exhibited much higher activity than that of 3 mM AG.

### 3.4. Longevity Extension of BALB/c and ICR Mice

For the 11-mon-old female BALB/c mice, an in-bred animal model, continuously fed with BPSP-supplemented normal diets for 750 days until the last death, survival cures are shown in Figure 4(a). Longevities of the mice fed with BPSP-supplemented normal diets were longer than mice fed with the control diet. As further comparison of overall life spans, average lifespans of the mice fed with BPSP-supplemented diets were longer than that of normal diet. For the mice fed with BPSP-supplemented diets at 0.5 g BPSP/kg·bw [Figure 4(b)], ca. 50 days of longevity was extended.

For the experiment of longevity extension with an out-bred animal model for both genders, 12-mon-old male and female ICR mice fed with the same above-described diets for 762 days. As generally observed, longevity of the mice as affected by gender was limited and the integrated survival curves are shown in Figure 4(c). It is obvious to observe that longevity of the mice fed with BPSP-supplemented basal diets were longer, with a dose dependent manner, than mice fed with the control basal diet. As compared, average lifespans of the mice fed with BPSP-supplemented diets was longer

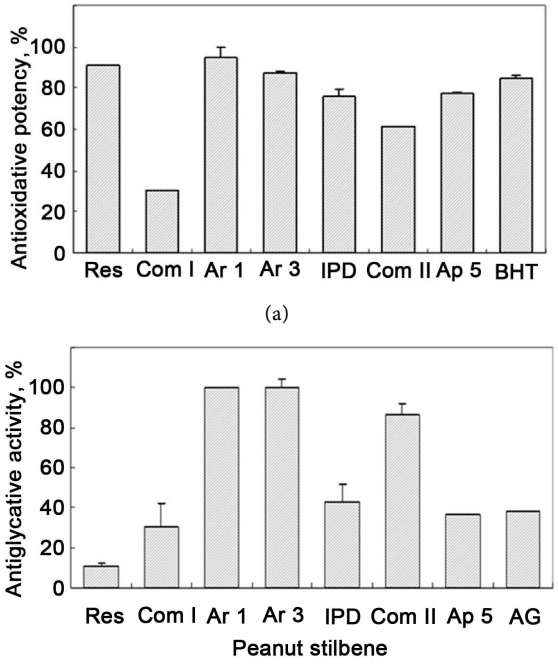



**Figure 3.** Antioxidative potencies (a) test at 100 μM of resveratrol (Res), compound I (Com I), arachidin-1 (Ar 1), arachidn-3 (Ar 3), isopentadienylresveratrol (IPD), compound II (Com II), arahypin-5 (Ap 5) and butylated hydroxytoluene (BHT) and antiglycative activities (b) test at 50 μM and aminoguanidine (AG) at 3 mM as a reference (n = 3).

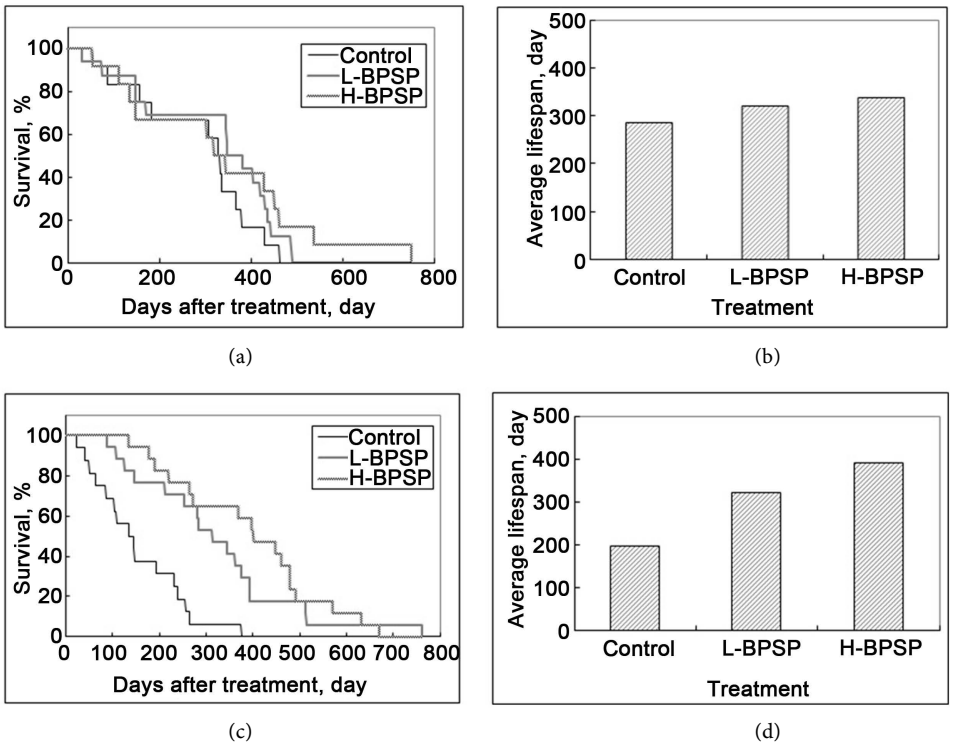



**Figure 4.** Survival curves and average lifespans of the 11-mon-old female BALB/c mice (a) and (b) and 12-mon-old male and female ICR mice (c) and (d) respectively fed daily with basal diets supplemented with bio-elicited peanut sprout powder (BPSP) for 750 and 762 days; control: 0 g BPSP/kg bw; L: 0.1 g BPSP/kg bw; H: 0.5 g BPSP/kg bw.

than that of basal diet [**Figure 4(d)**]. For the mice fed with 0.5 g BPSP/kg bw, ca. 96 days of the mouse lifespan was extended.

## 4. Discussion

With an attempt to effectively isolate the complex compositional compounds from BPSP (**Figure 1**), MPLC is capable of resolving whole overlapping compounds into fractions and enabling subsequent semi-pre HPLC purification to obtain 7 compounds (**Figure 1** and **Figure 2**). *Trans*-resveratrol (Res), *trans*-arachidin-1 (Ar 1), *trans*-arachdin-3 (Ar 3) and *trans*-isopentadieneyl resveratrol (IPD) have been previously reported in our laboratory [8]. Those stilbenes along with t*rans*-arahypin-5 (Ap 5) and many other stilbene compounds of peanut origins have also been discovered in different research laboratories [10] [12] [13] [17] [20] [22]. A new compound with prenylatedbenzenoid and but-2-enolide moieties, termed SB-1, has been isolated from peanut kernels challenged with species of *Aspergillus* [18]. Arahypin-5 (4,5'-dihydroxy-6",6"-dimethylpyran [2",3":3',4']stilbene) has been isolated from peanut seeds challenged with *Aspergillus caelatus* [20]. Other new arahypins and other stilbenes have also been isolated from fungal-challenged peanut seeds [12] [13]. Even peanut stilbene compounds have long been extensively and intensively investigated, two new peanut stilbene compounds, *i.e.*, 4, 5'-dihydroxy-6"-hydroxymethyl, 6"-methylpyrano [2", 3": 3', 4']stilbene (compound **I**) and 3, 4, 5'-trihydroxy-6", 6"-dimethylpyrano [2", 3": 3', 4']stilbene (compound **II**) (**Figure 2**) have been isolated, identified and assigned.

In addition to chemical characterization, a wide variety of biological activities of the selected natural and/or synthetic peanut-related stilbenes has been investigated and demonstrated [12] [13] [22] [25] [26] [27] [28]. In particular, presence of resveratrol and other bioactive phytochemicals related to consumer health has been highlighted [3] [6]. As subjection of the 7 peanut stilbenes isolated in this study to AOP and antiglycative activity characterization (**Figure 3**), all test compounds have exhibited potent activities and varied on structure-activity nature of each molecule. Antioxidant activity of a molecule is the most noticed characteristics of a molecule to be recognized as a bioactive candidate. Glycation of serum albumin and functional proteins under high-blood-sugar condition, such as enzymes and membrane proteins, leading to metabolic syndrome and subsequent chronic diseases is always an important issue of human health. Among the test stilbenoids shown in **Figure 3**, it is of importance to point out that arachidin-1 and arachidin-3 have exhibited considerably higher AOP and antiglycative activities than other stilbenes. As reported, arachidin-1 and arachidin-3 are major stilbenes of BPSP and bear potent antioxidant and anti-inflammatory activities have been demonstrated [8] [23]. In further, a broad spectrum of potent biological activities of arachidin-1 has been demonstrated [4] [8] [23] [24] [26] [28]. In the future, an intensive structure-activity investigation addressed on nature of each compound remained research interest. For BPSP, presence of a wide spectrum of various peanut stilbenes is no doubt closely related to reflect its unique properties.

In longevity experiments with the aged mice of in-bred and out-bred models for 750 and 762 days, it is obvious that longevity of the mice fed with BPSP-supplemented basal diets were longer than mice fed with the control basal diet (**Figure 4**). Overall average life spans of the mice fed with BPSP-supplemented diets at 0.5 g BPSP/kg·bw were

longer than that of basal diet by ca. 50 and 96 days for both mouse models, respectively. Based on the obtained survival curves or average life spans as science-based evidences, the effect of dietary supplementation of BPSP in longevity extension was also obvious. These findings are supporting an old Chinese tale that "Peanut also named life longer nut" as well as a phrase that "A longer life with peanuts" promoted by The Peanut Institute [3].

As expected, approaches by dietary interventions to extend mouse longevity by anti-oxidant supplementation, calorie restriction or complex dietary supplements are of virtue [36] [37] [38]. In this study, longevity extension of either in-bred or out-bred mouse models was achieved by dietary BPSP supplementation (**Figure 4**). This is of significance and perspective to raise the link that human longevity extended by dietary intake of the BPSP-like supplements is likely. As reported recently, arachidin-1 and resveratrol modulate regulatory T cell functions responsible for successful aging in aged ICR mice mainly due to their phytoestrogenic activities [28]. In that report, when 12-week-old ICR mice were fed with BPSP-supplemented diets for 48 weeks, their circulating Treg populations, the gene expression levels of CTLA-4 and TGF-β were significantly elevated. Arachdin-1 is one of the major stilbenes of BPSP [8]. Therefore, the studies support the beneficial roles of arachidin-1, resveratrol and other BPSP stilbenes in facilitating a successful aging immune status and contribute longevity extension.

## 5. Conclusion

It is of novelty that the subjection of peanut kernels to germination and wound-stress is remarkably activating biosynthesis of peanut stilbenes. Through MPLC to facilitate the followed isolation and recovery of the stilbenes from BPSP, high yield up to ca. 1.0% (w/w) of two new stilbenes, identified for the first time, and 5 new compounds were recovered. All recovered stilbenes exhibited potent anti-oxidative and anti-glycative activities. With an attempt to use BPSP as a dietary supplement, it is of merit to demonstrate that BPSP is effective to extend mouse longevity as science-evidenced by the two long-term animal experiments. In addition to the fact that phytoestrogenic stilbenes, arachidin-1 and resveratrol exhibit phytoestrogenic activities and modulate regulatory T cell functions responsible for successful aging in aged ICR mice [28], co-present components of BPSP including other phenolic compounds all contribute to the observed effectiveness of lifespan extension. In the future, continuous investigations with BPSP addressed on investigations of the specified longevity involved mechanisms and related nutraceutical products development are of worth.

## Acknowledgements

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## Supplementary Material

Supplementary data associated with this article are presented in the **Appendix**.

## Declarations

**1) Ethics approval and consent to participate**

Not applicable.

**2) Availability of data and material**

Datasets generated or analyzed in this study are included in this published article and raw data of the current study are available from the corresponding author on reasonable request.

## Authors' Contributions

R.Y.Y.C. supervised, designed experiments and written manuscript; P.C.C. purified new compound and instrumental analyses; J.C.C. conducted and managed progress of experiments; Y.J.L. elucidated chemical structure; C.W.H. conducted product preparation and characterization; J.Y.W carried NMR analysis and data collection; S.M.L. carried out parts of longevity animal experiments; Y.L.L. carried out parts of new compound elucidation; B.B.C.W. carried out parts of animal experiments

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## Abbreviations

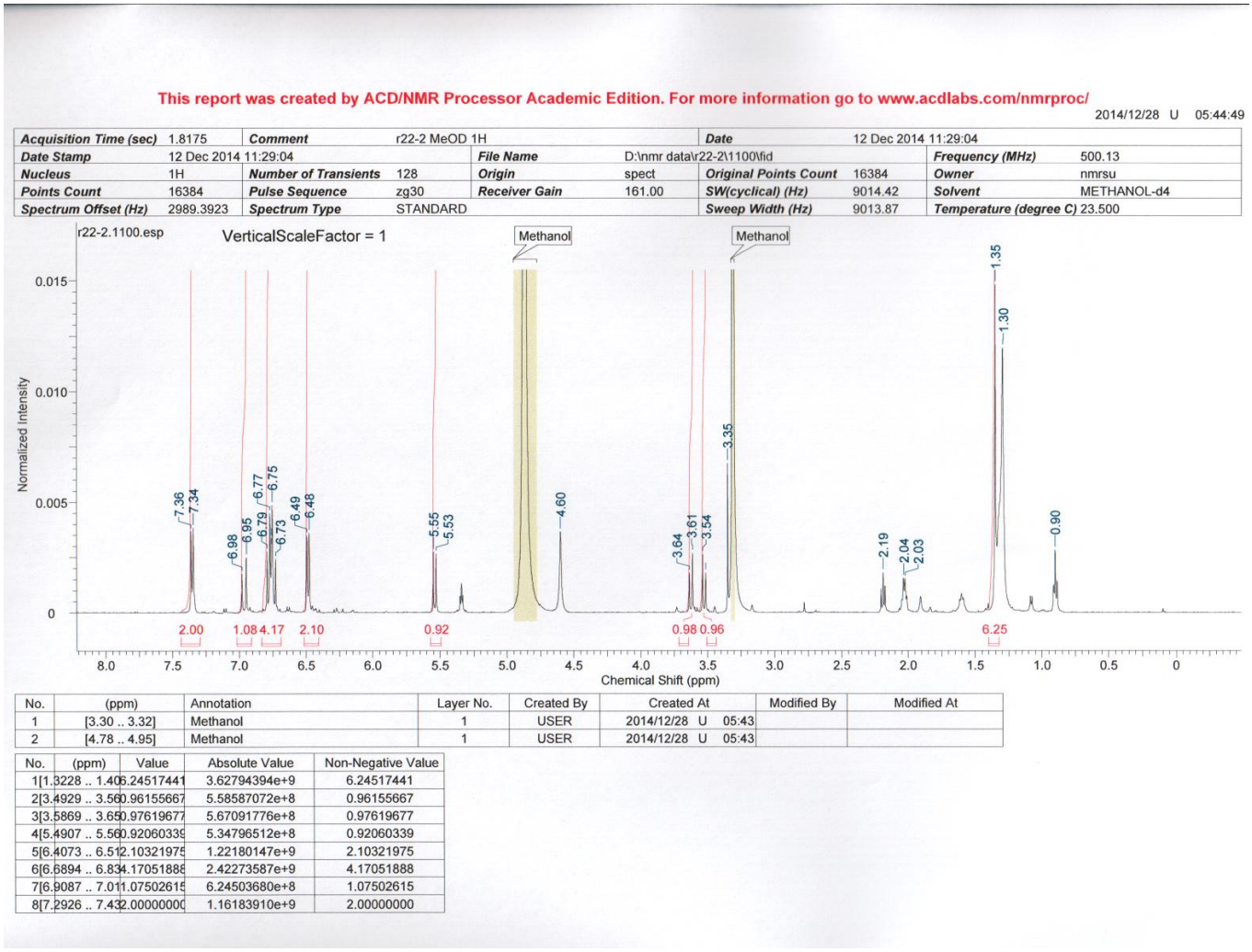
AG, aminoguanidine;
Ar 1, arachidin-1;
Ar 3, arachidin-3;
IPD, isopentadienylresveratrol;
Ap 5, arahypin-5;
AOP, antioxidative potency;
BPSP, bio-elicited peanut sprout powder;
MPLC, medium pressure liquid chromatography;
NMR, nuclear magnetic resonance;
Res, resveratrol.

## Appendix

**Table S1.** Composition of basal murine diets.

| Ingredients | % | 1 kg |
|---|---|---|
| Soybean protein | 10.0 | 100 g |
| Full-fat soybean powder | 27.0 | 270 g |
| Corn starch | 20.0 | 200 g |
| Fish meal | 5.0 | 50 g |
| Wheat bran | 8.0 | 80 g |
| Sucrose | 19.54 | 195.4 g |
| Corn oil | 4.8 | 49 g |
| Salt | 0.3 | 3 g |
| Limestone powder | 0.29 | 2.9 g |
| L-lysine | 0.17 | 1.7 g |
| DL-methionine | 0.2 | 2 g |
| AIN-Vitamin premix[a] | 1.0 | 10 g |
| AIN-Mineral premix[b] | 3.5 | 35 g |
| Choline bitartrate | 0.2 | 2 g |

[a]AIN-76; [b]AIN-76.

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| Spectrum Offset (Hz) | 2989.3923 | Spectrum Type | STANDARD | | | Sweep Width (Hz) | 9013.87 | Temperature (degree C) | 23.500 |

| No. | (ppm) | Annotation | Layer No. | Created By | Created At | Modified By | Modified At |
|---|---|---|---|---|---|---|---|
| 1 | [3.30 .. 3.32] | Methanol | 1 | USER | 2014/12/28 U 05:43 | | |
| 2 | [4.78 .. 4.95] | Methanol | 1 | USER | 2014/12/28 U 05:43 | | |

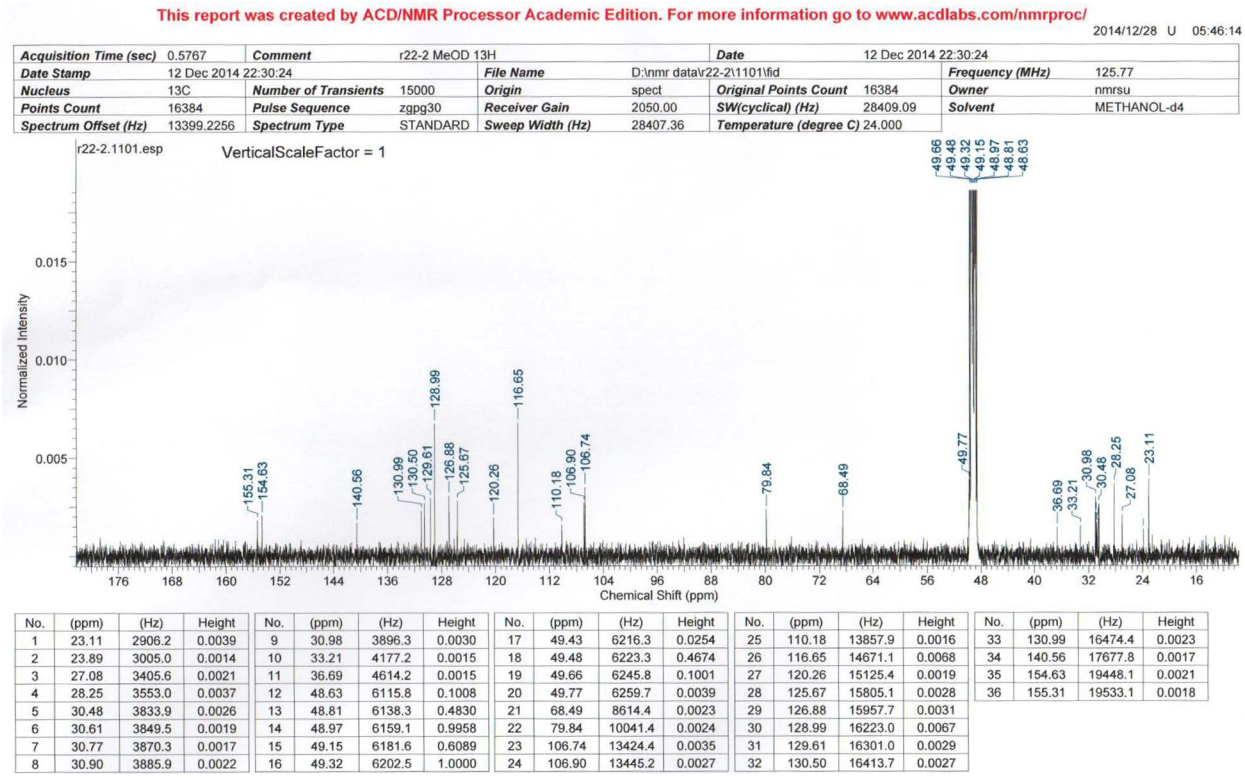
| No. | (ppm) | Value | Absolute Value | Non-Negative Value |
|---|---|---|---|---|
| 1 | [1.3228 .. 1.40 | 6.24517441 | 3.62794394e+9 | 6.24517441 |
| 2 | [3.4929 .. 3.56 | 0.96155667 | 5.58587072e+8 | 0.96155667 |
| 3 | [3.5869 .. 3.65 | 0.97619677 | 5.67091776e+8 | 0.97619677 |
| 4 | [5.4907 .. 5.56 | 0.92060339 | 5.34796512e+8 | 0.92060339 |
| 5 | [6.4073 .. 6.51 | 2.10321975 | 1.22180147e+9 | 2.10321975 |
| 6 | [6.6894 .. 6.83 | 4.17051888 | 2.42273587e+9 | 4.17051888 |
| 7 | [6.9087 .. 7.01 | 1.07502615 | 6.24503680e+8 | 1.07502615 |
| 8 | [7.2926 .. 7.43 | 2.00000000 | 1.16183910e+9 | 2.00000000 |



| | (ppm) | (Hz) | Height |
|---|---|---|---|
| 1 | 0.90 | 451.6 | 0.0028 |
| 2 | 1.30 | 649.7 | 0.0120 |
| 3 | 1.35 | 676.6 | 0.0148 |
| 4 | 2.03 | 1012.8 | 0.0015 |
| 5 | 2.04 | 1018.3 | 0.0015 |
| 6 | 2.19 | 1095.9 | 0.0018 |
| 7 | 3.35 | 1674.7 | 0.0068 |
| 8 | 3.51 | 1757.8 | 0.0018 |
| 9 | 3.54 | 1769.3 | 0.0026 |
| 10 | 3.61 | 1807.3 | 0.0027 |
| 11 | 3.64 | 1818.8 | 0.0018 |
| 12 | 4.60 | 2300.3 | 0.0037 |
| 13 | 5.53 | 2765.7 | 0.0026 |
| 14 | 5.55 | 2775.6 | 0.0027 |
| 15 | 6.48 | 3240.0 | 0.0036 |
| 16 | 6.49 | 3247.7 | 0.0035 |
| 17 | 6.73 | 3366.0 | 0.0024 |
| 18 | 6.75 | 3377.6 | 0.0048 |
| 19 | 6.76 | 3380.3 | 0.0033 |
| 20 | 6.77 | 3386.4 | 0.0045 |
| 21 | 6.79 | 3396.8 | 0.0028 |
| 22 | 6.95 | 3474.4 | 0.0025 |
| 23 | 6.98 | 3490.9 | 0.0018 |
| 24 | 7.34 | 3671.9 | 0.0038 |
| 25 | 7.36 | 3680.7 | 0.0037 |

$^{1}$H compound **I**

**Figure S1.** NMR data for compound **I**: $^{1}$H.

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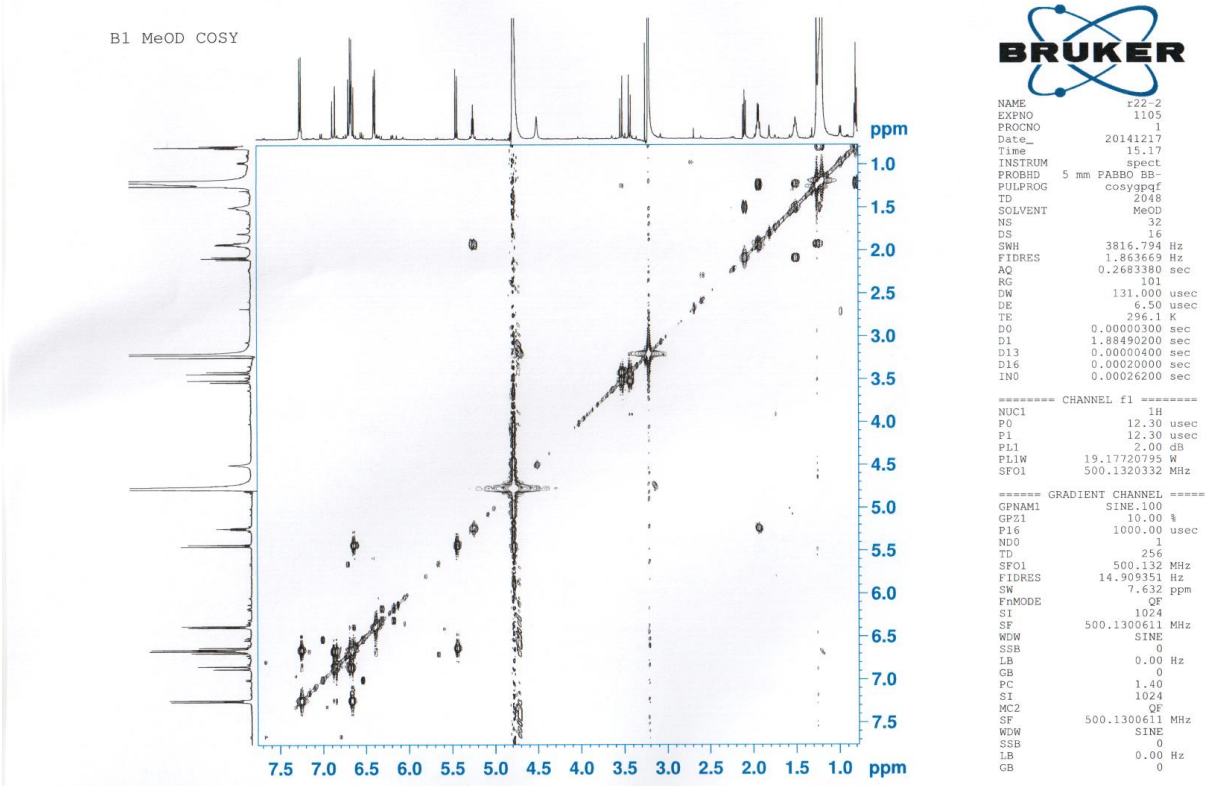
| *Points Count* | 16384 | *Pulse Sequence* | zgpg30 | *Receiver Gain* | 2050.00 | *SW(cyclical) (Hz)* | 28409.09 | *Solvent* | METHANOL-d4 | | | | | | |
| *Spectrum Offset (Hz)* | 13399.2256 | *Spectrum Type* | STANDARD | *Sweep Width (Hz)* | 28407.36 | *Temperature (degree C)* | 24.000 | | | | | | | | |

| No. | (ppm) | (Hz) | Height | No. | (ppm) | (Hz) | Height | No. | (ppm) | (Hz) | Height | No. | (ppm) | (Hz) | Height | No. | (ppm) | (Hz) | Height |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 23.11 | 2906.2 | 0.0039 | 9 | 30.98 | 3896.3 | 0.0030 | 17 | 49.43 | 6216.3 | 0.0254 | 25 | 110.18 | 13857.9 | 0.0016 | 33 | 130.99 | 16474.4 | 0.0023 |
| 2 | 23.89 | 3005.0 | 0.0014 | 10 | 33.21 | 4177.2 | 0.0015 | 18 | 49.48 | 6223.3 | 0.4674 | 26 | 116.65 | 14671.1 | 0.0068 | 34 | 140.56 | 17677.8 | 0.0017 |
| 3 | 27.08 | 3405.6 | 0.0021 | 11 | 36.69 | 4614.2 | 0.0015 | 19 | 49.66 | 6245.8 | 0.1001 | 27 | 120.26 | 15125.4 | 0.0019 | 35 | 154.63 | 19448.1 | 0.0021 |
| 4 | 28.25 | 3553.0 | 0.0037 | 12 | 48.63 | 6115.8 | 0.1008 | 20 | 49.77 | 6259.7 | 0.0039 | 28 | 125.67 | 15805.1 | 0.0028 | 36 | 155.31 | 19533.1 | 0.0018 |
| 5 | 30.48 | 3833.9 | 0.0026 | 13 | 48.81 | 6138.3 | 0.4830 | 21 | 68.49 | 8614.4 | 0.0023 | 29 | 126.88 | 15957.7 | 0.0031 | | | | |
| 6 | 30.61 | 3849.5 | 0.0019 | 14 | 48.97 | 6159.1 | 0.9958 | 22 | 79.84 | 10041.4 | 0.0024 | 30 | 128.99 | 16223.0 | 0.0067 | | | | |
| 7 | 30.77 | 3870.3 | 0.0017 | 15 | 49.15 | 6181.6 | 0.6089 | 23 | 106.74 | 13424.4 | 0.0035 | 31 | 129.61 | 16301.0 | 0.0029 | | | | |
| 8 | 30.90 | 3885.9 | 0.0022 | 16 | 49.32 | 6202.5 | 1.0000 | 24 | 106.90 | 13445.2 | 0.0027 | 32 | 130.50 | 16413.7 | 0.0027 | | | | |

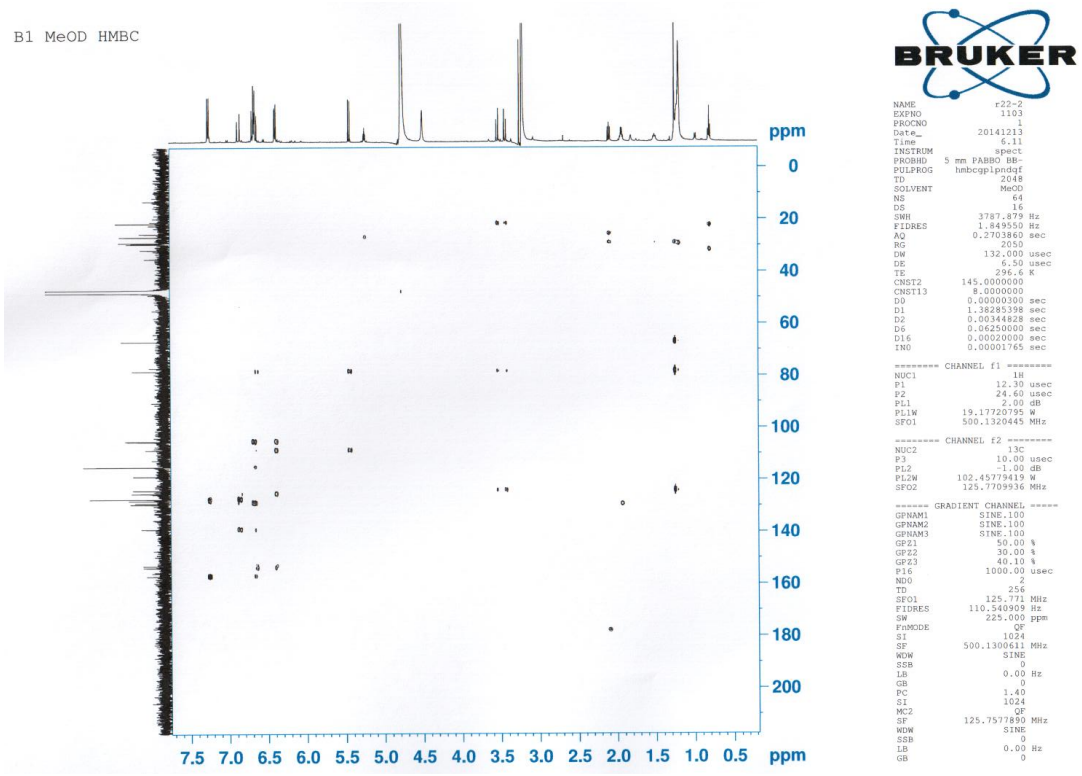
$^{13}$C-Compound I

**Figure S2.** NMR data for compound **I**: $^{13}$C.



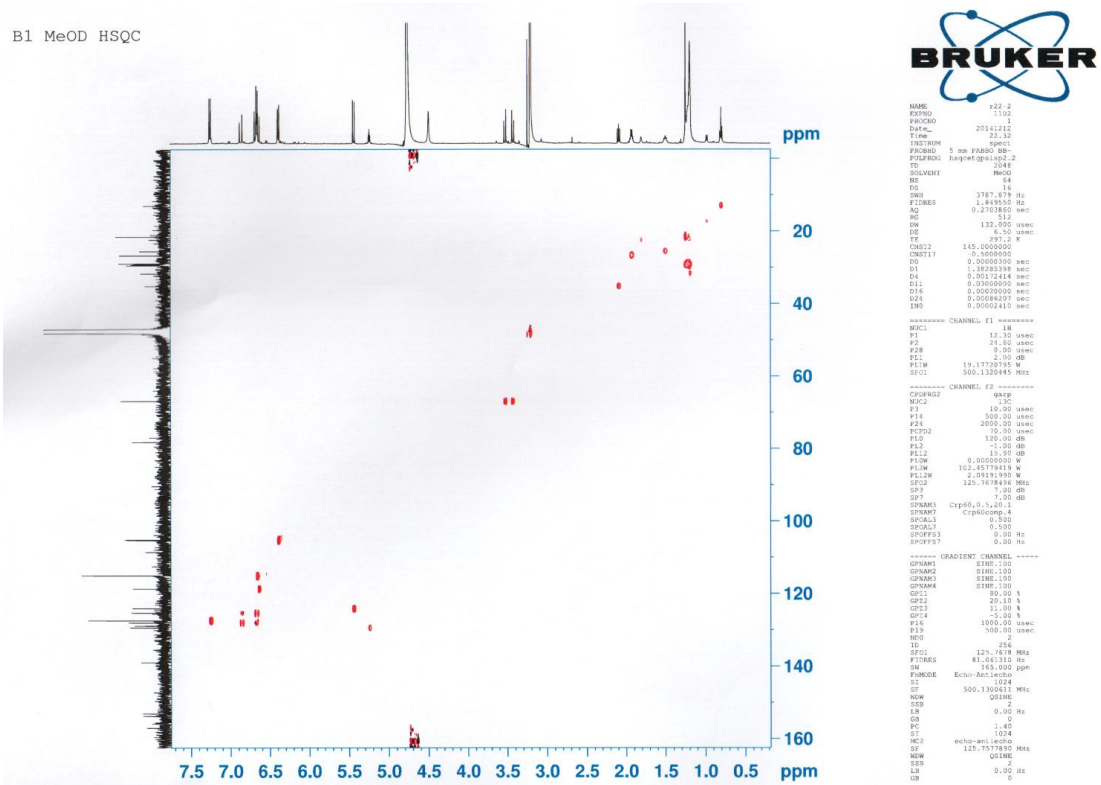

COSY-Compound I

**Figure S3.** NMR data for compound **I**: COSY.



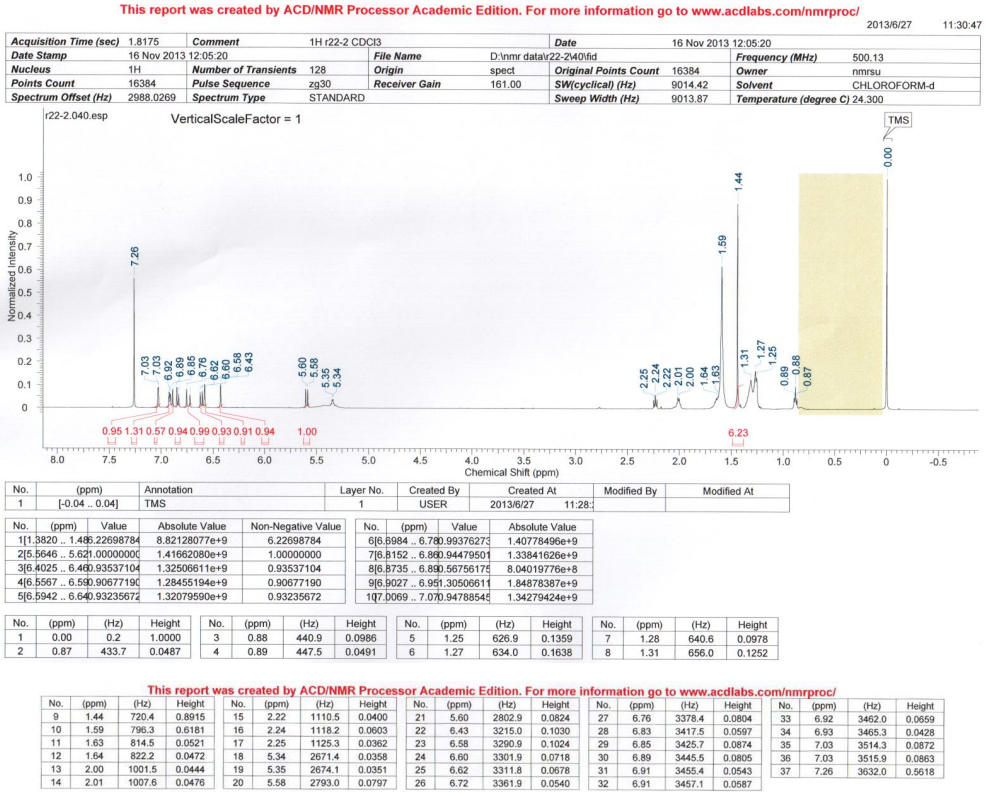

HMBC-Compound I

**Figure S4.** NMR data for Compound **I**: HMBC.




HSQC-Compound I

**Figure S5.** NMR data for Compound **I**: HSQC.




| Acquisition Time (sec) | 1.8175 | Comment | 1H r22-2 CDCl3 | | | Date | 16 Nov 2013 12:05:20 | | |
|---|---|---|---|---|---|---|---|---|---|
| Date Stamp | 16 Nov 2013 12:05:20 | | | File Name | D:\nmr data\r22-2\40\fid | | | Frequency (MHz) | 500.13 |
| Nucleus | 1H | Number of Transients | 128 | Origin | spect | Original Points Count | 16384 | Owner | nmrsu |
| Points Count | 16384 | Pulse Sequence | zg30 | Receiver Gain | 161.00 | SW(cyclical) (Hz) | 9014.42 | Solvent | CHLOROFORM-d |
| Spectrum Offset (Hz) | 2988.0269 | Spectrum Type | STANDARD | | | Sweep Width (Hz) | 9013.87 | Temperature (degree C) | 24.300 |

| No. | (ppm) | Annotation | Layer No. | Created By | Created At | Modified By | Modified At |
|---|---|---|---|---|---|---|---|
| 1 | [-0.04 .. 0.04] | TMS | 1 | USER | 2013/6/27 11:28: | | |

| No. | (ppm) | Value | Absolute Value | Non-Negative Value |
|---|---|---|---|---|
| 1 | [1.3820 .. 1.48 | 6.22698784 | 8.82128077e+9 | 6.22698784 |
| 2 | [5.5646 .. 5.62 | 1.00000000 | 1.41662080e+9 | 1.00000000 |
| 3 | [6.4025 .. 6.46 | 0.93537104 | 1.32506611e+9 | 0.93537104 |
| 4 | [6.5567 .. 6.59 | 0.90677190 | 1.28455194e+9 | 0.90677190 |
| 5 | [6.5942 .. 6.64 | 0.93235672 | 1.32079590e+9 | 0.93235672 |

| No. | (ppm) | Value | Absolute Value |
|---|---|---|---|
| 6 | [6.6984 .. 6.78 | 0.99376273 | 1.40778496e+9 |
| 7 | [6.8152 .. 6.86 | 0.94479501 | 1.33841626e+9 |
| 8 | [6.8735 .. 6.89 | 0.56756175 | 8.04019776e+8 |
| 9 | [6.9027 .. 6.95 | 1.30506611 | 1.84878387e+9 |
| 10 | [7.0069 .. 7.07 | 0.94788545 | 1.34279424e+9 |

| No. | (ppm) | (Hz) | Height |
|---|---|---|---|
| 1 | 0.00 | 0.2 | 1.0000 |
| 2 | 0.87 | 433.7 | 0.0487 |
| 3 | 0.88 | 440.9 | 0.0986 |
| 4 | 0.89 | 447.5 | 0.0491 |
| 5 | 1.25 | 626.9 | 0.1359 |
| 6 | 1.27 | 634.0 | 0.1638 |
| 7 | 1.28 | 640.6 | 0.0978 |
| 8 | 1.31 | 656.0 | 0.1252 |

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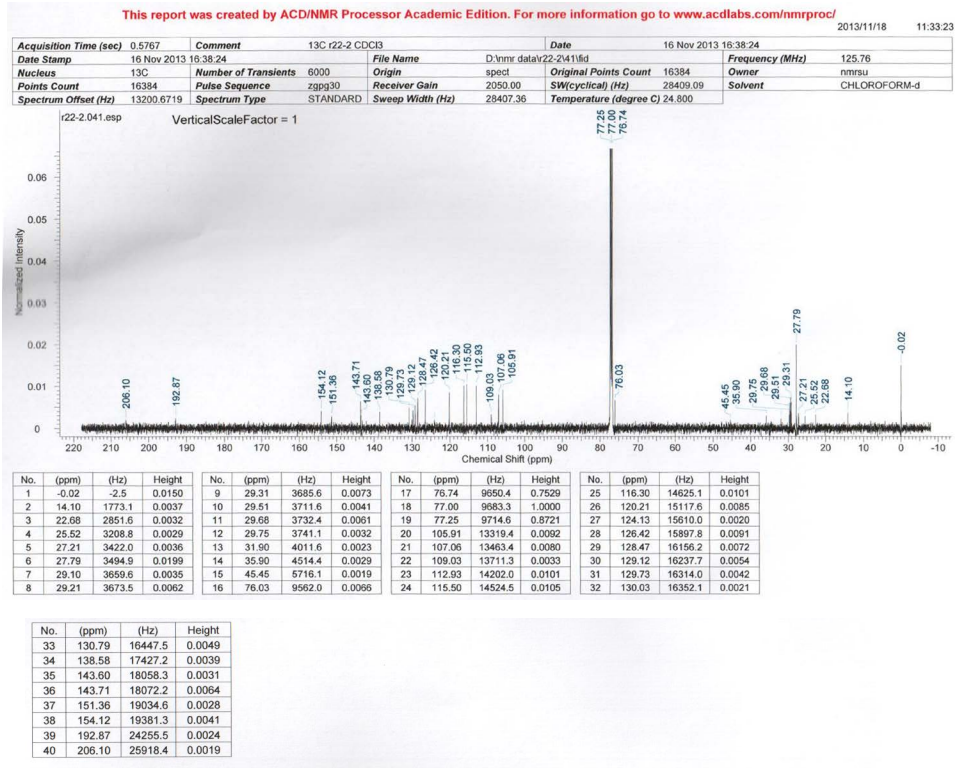
| No. | (ppm) | (Hz) | Height |
|---|---|---|---|
| 9 | 1.44 | 720.4 | 0.8915 |
| 10 | 1.59 | 796.3 | 0.6181 |
| 11 | 1.63 | 814.5 | 0.0521 |
| 12 | 1.64 | 822.2 | 0.0472 |
| 13 | 2.00 | 1001.5 | 0.0444 |
| 14 | 2.01 | 1007.6 | 0.0476 |
| 15 | 2.22 | 1110.5 | 0.0400 |
| 16 | 2.24 | 1118.2 | 0.0603 |
| 17 | 2.25 | 1125.3 | 0.0362 |
| 18 | 5.34 | 2671.4 | 0.0358 |
| 19 | 5.35 | 2674.1 | 0.0351 |
| 20 | 5.58 | 2793.0 | 0.0797 |
| 21 | 5.60 | 2802.9 | 0.0824 |
| 22 | 6.43 | 3215.0 | 0.1030 |
| 23 | 6.58 | 3290.9 | 0.1024 |
| 24 | 6.60 | 3301.9 | 0.0718 |
| 25 | 6.62 | 3311.8 | 0.0678 |
| 26 | 6.72 | 3361.9 | 0.0540 |
| 27 | 6.76 | 3378.4 | 0.0804 |
| 28 | 6.83 | 3417.5 | 0.0597 |
| 29 | 6.85 | 3425.7 | 0.0874 |
| 30 | 6.89 | 3445.5 | 0.0805 |
| 31 | 6.91 | 3455.4 | 0.0543 |
| 32 | 6.91 | 3457.1 | 0.0587 |
| 33 | 6.92 | 3462.0 | 0.0659 |
| 34 | 6.93 | 3465.3 | 0.0428 |
| 35 | 7.03 | 3514.3 | 0.0872 |
| 36 | 7.03 | 3515.9 | 0.0863 |
| 37 | 7.26 | 3632.0 | 0.5618 |

$^{1}$H compound II

**Figure S6.** NMR data for compound **II**: $^{1}$H.

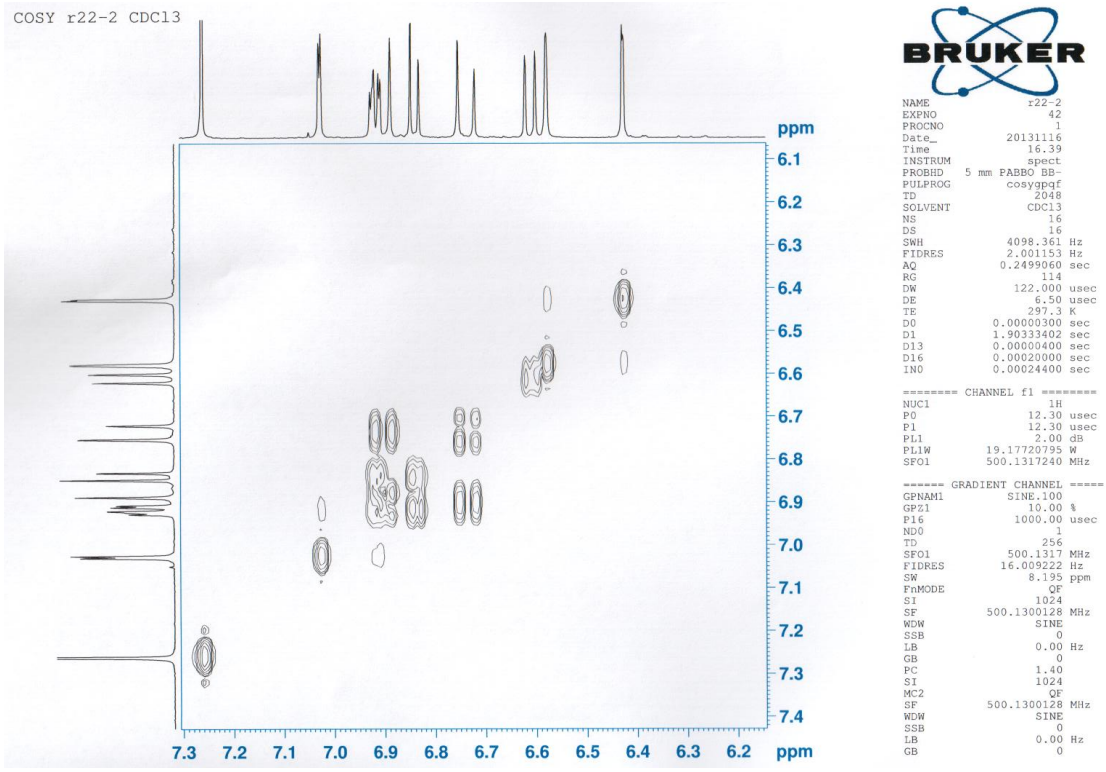



| Acquisition Time (sec) | 0.5767 | Comment | 13C r22-2 CDCl3 | | | Date | 16 Nov 2013 16:38:24 | | |
|---|---|---|---|---|---|---|---|---|---|
| Date Stamp | 16 Nov 2013 16:38:24 | | | File Name | D:\nmr data\r22-2\41\fid | | | Frequency (MHz) | 125.76 |
| Nucleus | 13C | Number of Transients | 6000 | Origin | spect | Original Points Count | 16384 | Owner | nmrsu |
| Points Count | 16384 | Pulse Sequence | zgpg30 | Receiver Gain | 2050.00 | SW(cyclical) (Hz) | 28409.09 | Solvent | CHLOROFORM-d |
| Spectrum Offset (Hz) | 13200.6719 | Spectrum Type | STANDARD | Sweep Width (Hz) | 28407.36 | Temperature (degree C) | 24.800 | | |

| No. | (ppm) | (Hz) | Height |
|---|---|---|---|
| 1 | -0.02 | -2.5 | 0.0150 |
| 2 | 14.10 | 1773.1 | 0.0037 |
| 3 | 22.68 | 2851.6 | 0.0032 |
| 4 | 25.52 | 3208.8 | 0.0029 |
| 5 | 27.21 | 3422.0 | 0.0036 |
| 6 | 27.79 | 3494.9 | 0.0199 |
| 7 | 29.10 | 3659.6 | 0.0035 |
| 8 | 29.21 | 3673.5 | 0.0062 |
| 9 | 29.31 | 3685.6 | 0.0073 |
| 10 | 29.51 | 3711.6 | 0.0041 |
| 11 | 29.68 | 3732.4 | 0.0061 |
| 12 | 29.75 | 3741.1 | 0.0032 |
| 13 | 31.90 | 4011.6 | 0.0023 |
| 14 | 35.90 | 4514.4 | 0.0029 |
| 15 | 45.45 | 5716.1 | 0.0019 |
| 16 | 76.03 | 9562.0 | 0.0066 |
| 17 | 76.74 | 9650.4 | 0.7529 |
| 18 | 77.00 | 9683.3 | 1.0000 |
| 19 | 77.25 | 9714.6 | 0.8721 |
| 20 | 105.91 | 13319.4 | 0.0092 |
| 21 | 107.06 | 13463.4 | 0.0080 |
| 22 | 109.03 | 13711.3 | 0.0033 |
| 23 | 112.93 | 14202.0 | 0.0101 |
| 24 | 115.50 | 14524.5 | 0.0105 |
| 25 | 116.30 | 14625.1 | 0.0101 |
| 26 | 120.21 | 15117.6 | 0.0085 |
| 27 | 124.13 | 15610.0 | 0.0020 |
| 28 | 126.42 | 15897.8 | 0.0091 |
| 29 | 128.47 | 16156.2 | 0.0072 |
| 30 | 129.12 | 16237.7 | 0.0054 |
| 31 | 129.73 | 16314.0 | 0.0042 |
| 32 | 130.03 | 16352.1 | 0.0021 |
| 33 | 130.79 | 16447.5 | 0.0049 |
| 34 | 138.58 | 17427.2 | 0.0039 |
| 35 | 143.60 | 18058.3 | 0.0031 |
| 36 | 143.71 | 18072.2 | 0.0064 |
| 37 | 151.36 | 19034.6 | 0.0028 |
| 38 | 154.12 | 19381.3 | 0.0041 |
| 39 | 192.87 | 24255.5 | 0.0024 |
| 40 | 206.10 | 25918.4 | 0.0019 |

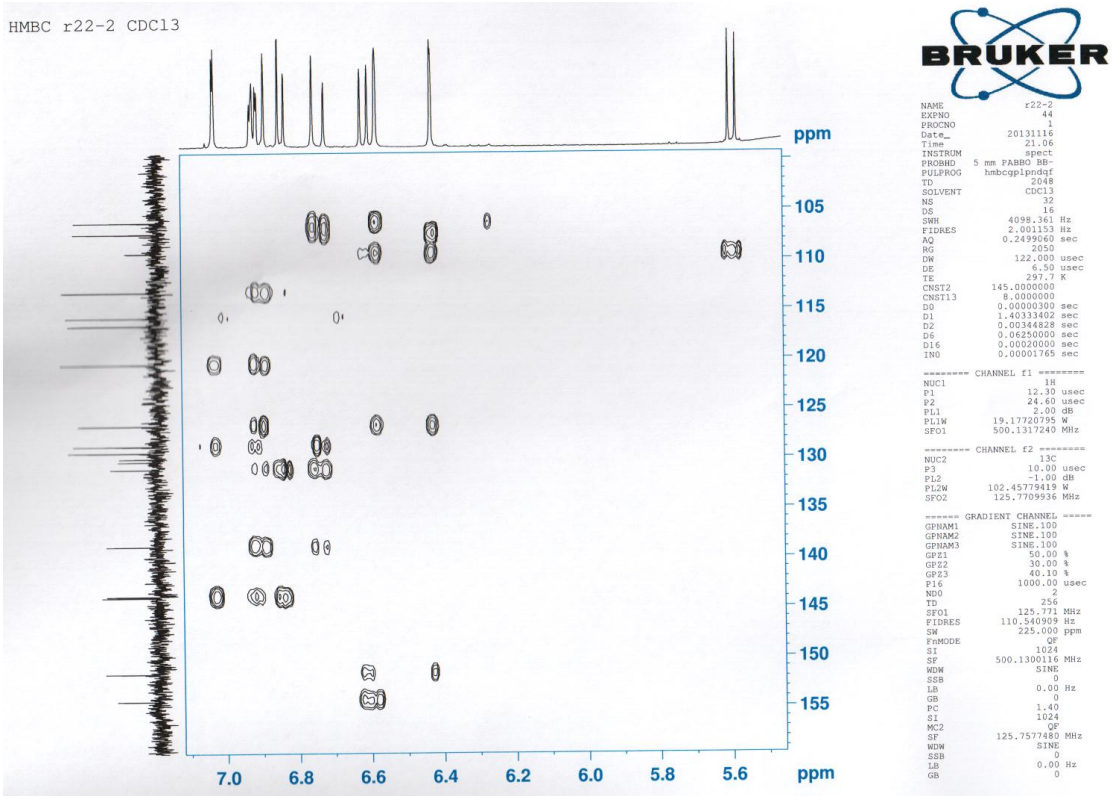
$^{13}$C-Compound II

**Figure S7.** NMR data for compound **II**: $^{13}$C.



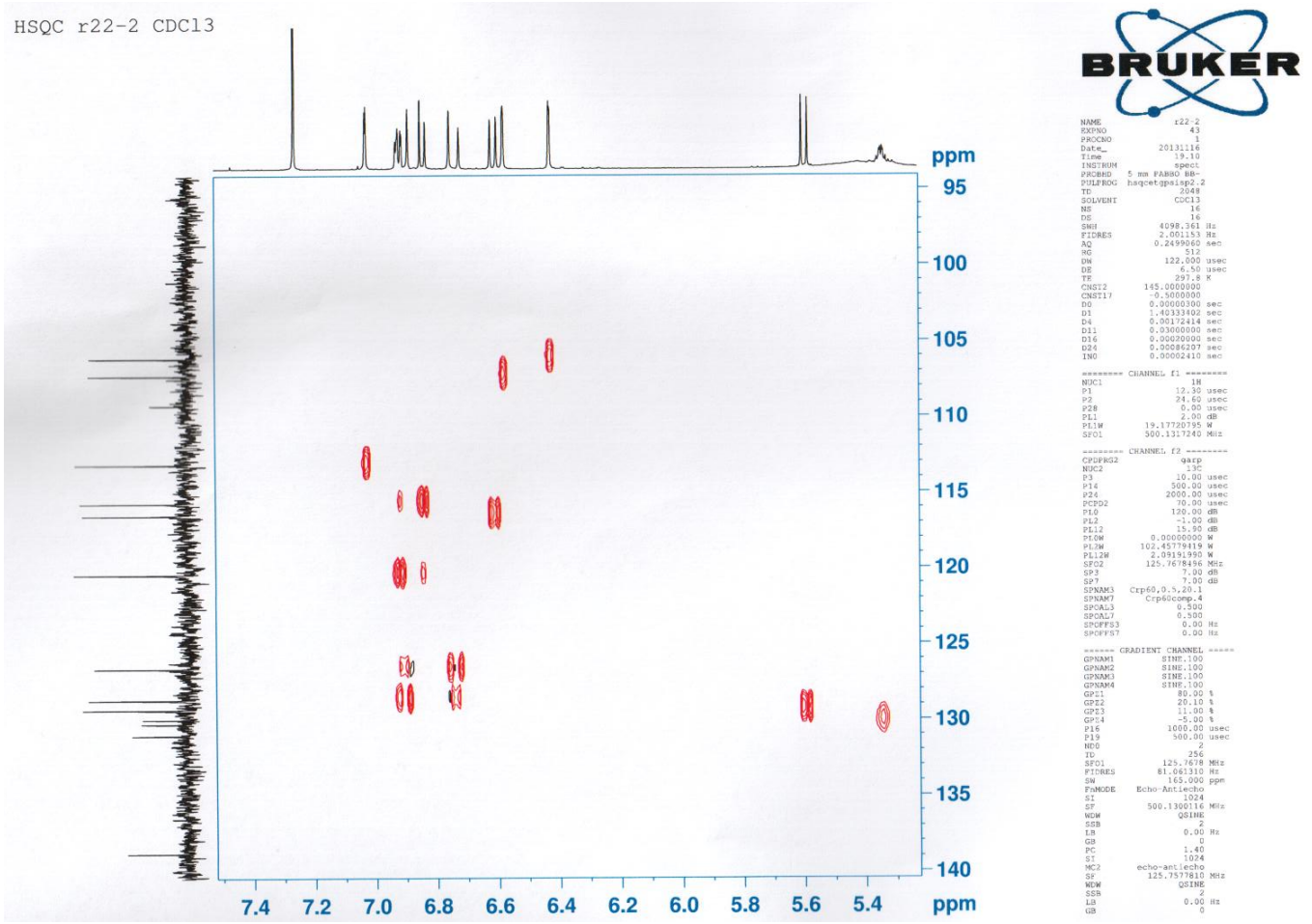

COSY Compound II

**Figure S8.** NMR data for Compound **II**: COSY.




HMBC-Compound II

**Figure S9.** NMR data for Compound **II**: HMBC.




HSQC–Compound II

**Figure S10.** NMR data for Compound **II**: HSQC.