Supporting Information

Stereocontrolled synthesis of boranophosphate DNA by an oxazaphospholidine approach and evaluation of its properties

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General Procedure for Manual Solid-Phase Synthesis of PB-DNA

PB-DNA was conducted manually using a small glass filter with a stopper at the top and a stopcock at the bottom as a reaction vessel. CPG was used as a solid support. Synthesis was conducted according to Table S1 from DMTr-T-succinyl-CPG (39 µmol/g) as the starting material.

Table S1 Standard conditions for manual solid-phase synthesis of PB-DNA

step	manipulation	reagents and solvents	time
1	detritylation	1% TFA in CH_2Cl_2	15 s × 4
2	wash	(1) CH_2Cl_2 (2) CH_3CN	---
3	drying	---	5 min
4	condensation	monomer units (0.2 M), CMPT (1.0 M) /CH_3CN	5 min
5	wash	(1) CH_3CN (2) CH_2Cl_2	---
6	drying	---	5 min
7	removal of DMTr, auxiliary and PRO	1% TFA in CH_2Cl_2-Et_3SiH (1:1, v/v)	30 s × 4
8	wash	(1) CH_2Cl_2 (2) CH_3CN	---
9	drying	---	5 min
repeat steps 4-9 in order to synthesize the objective sequence			
10	boronation	$BH_3 \cdot SMe_2$-BSA-DMAc (1:1:8, v/v/v)	15 min
11	wash	(1) DMAc (2) CH_3CN	---
12	release from the CPG	conc NH_3aq-EtOH (3:1, v/v, 25 ℃ or 55 ℃)	3 h or 12 h

Conditions for UV Melting Analyses

The absorbance-versus-temperature profiles were measured using an eight-sample cell changer, in quartz cells of 1 cm path length. All experiments were conducted in a 10 mM phosphate buffer containing 100 mM NaCl at pH 7.0. The UV absorbance at 260 and 320 nm was monitored with temperature. Samples containing oligonucleotides were first rapidly heated to 95 °C followed by cooling down to 0 °C at a rate of 0.5 °C/min. Dissociation was recorded by heating to 95 °C at a rate of 0.2 °C/min.

SVPDE assay

In the SVPDE assay, phosphodiesterase I from *Crotalus adamanteus* venom was used. SVPDE solution (2.0×10^{-3} in 45 µL) and 200 mM Tris-HCl buffer containing 30 mM $MgCl_2$ (50 µL) was added (pH 8.5, 37 °C) to 0.1 mM aqueous solution of PO-, PB-, or PS-DNA (5 µL). After 12 h, the solution was diluted with 0.1 M triethylammonium acetate buffer (80 µL) acetonitrile (20 µL), and heated to 100 °C for 1 h. The mixture was then analyzed using RP-HPLC.

nP1 assay

In the nP1 assay, nP1 from *Penicillium citrinum* was used. nP1 solution (1 unit in 45 μL) and 200 mM Tris-HCl buffer containing 2 mM mM ZnCl (50 μL) was added (pH 7.5, 37 °C) to 0.1 mM aqueous solution of PO-, PB-, or PS-DNA (5 μL). After 12 h, the solution was diluted with 0.1 M triethylammonium acetate buffer (80 μL) and acetonitrile (20 μL), and heated to 100 °C for 1 h. The mixture was then analyzed using RP-HPLC.

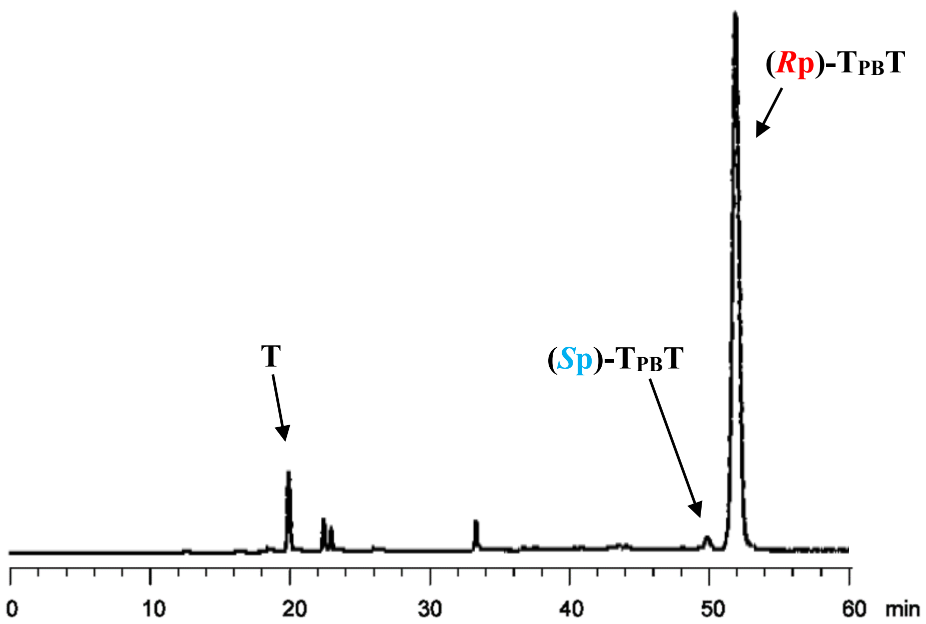

Figure S1 HPLC profile at 260nm of crude (*R*p)-T$_{PB}$T (Table 2, Entry 1). RP-HPLC was performed with a linear gradient of 0–10% acetonitrile for 30 min then 10% acetonitrile for 30 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 × 150 mm) (Waters).

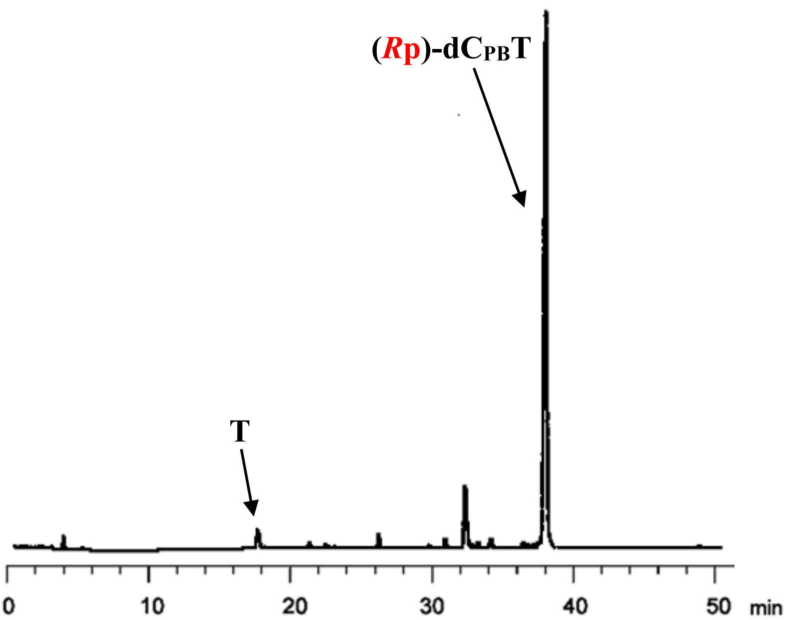

Figure S2 HPLC profile at 260 nm of crude (*R*p)-dC$_{PB}$T (Table 2, Entry 2). RP-HPLC was performed with a linear gradient of 0–10% acetonitrile for 30 min then 10% acetonitrile for 20 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 × 150 mm) (Waters).

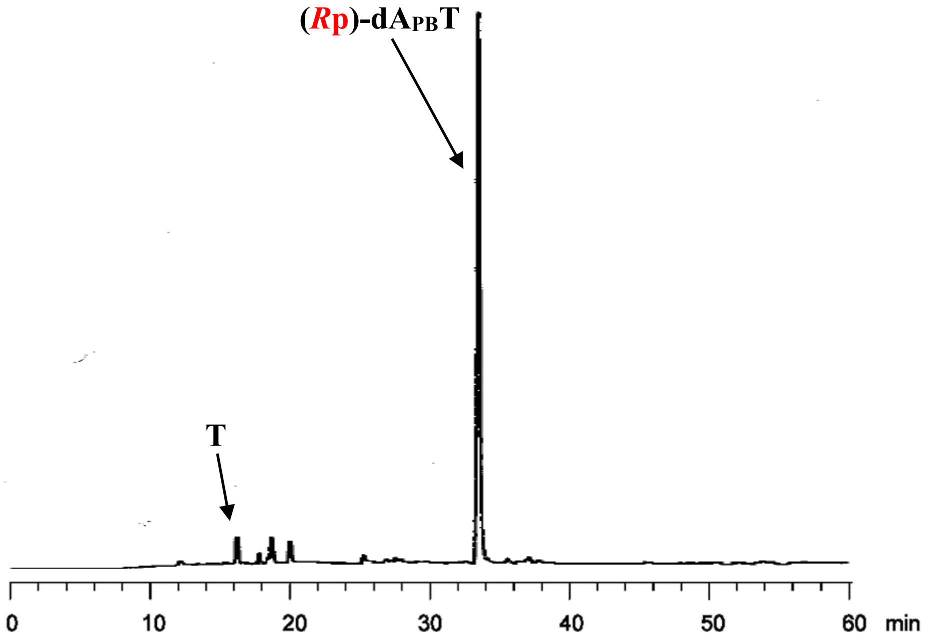

Figure S3 HPLC profile at 260 nm of crude (*R*p)-dA$_{PB}$T (Table 2, Entry 3). RP-HPLC was performed with a linear gradient of 0–30% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 mm × 150 mm) (Waters).

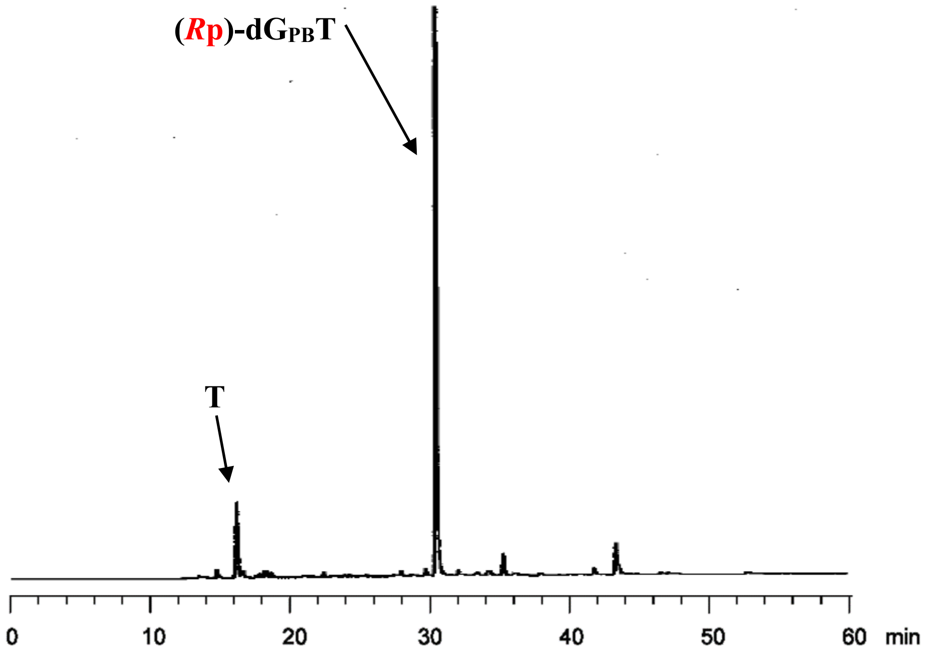

Figure S4 HPLC profile of crude (*R*p)-dG$_{PB}$T (Table 2, Entry 4). RP-HPLC was performed with a linear gradient of 0–30% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 mm × 150 mm) (Waters).

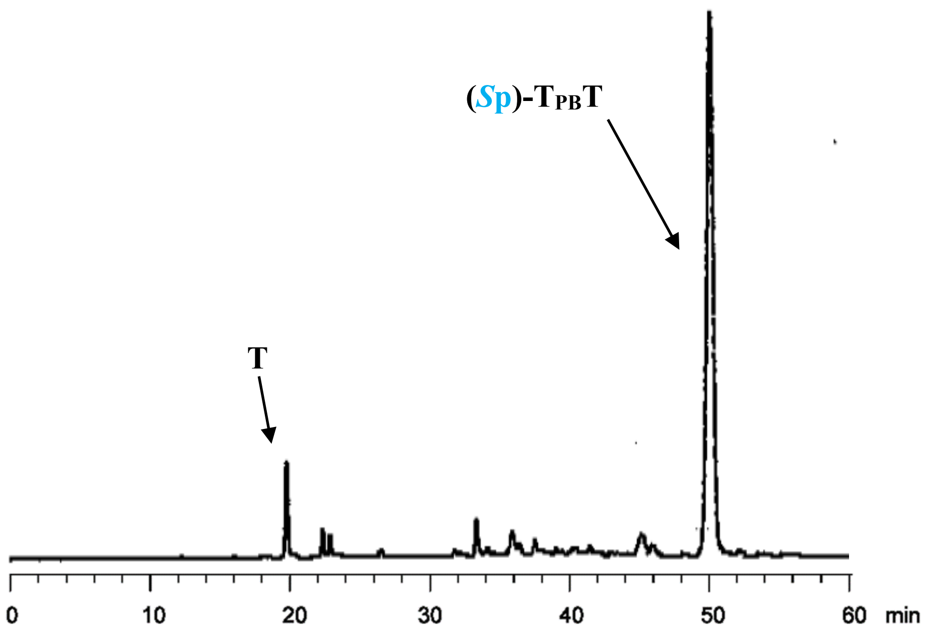

Figure S5 HPLC profile at 260 nm of crude (*Sp*)-T$_{PB}$T (Table 2, Entry 5). RP-HPLC was performed with a linear gradient of 0–10% acetonitrile for 30 min then 10% acetonitrile for 30 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 × 150 mm) (Waters).

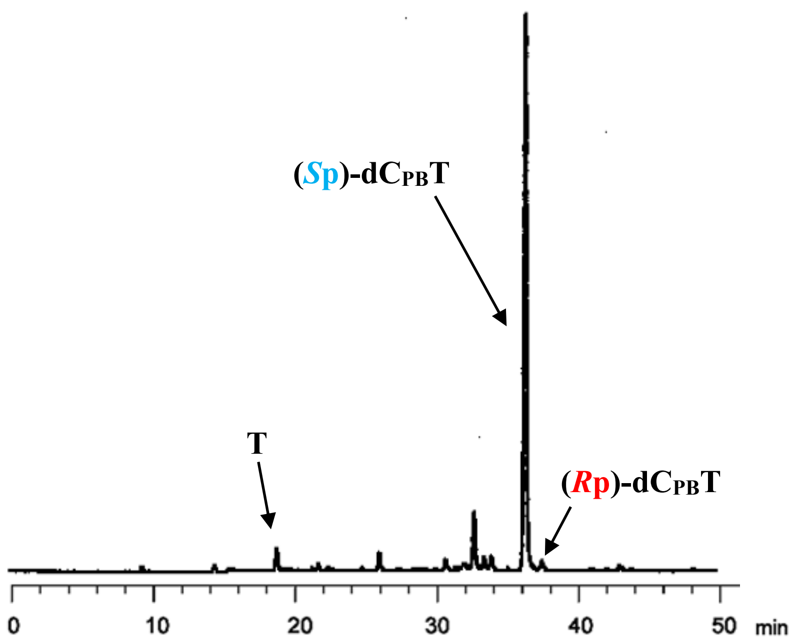

Figure S6 HPLC profile at 260 nm of crude (*Sp*)-dC$_{PB}$T (Table 2, Entry 6). RP-HPLC was performed with a linear gradient of 0–10% acetonitrile for 30 min then 10% acetonitrile for 20 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 × 150 mm) (Waters).

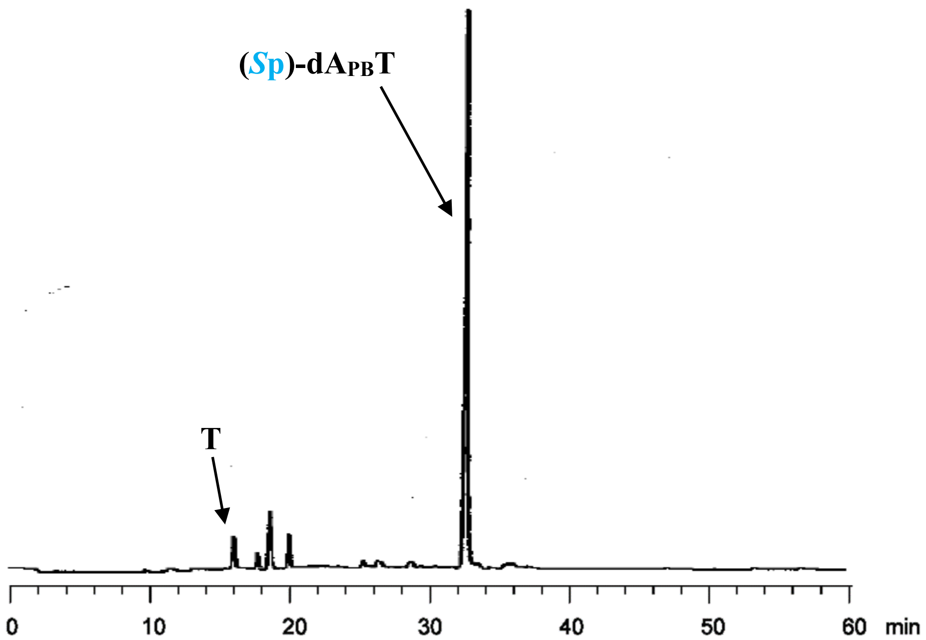

Figure S7 HPLC profile of at 260 nm crude (*Sp*)-dA$_{PB}$T (Table 2, Entry 7). RP-HPLC was performed with a linear gradient of 0–30% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 mm × 150 mm) (Waters).

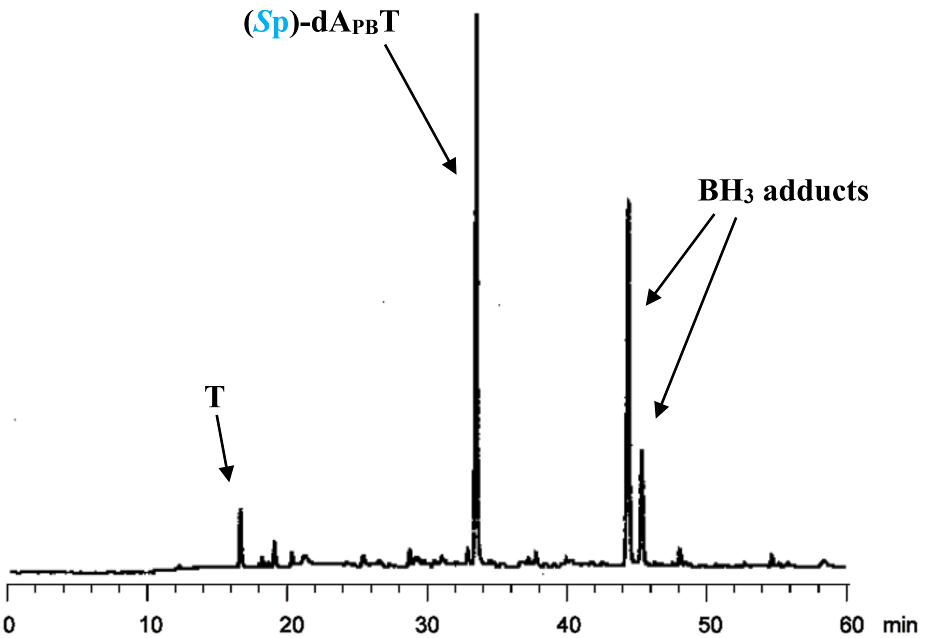

Figure S8 HPLC profile of at 260 nm crude (*Sp*)-dA$_{PB}$T after ammonia treatment for 25 °C, 3 h. RP-HPLC was performed with a linear gradient of 0–30% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 mm × 150 mm) (Waters)

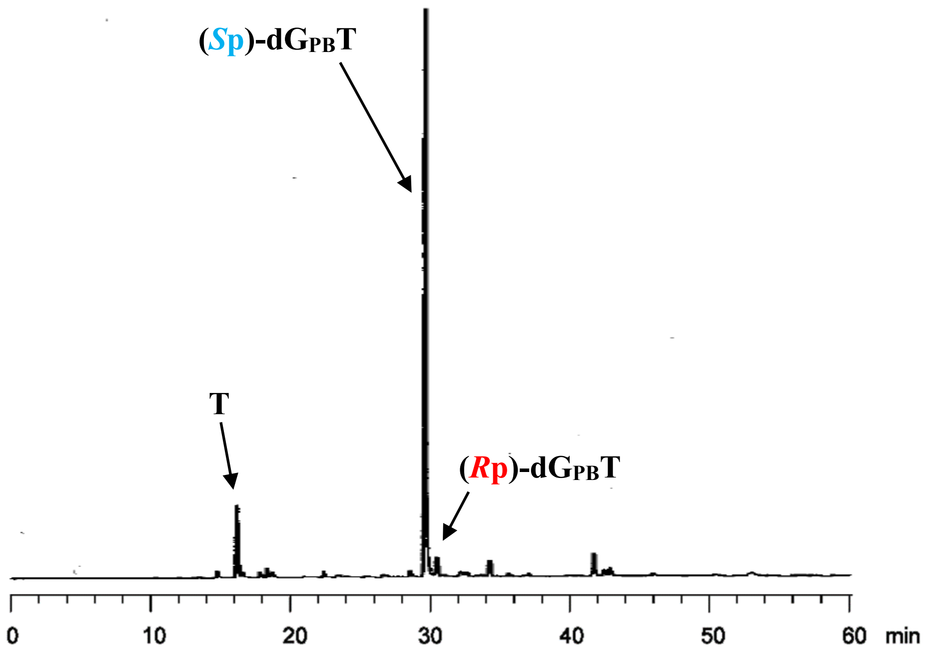

Figure S9 HPLC profile at 260 nm of crude (*S*p)-dG$_{PB}$T (Table 2, Entry 8). RP-HPLC was performed with a linear gradient of 0–30% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 mm × 150 mm) (Waters).

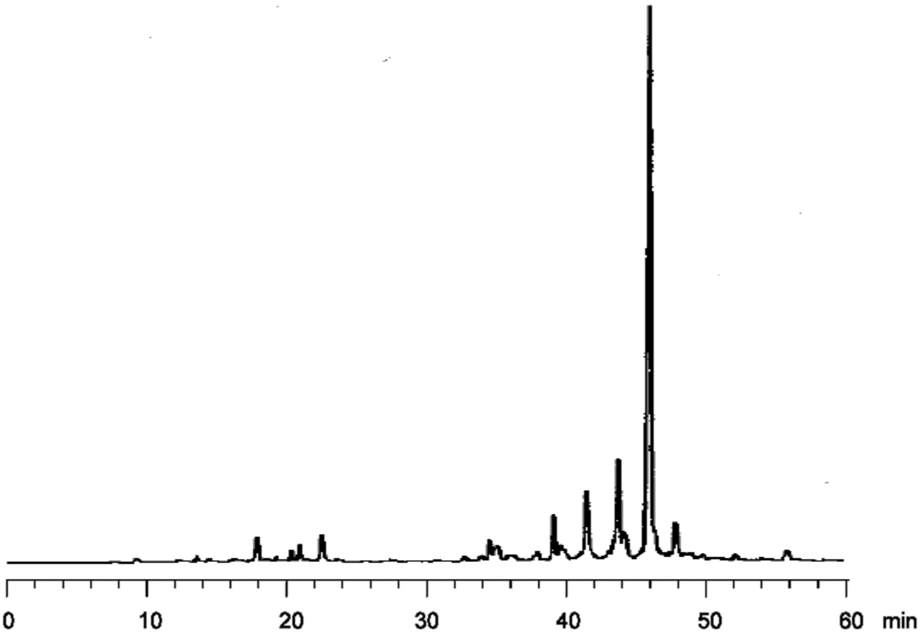

Figure S10 HPLC profile at 260 nm of crude all-(*R*p)-d($C_{PB}A_{PB}G_{PB}T$). RP-HPLC was performed with a linear gradient of 0–20% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 mm × 150 mm) (Waters).

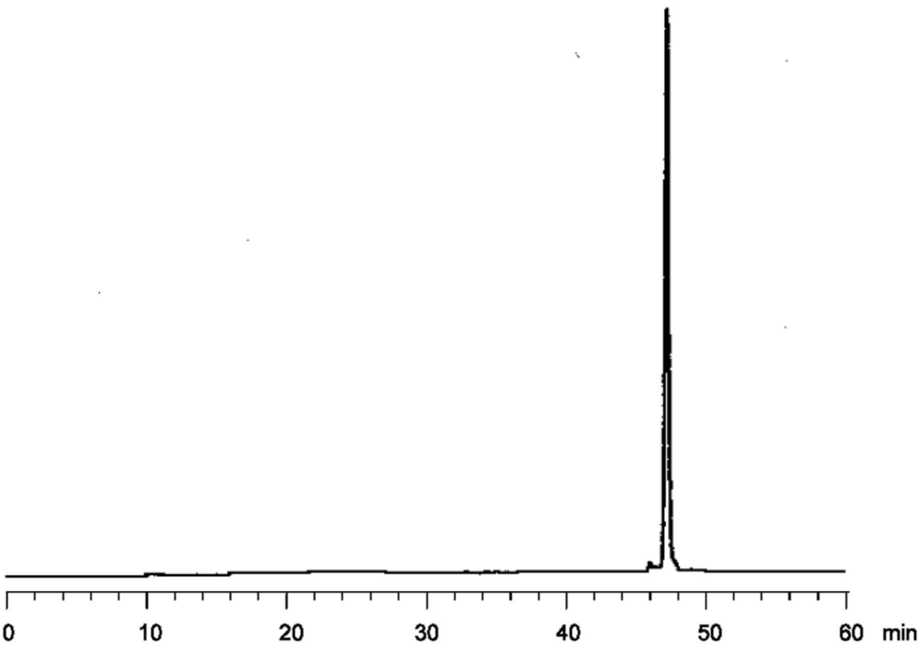

Figure S11 HPLC profile at 260 nm of purified all-(*R*p)-d($C_{PB}A_{PB}G_{PB}T$). RP-HPLC was performed with a linear gradient of 0–20% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 mm × 150 mm) (Waters).

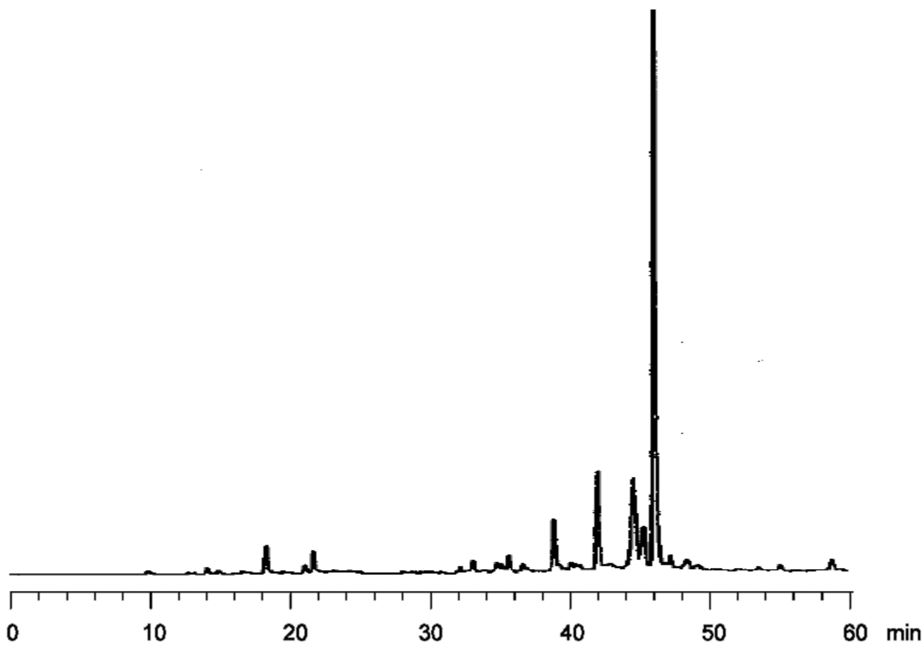

Figure S12 HPLC profile at 260 nm of crude all-(*Sp*)-d($C_{PB}A_{PB}G_{PB}T$). RP-HPLC was performed with a linear gradient of 0–20% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 mm × 150 mm) (Waters).

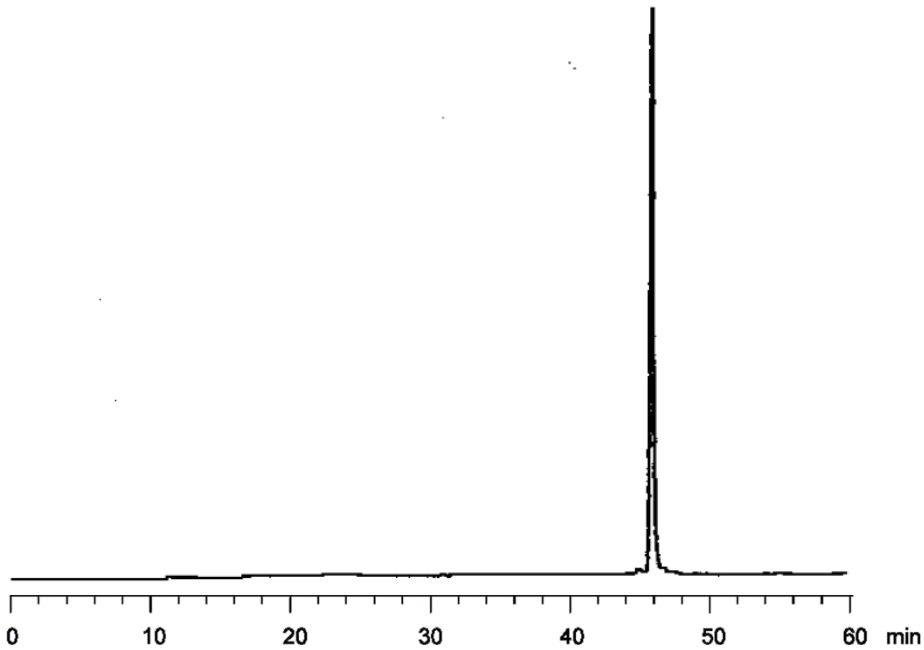

Figure S13 HPLC profile at 260 nm of purified all-(*Sp*)-d($C_{PB}A_{PB}G_{PB}T$). RP-HPLC was performed with a linear gradient of 0–20% acetonitrile for 60 min in 0.1 M triethylammonium acetate buffer (pH 7.0) at 30 °C at a flow rate of 0.5 mL/min using a μBondasphere 5 μm C18 column (100 Å, 3.9 mm × 150 mm) (Waters).

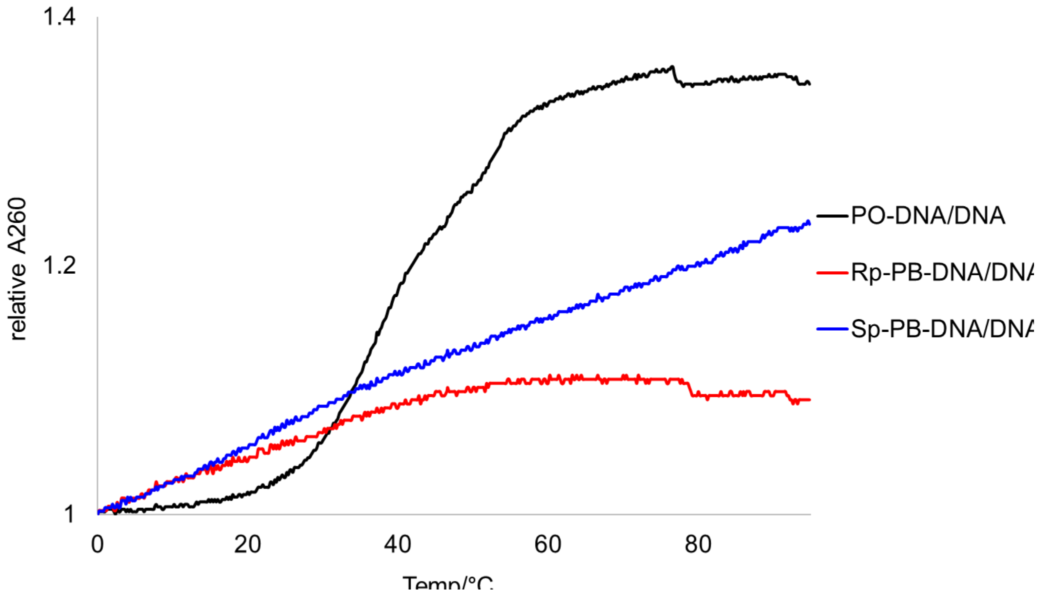

Figure S14 UV melting curves of the duplexes of PO- or PB- DNA 12mer and their complementary RNA 12mer.

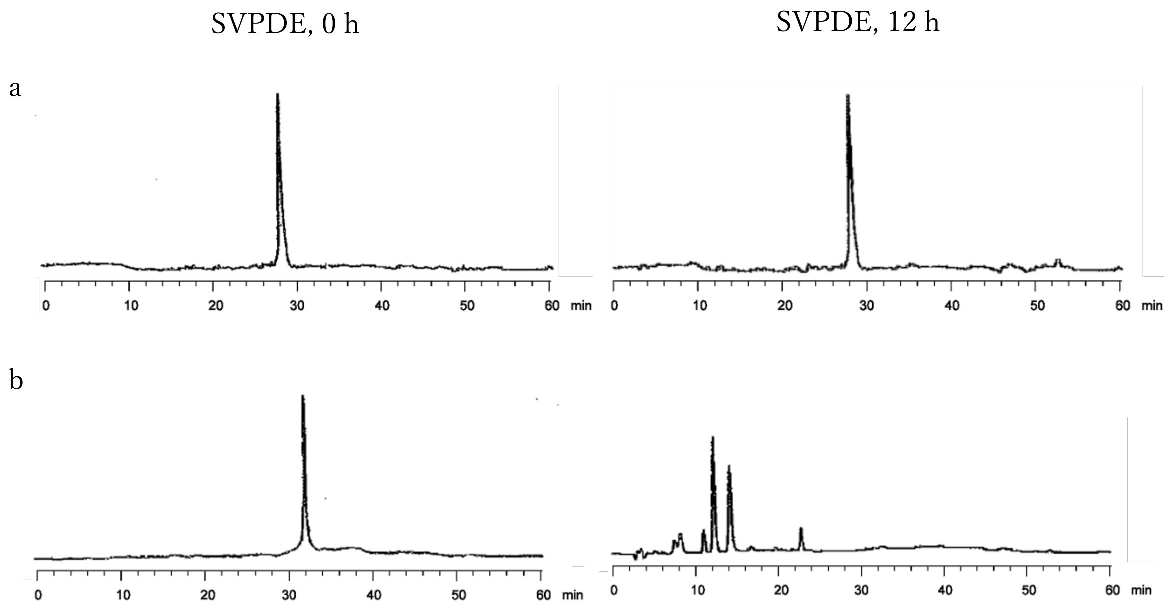

Figure S15 RP-HPLC profiles of DNA 12mers after treatment with SVPDE: (a) all-(*R*p)-PS-DNA 12mer (b) all-(*S*p)-PS-DNA 12mer

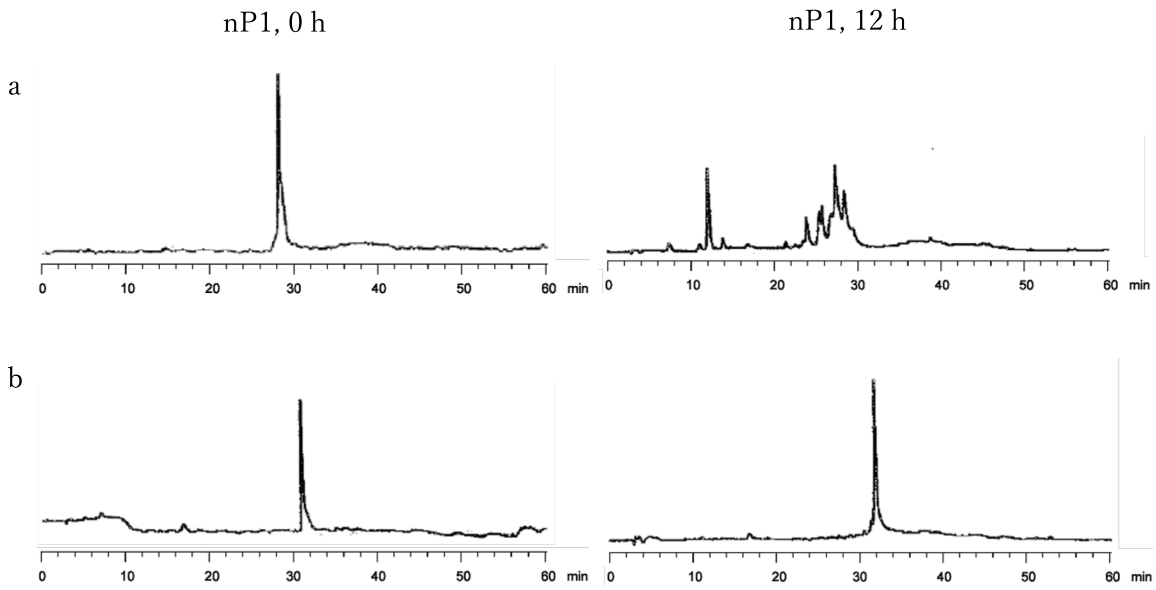

Figure S16 RP-HPLC profiles of DNA 12mers after treatment with nP1: (a) all-(*R*p)-PS-DNA 12mer (b) all-(*S*p)-PS-DNA 12mer

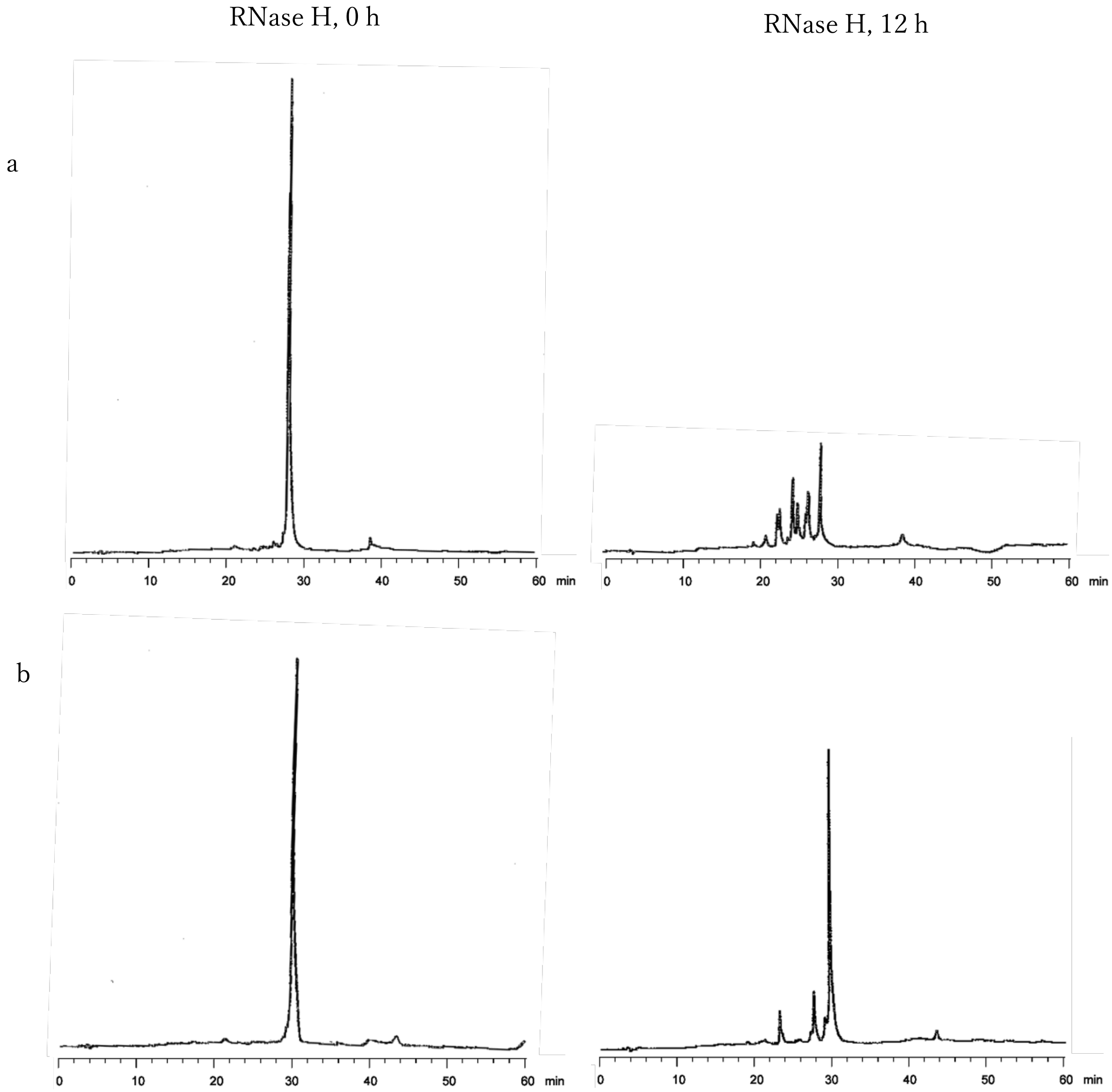

Figure S17 RP-HPLC profiles of RNA 12mers after treatment with nP1 in the presence of (a) all-(*R*p)-PS-DNA 12mer (c) all-(*S*p)-PS-DNA 12mer.

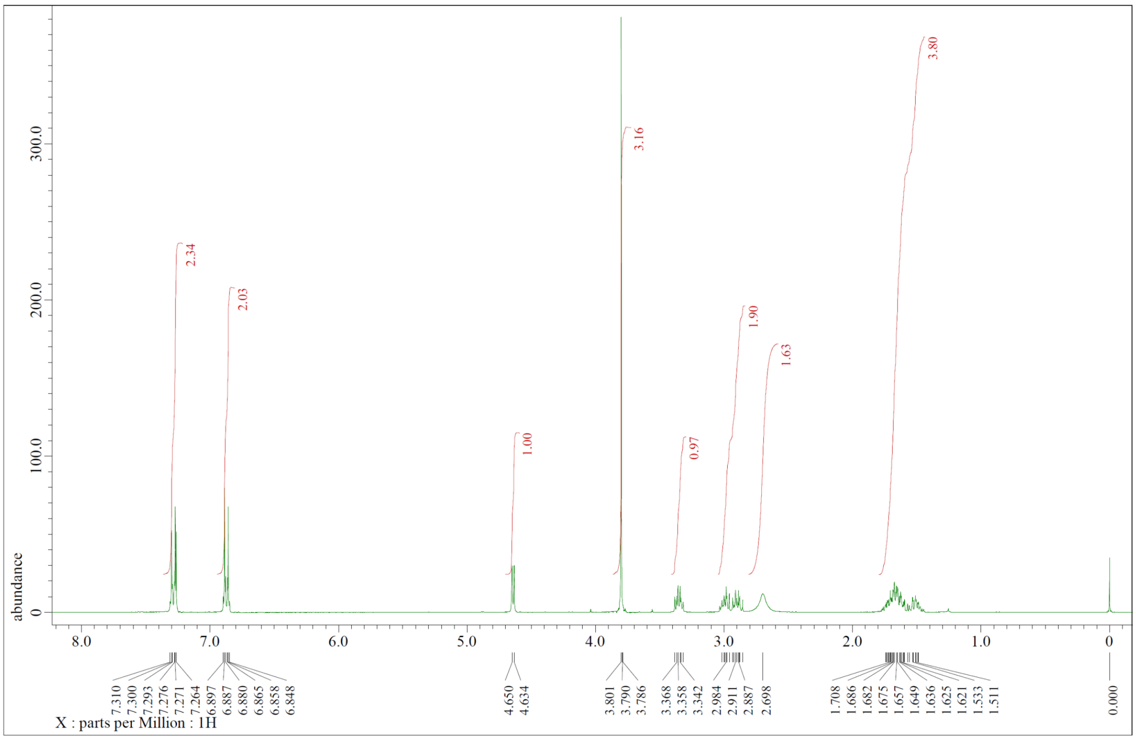

Figure S18 ^{1}H NMR spectra of (α*R*,2*S*)-**7** ($CDCl_3$, 300 MHz)

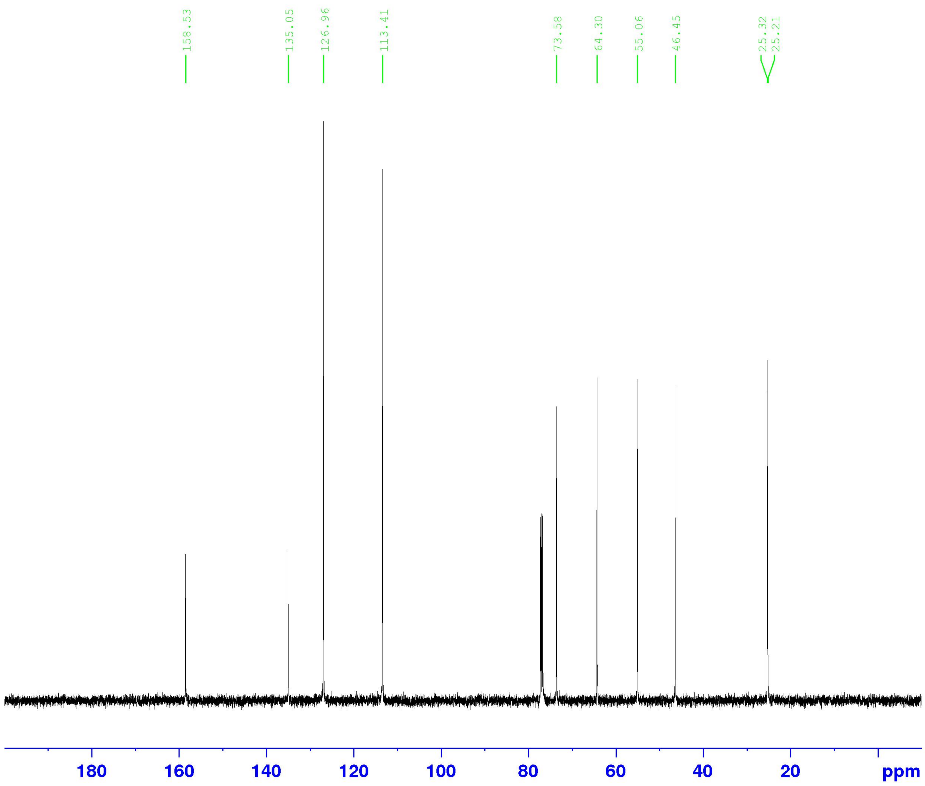

Figure S19 $^{13}C\{^{1}H\}$ NMR spectra of (α*R*,2*S*)-**7** ($CDCl_3$, 125 MHz)

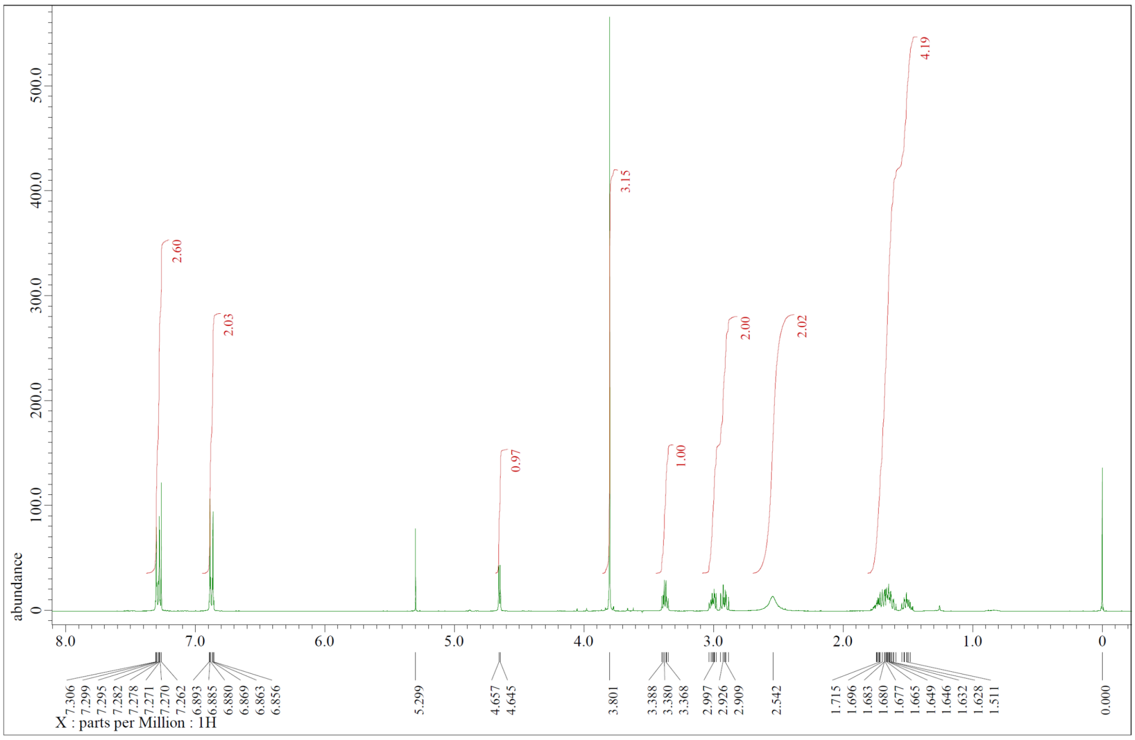

Figure S20 ^{1}H NMR spectra of (α*S*,2*R*)-**7** ($CDCl_3$, 300 MHz)

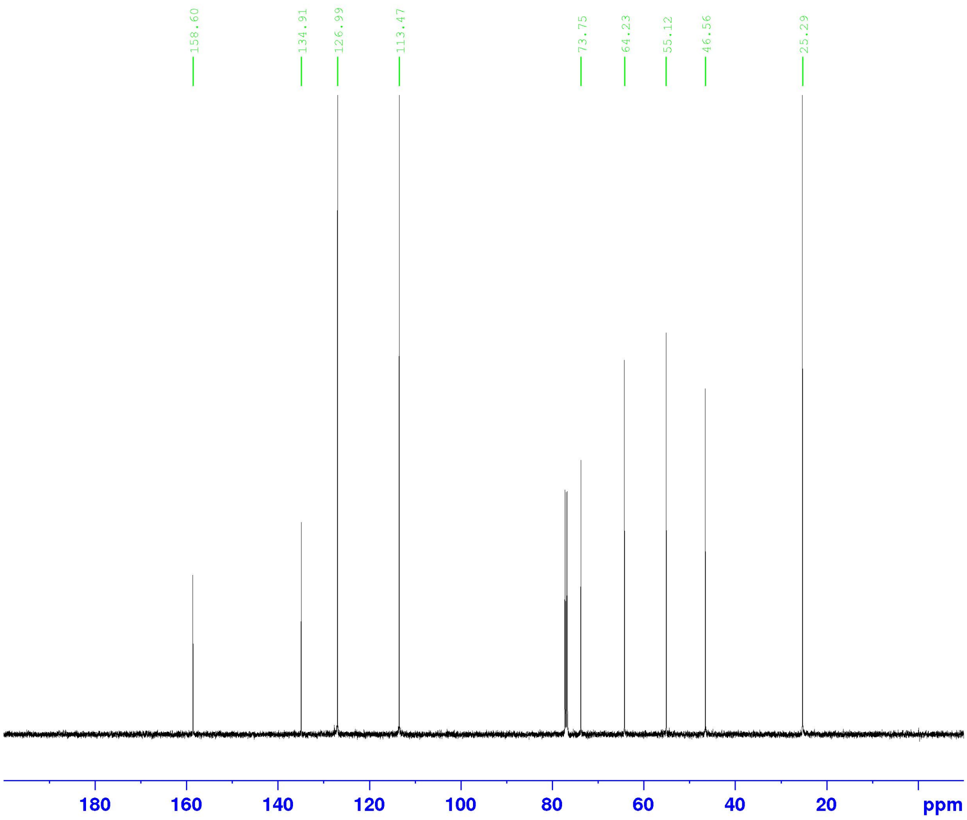

Figure S21 $^{13}C\{^{1}H\}$ NMR spectra of (α*S*,2*R*)-**7** ($CDCl_3$, 125 MHz)

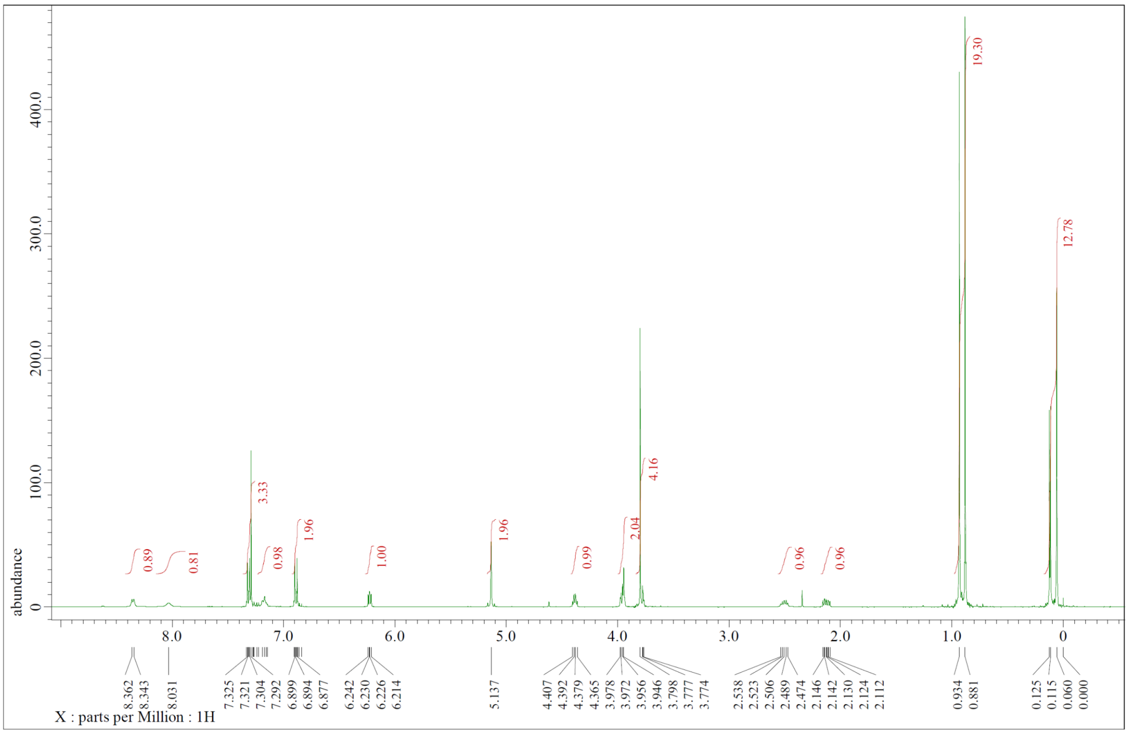

Figure S22 ^{1}H NMR spectra of **9** ($CDCl_3$, 300 MHz)

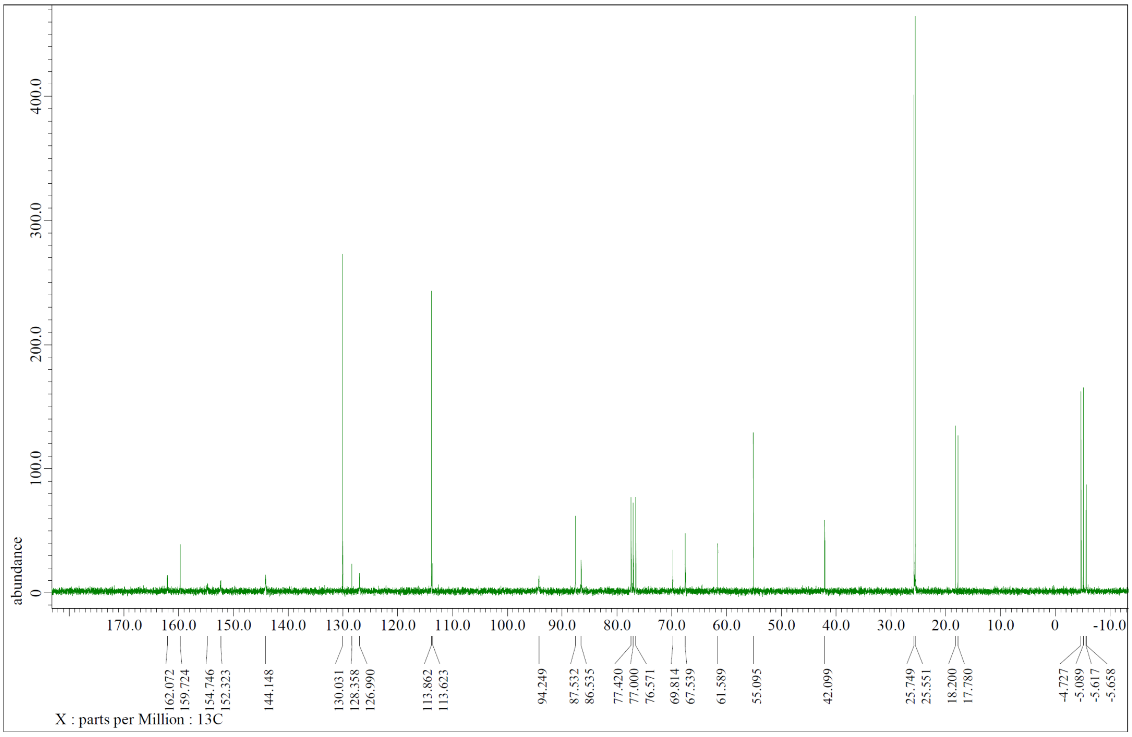

Figure S23 $^{13}C\{^{1}H\}$ NMR spectra of **9** ($CDCl_3$, 75 MHz)

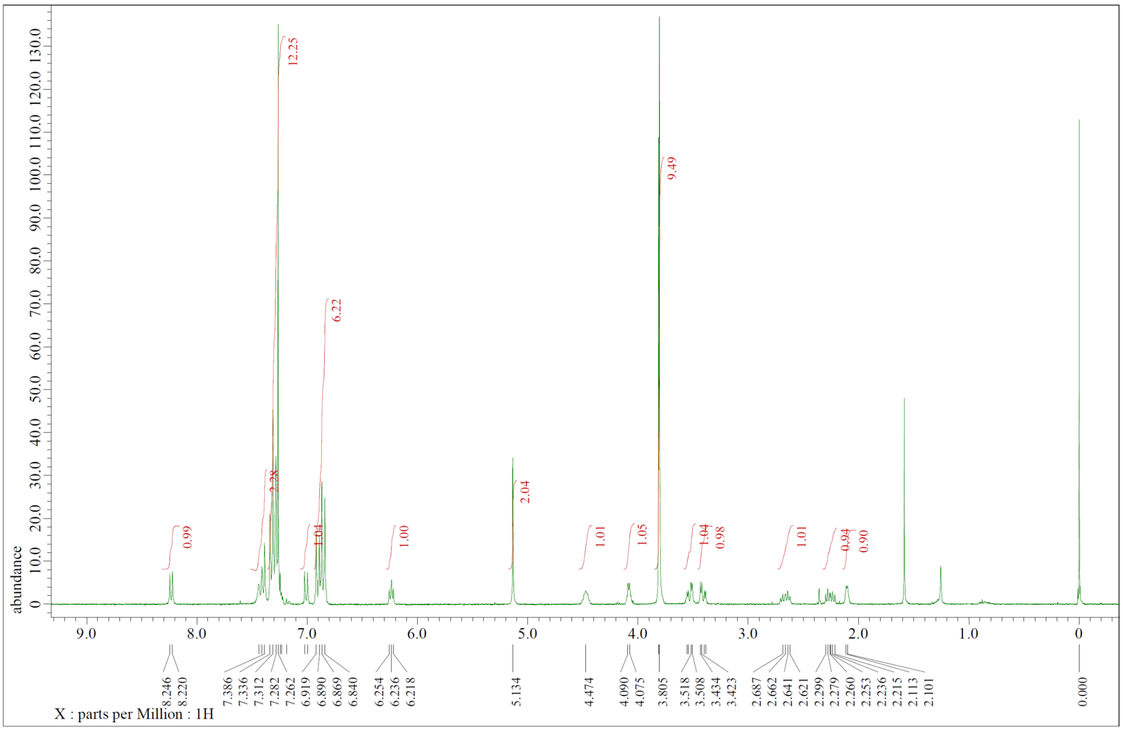

Figure S24 ^{1}H NMR spectra of **10** ($CDCl_3$, 300 MHz)

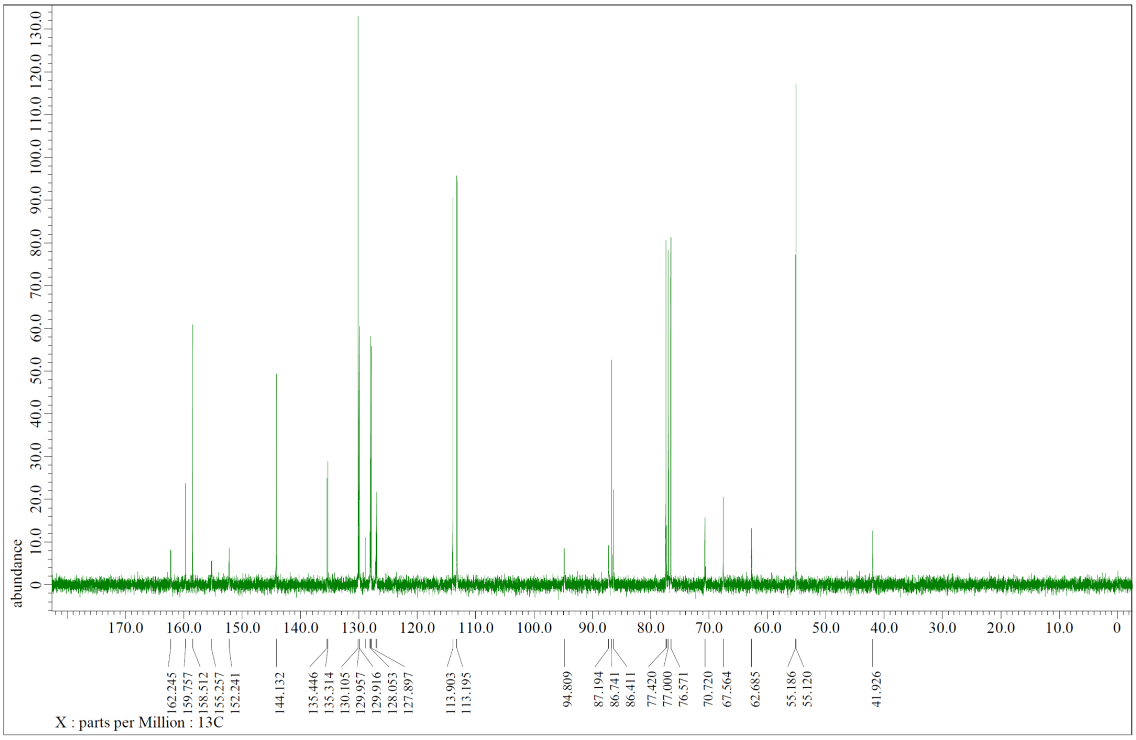

Figure S25 $^{13}C\{^{1}H\}$ NMR spectra of **10** ($CDCl_3$, 75 MHz)

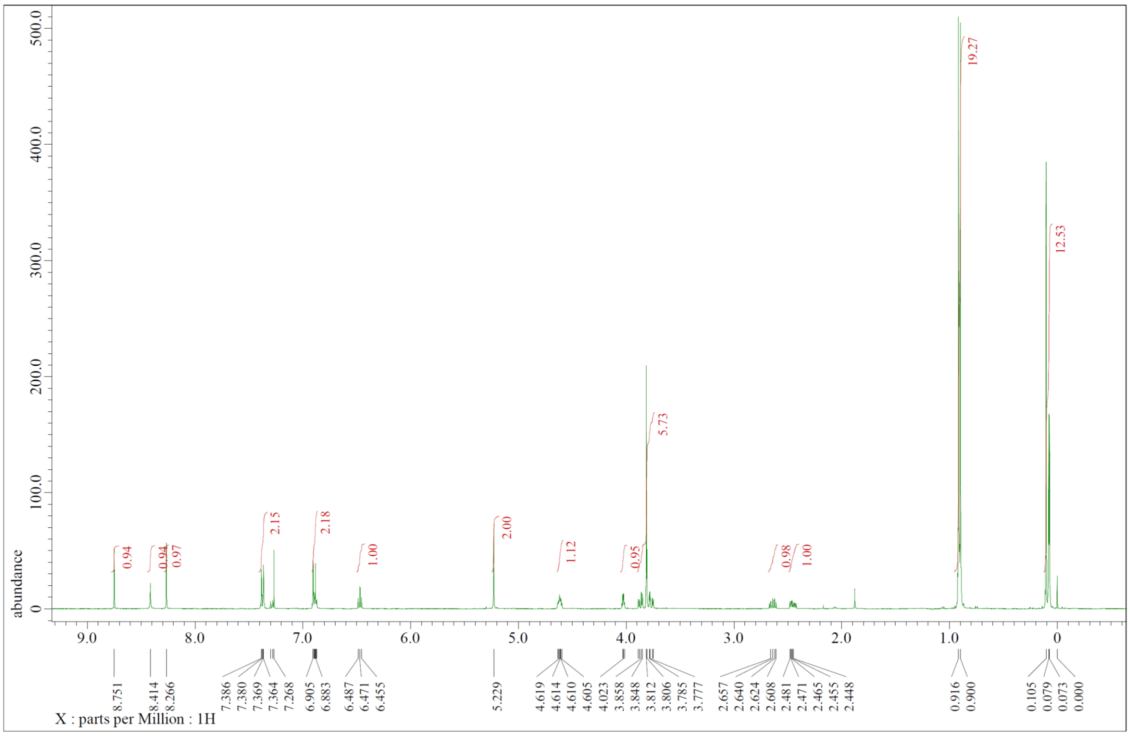

Figure S26 ^{1}H NMR spectra of **12** ($CDCl_3$, 300 MHz)

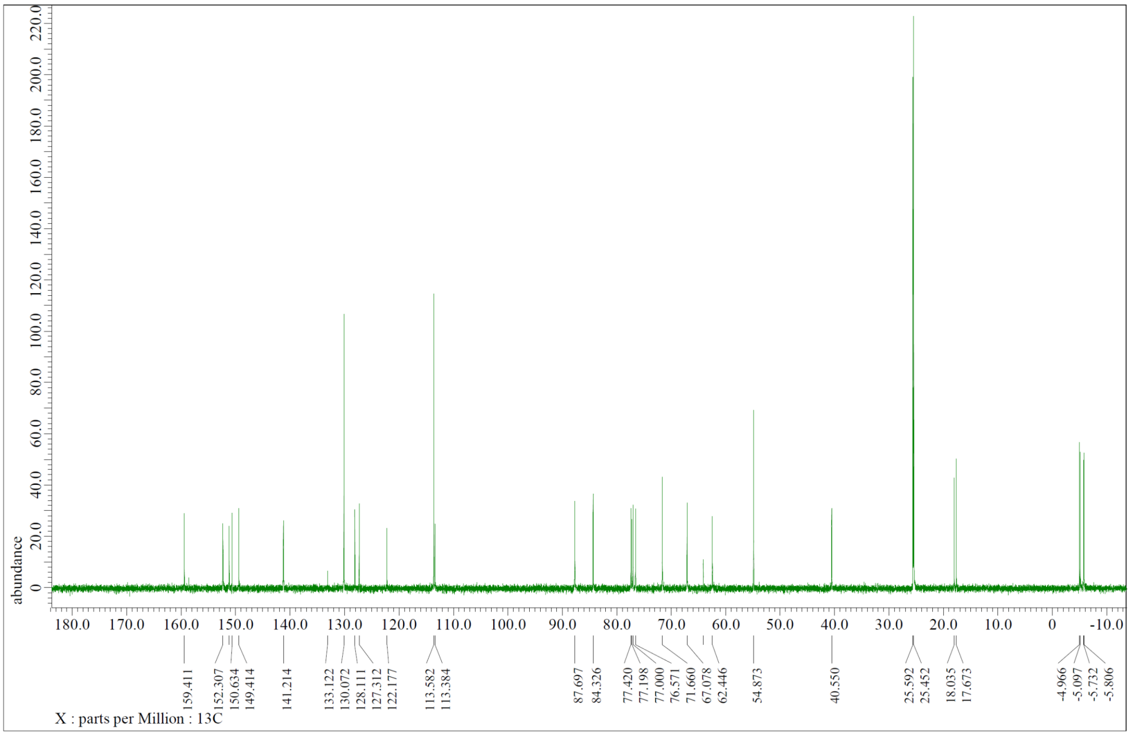

Figure S27 $^{13}C\{^{1}H\}$ NMR spectra of **12** ($CDCl_3$, 75 MHz)

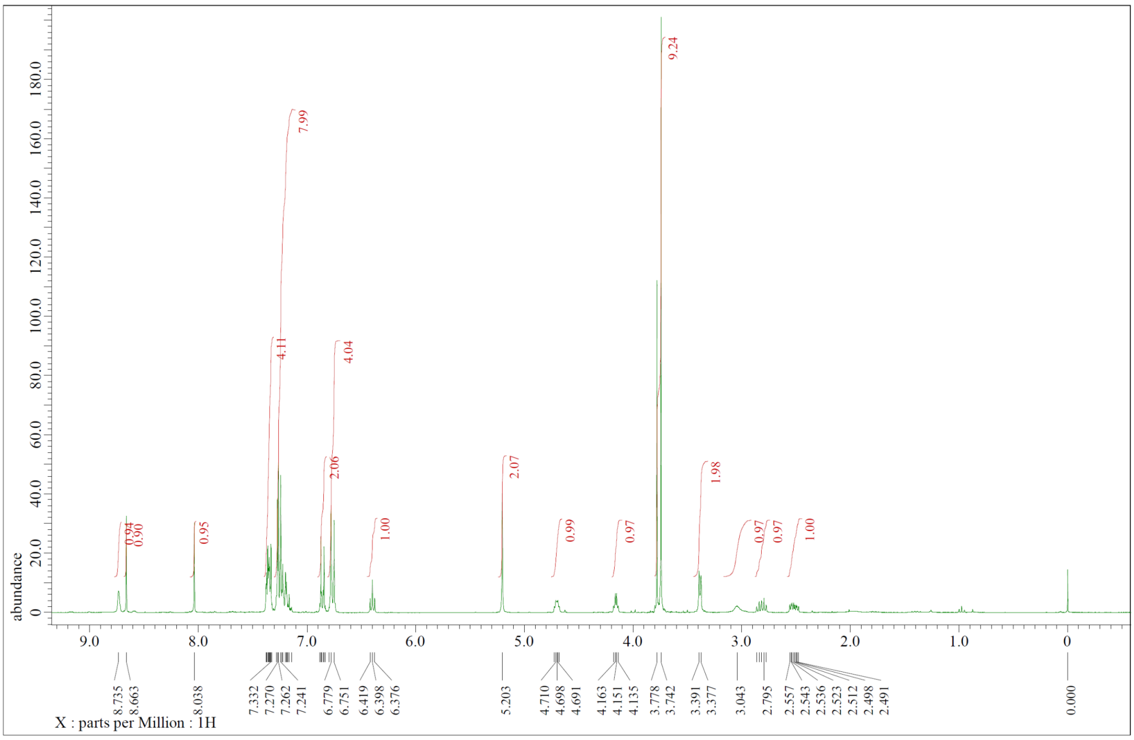

Figure S28 ^{1}H NMR spectra of **13** ($CDCl_3$, 300 MHz)

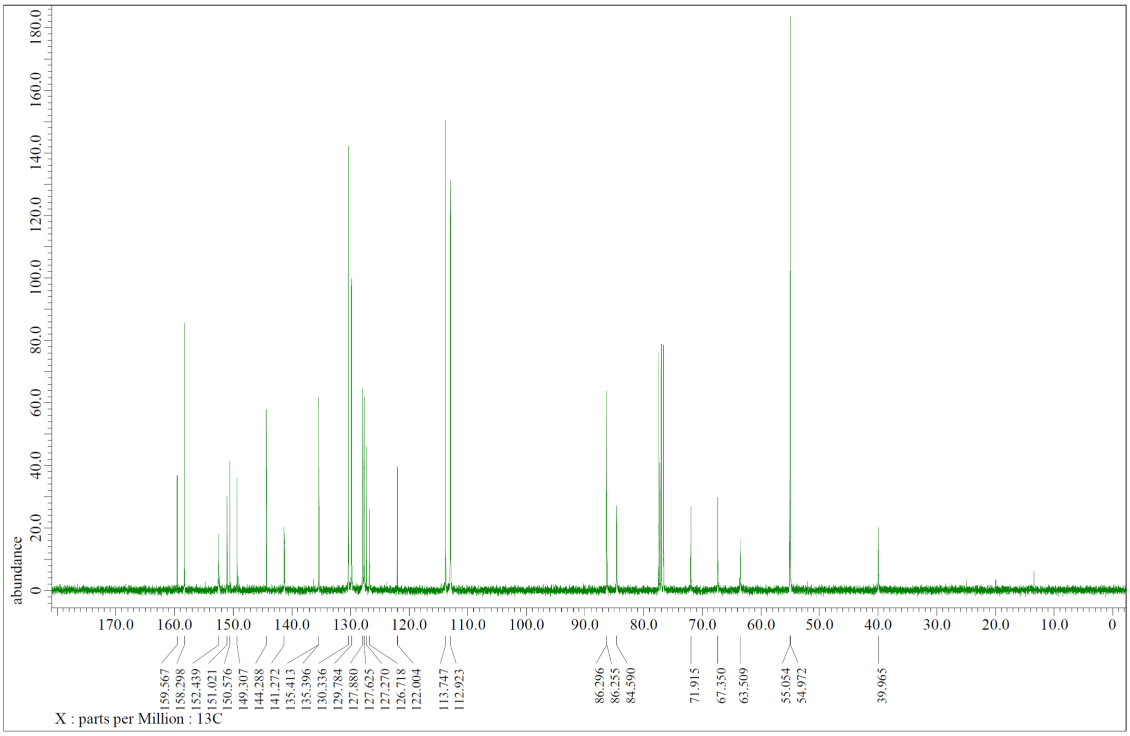

Figure S29 $^{13}C\{^{1}H\}$ NMR spectra of **13** ($CDCl_3$, 75 MHz)

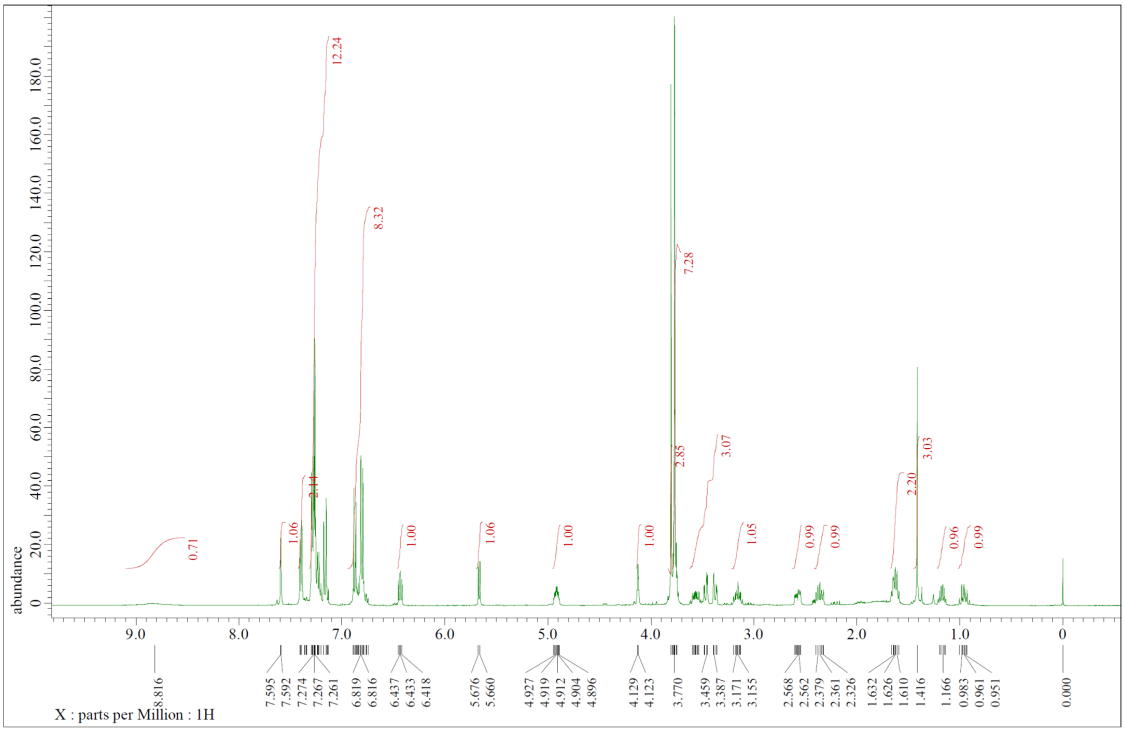

Figure S30 ^{1}H NMR spectra of (*R*p)-**15** ($CDCl_3$, 400 MHz)

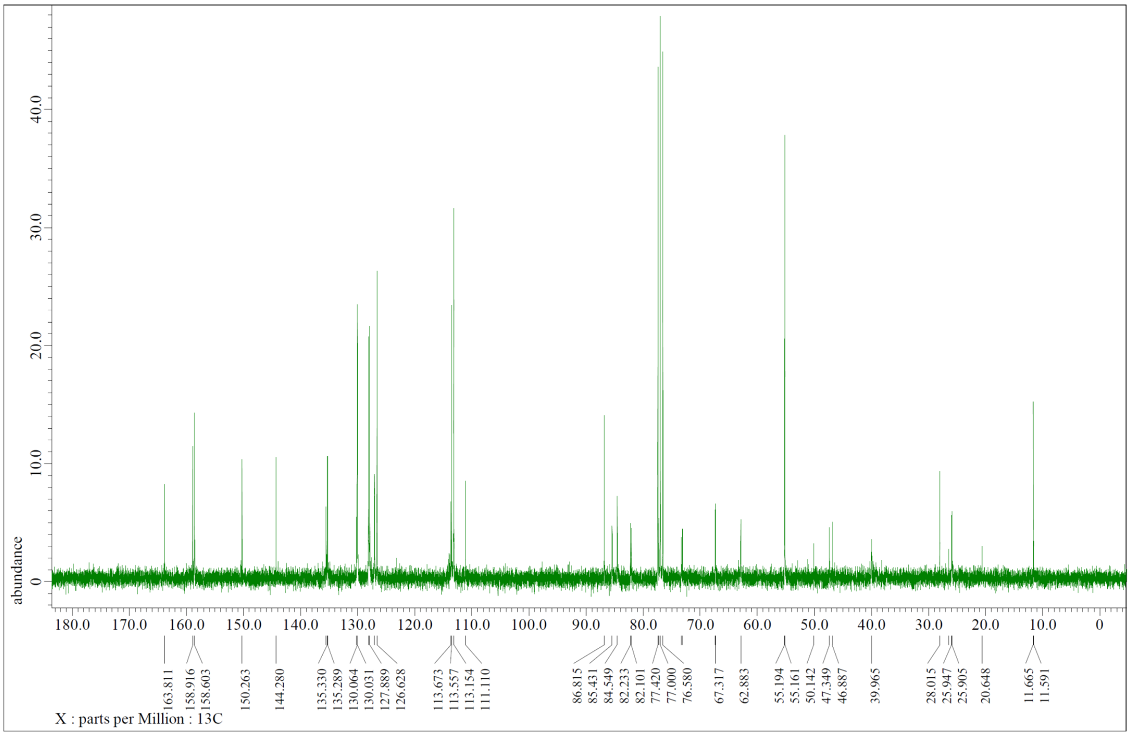

Figure S31 $^{13}C\{^{1}H\}$ NMR spectra of (*R*p)-**15** ($CDCl_3$, 75 MHz)

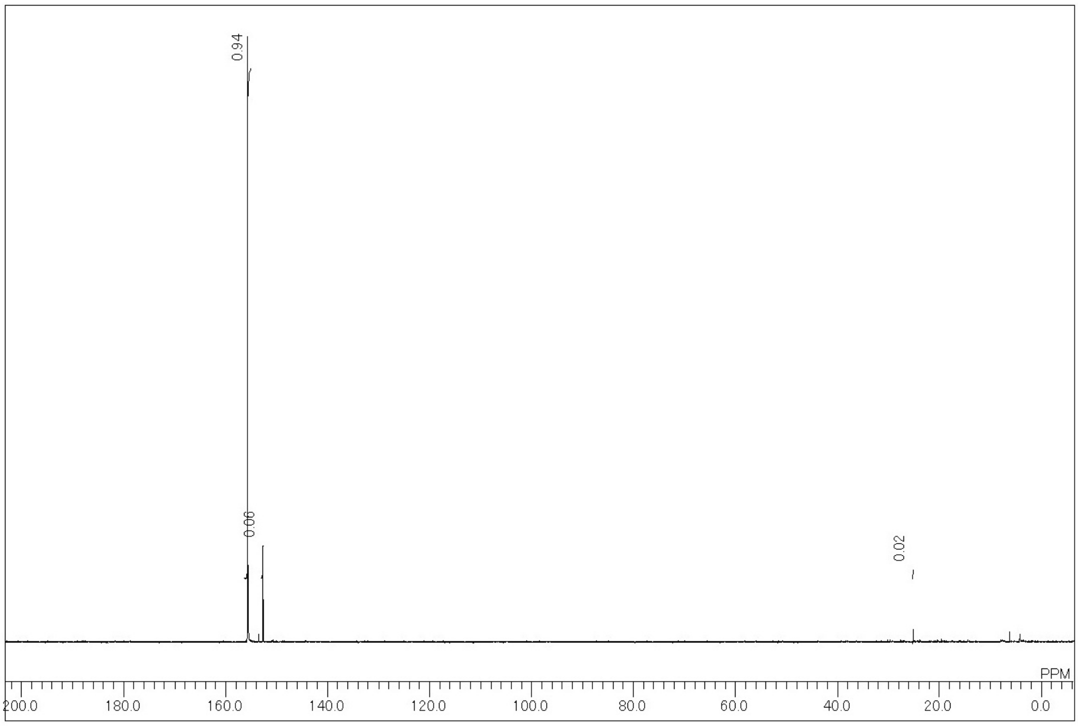

Figure S32 $^{31}P\{^{1}H\}$ NMR spectra of (*R*p)-**15** ($CDCl_3$, 162 MHz)

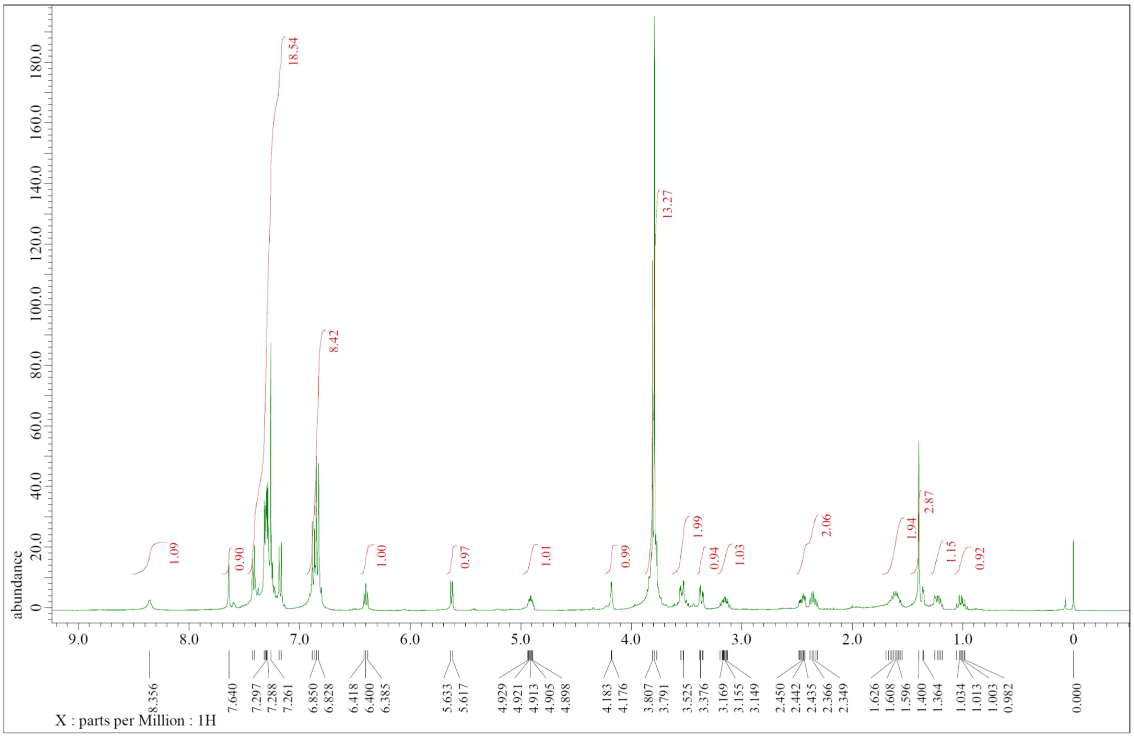

Figure S33 ^{1}H NMR spectra of (*S*p)-**15** ($CDCl_3$, 400 MHz)

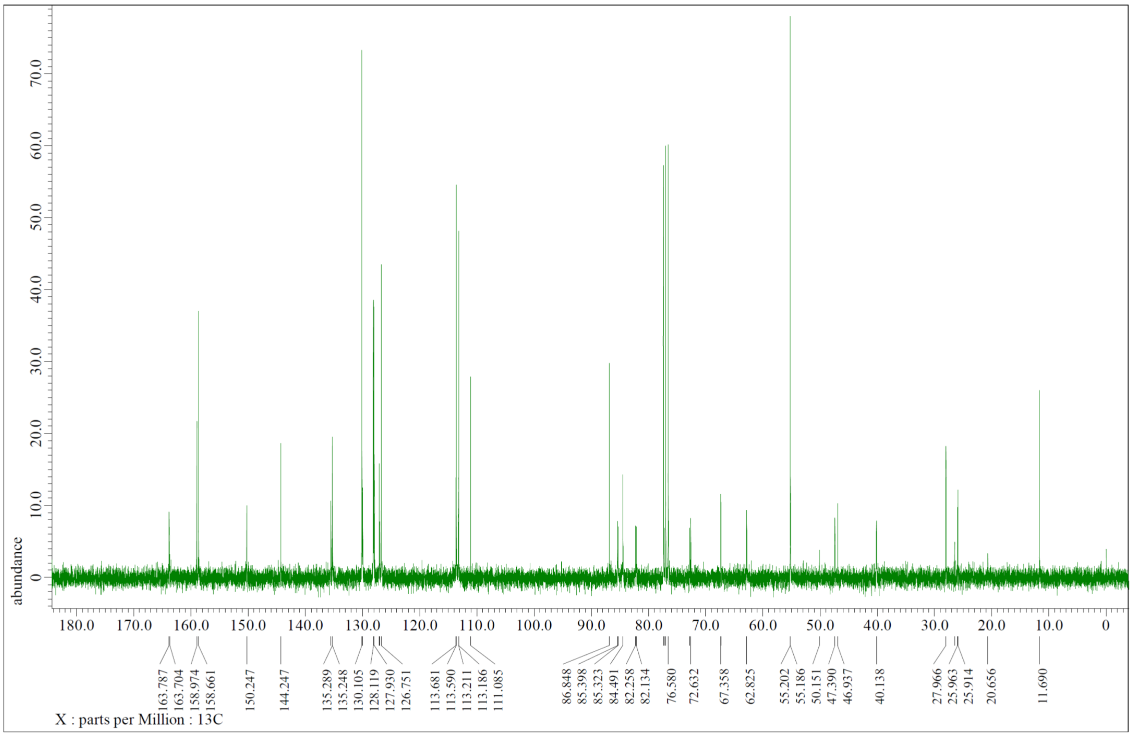

Figure S34 $^{13}C\{^{1}H\}$ NMR spectra of (*S*p)-**15** ($CDCl_3$, 75 MHz)

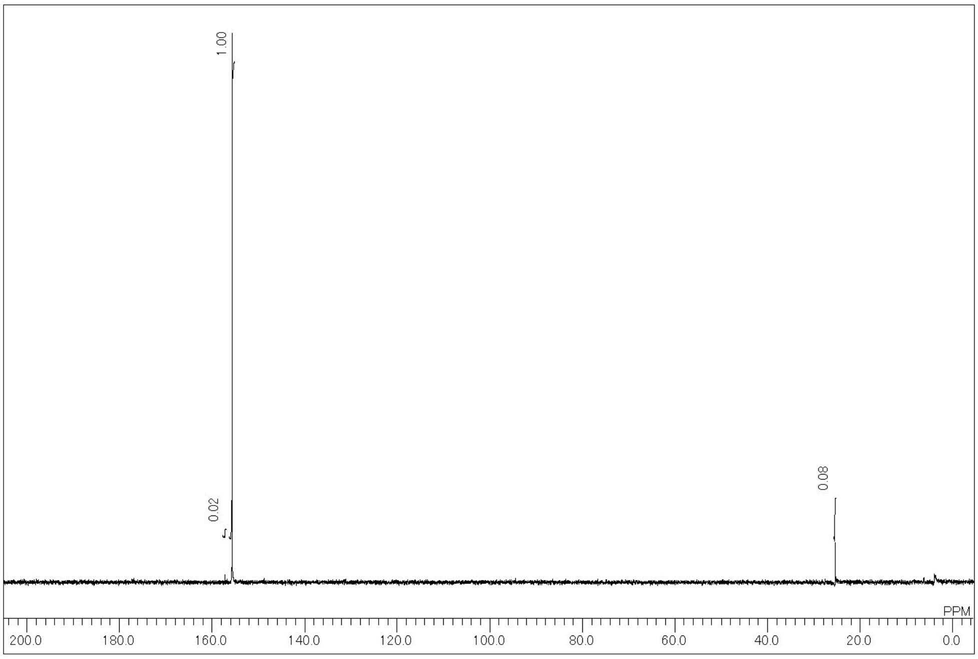

Figure S35 $^{31}P\{^1H\}$ NMR spectra of (*S*p)-**15** ($CDCl_3$, 162 MHz)

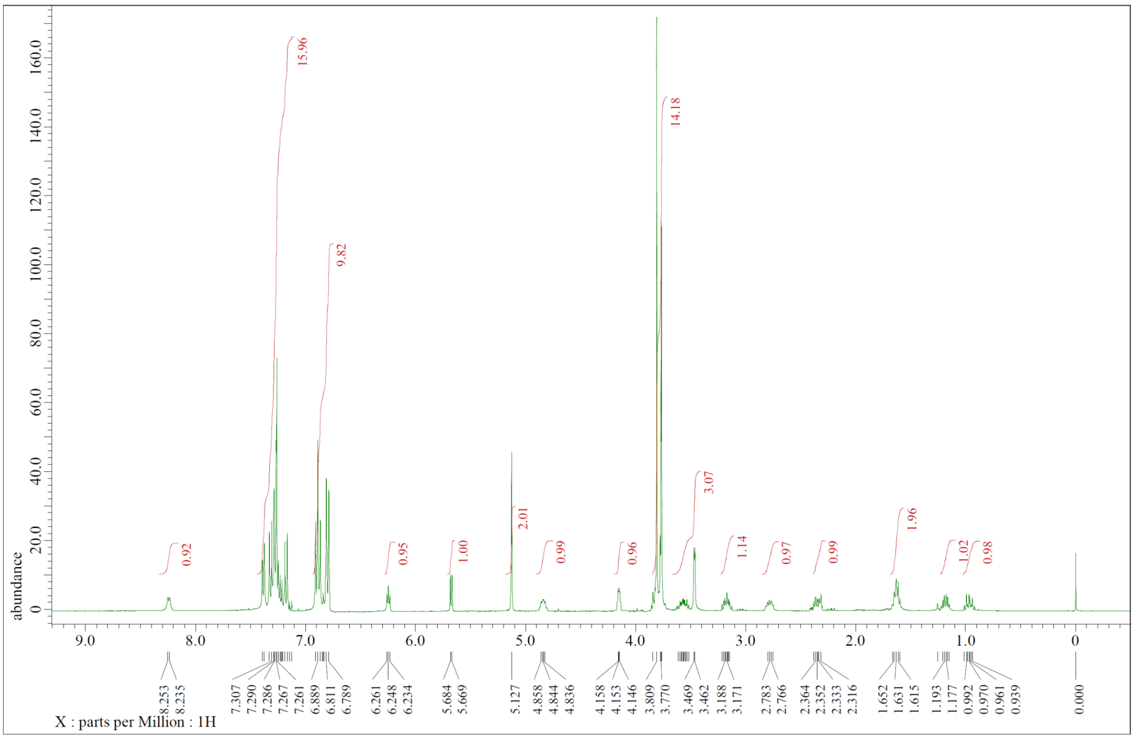

Figure S36 ^{1}H NMR spectra of (*R*p)-**16** ($CDCl_3$, 400 MHz)

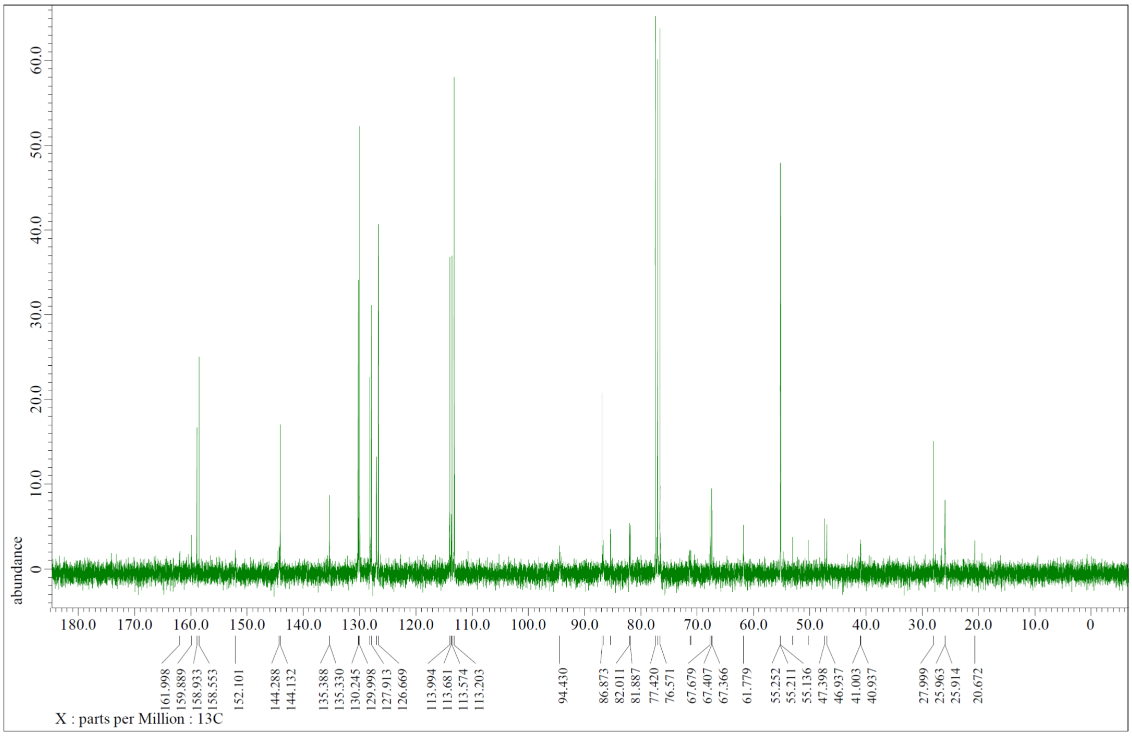

Figure S37 $^{13}C\{^{1}H\}$ NMR spectra of (*R*p)-**16** ($CDCl_3$, 75 MHz)

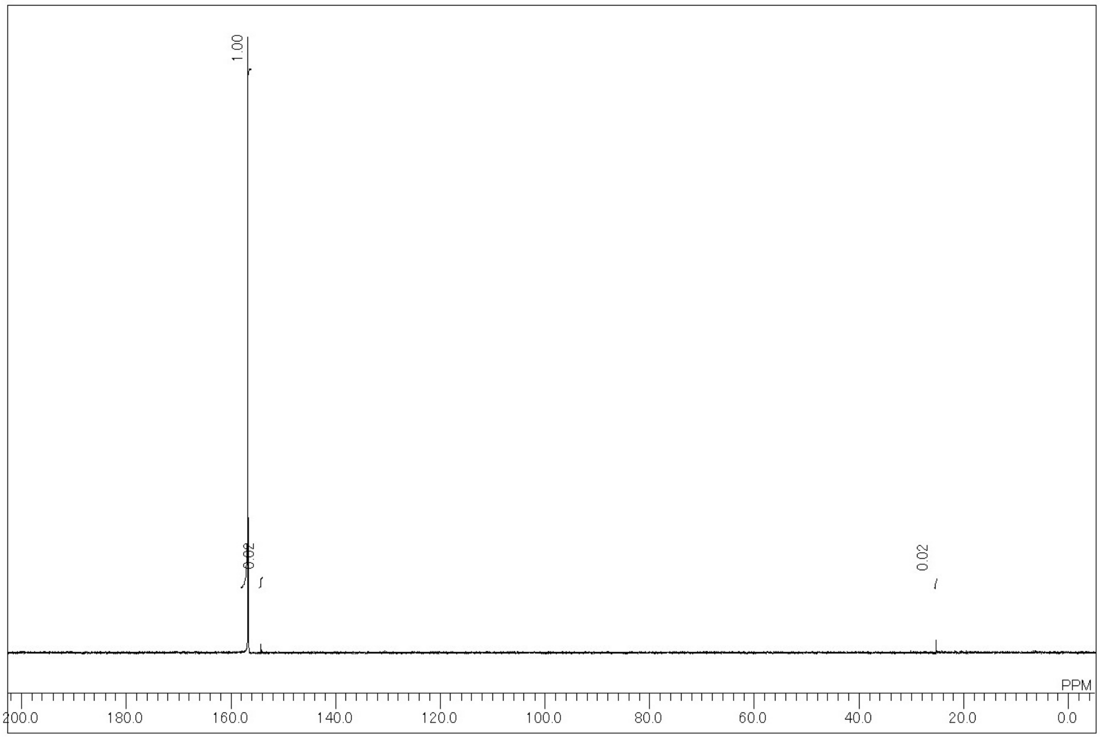

Figure S38 $^{31}P\{^{1}H\}$ NMR spectra of (*R*p)-**16** ($CDCl_3$, 162 MHz)

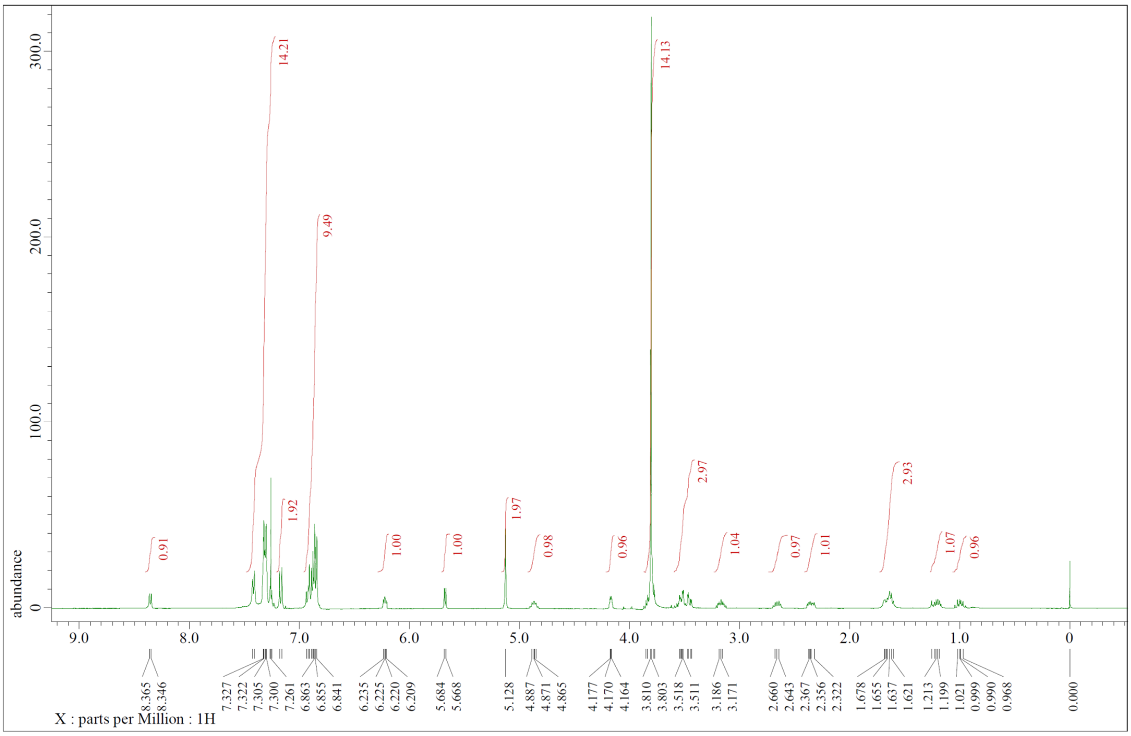

Figure S39 ^{1}H NMR spectra of (*S*p)-**16** ($CDCl_3$, 400 MHz)

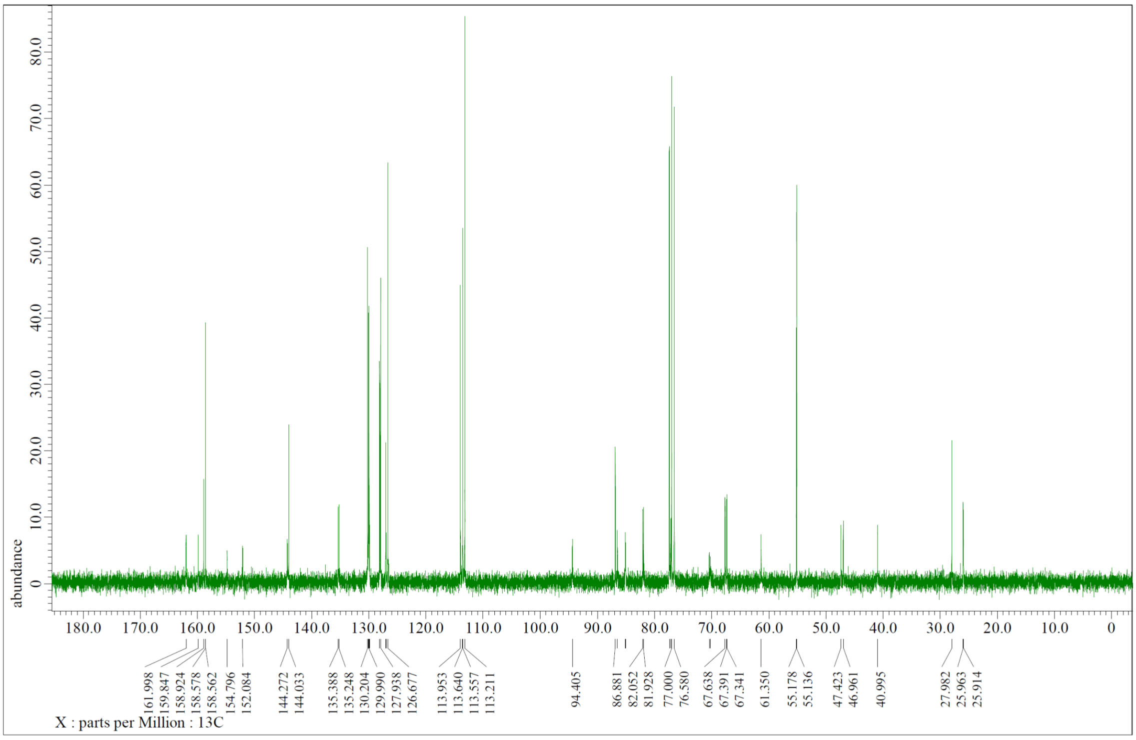

Figure S40 $^{13}C\{^{1}H\}$ NMR spectra of (*S*p)-**16** ($CDCl_3$, 75 MHz)

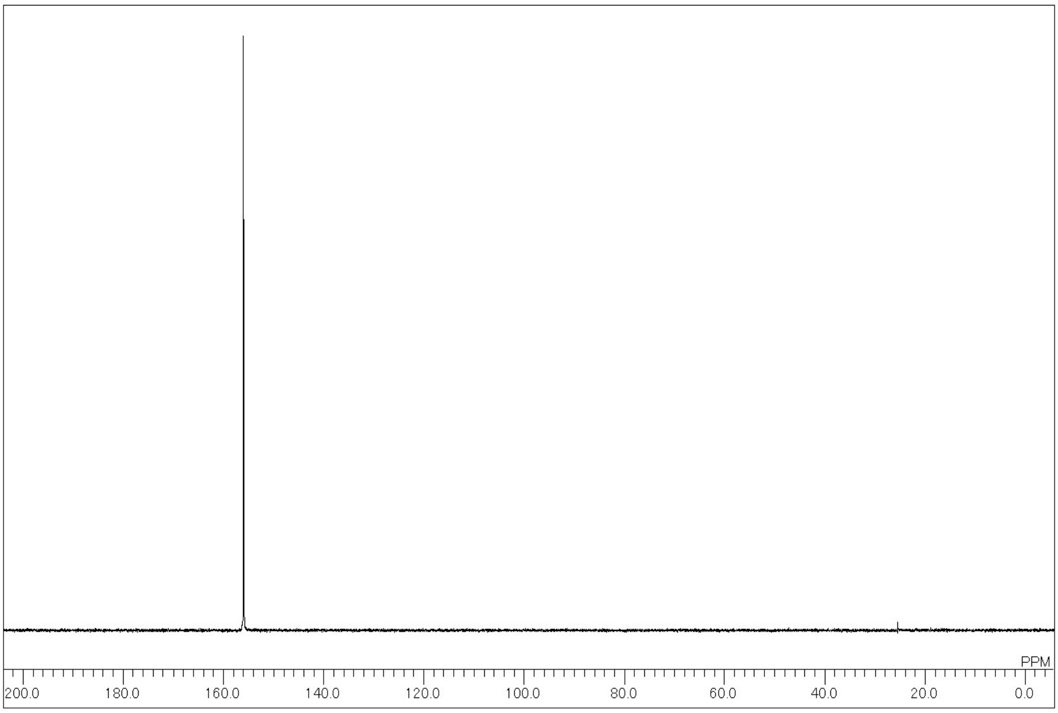

Figure S41 $^{31}P\{^{1}H\}$ NMR spectra of (*S*p)-**16** ($CDCl_3$, 162 MHz)

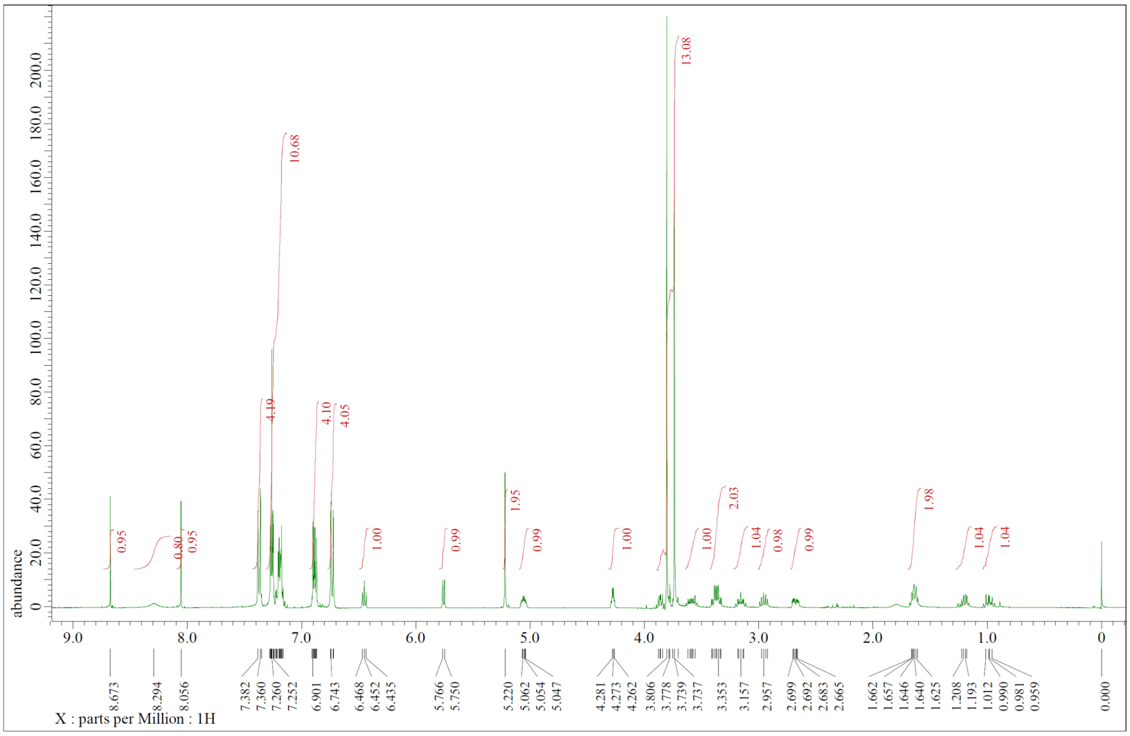

Figure S42 ^{1}H NMR spectra of (*R*p)-**17** ($CDCl_3$, 400 MHz)

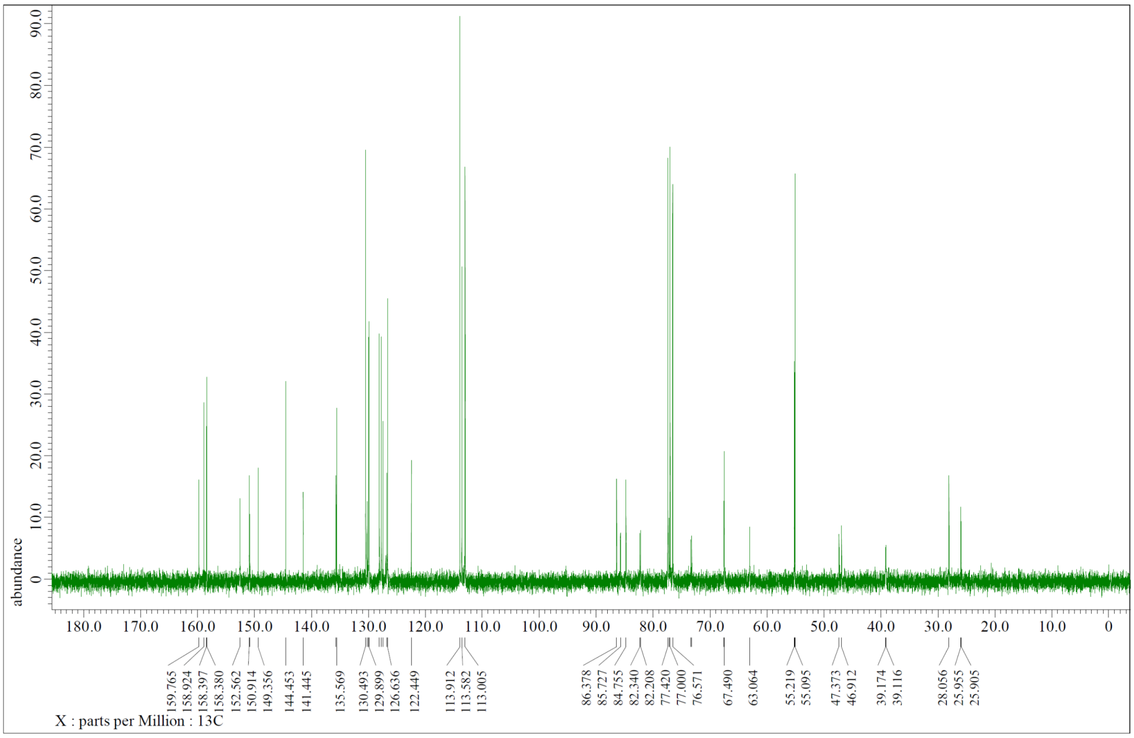

Figure S43 $^{13}C\{^{1}H\}$ NMR spectra of (*R*p)-**17** ($CDCl_3$, 75 MHz)

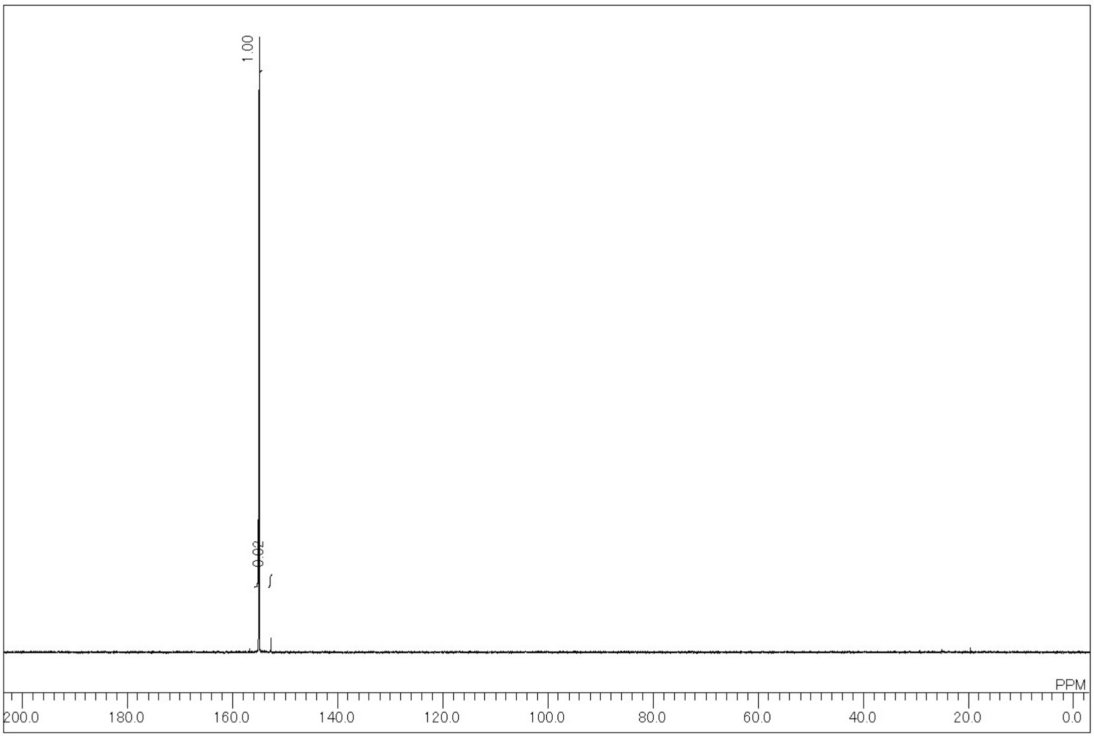

Figure S44 $^{31}P\{^{1}H\}$ NMR spectra of (*R*p)-**17** ($CDCl_3$, 162 MHz)

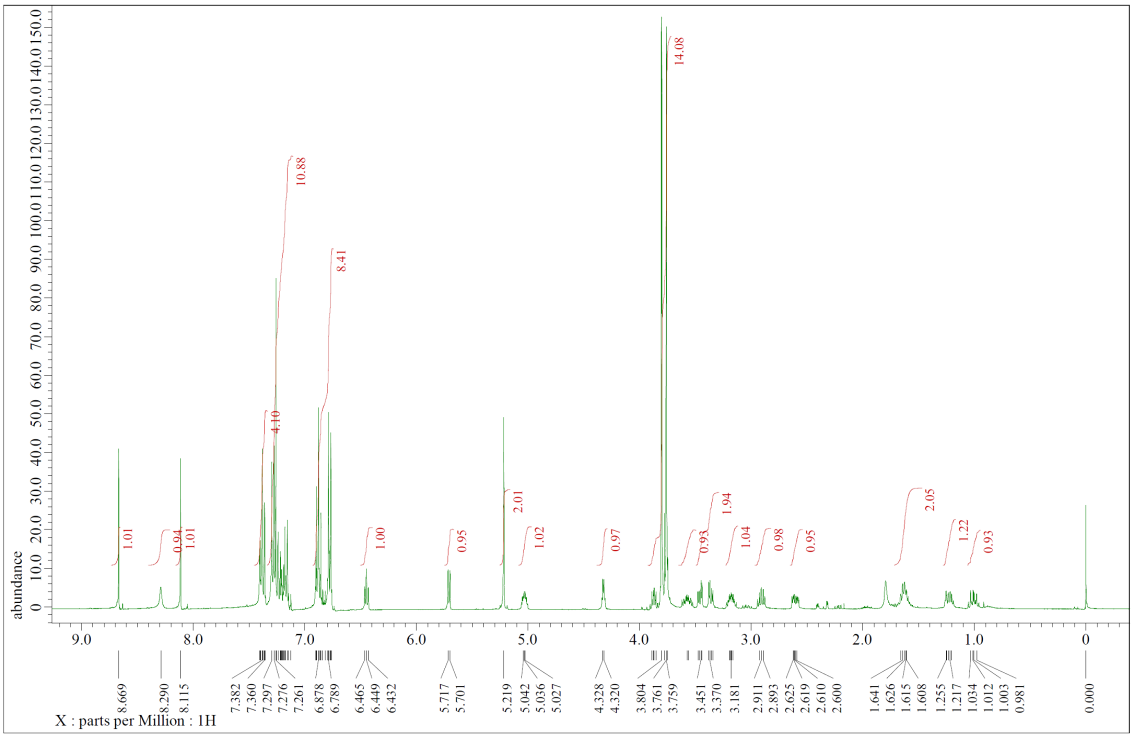

Figure S45 ^{1}H NMR spectra of (*S*p)-**17** ($CDCl_3$, 400 MHz)

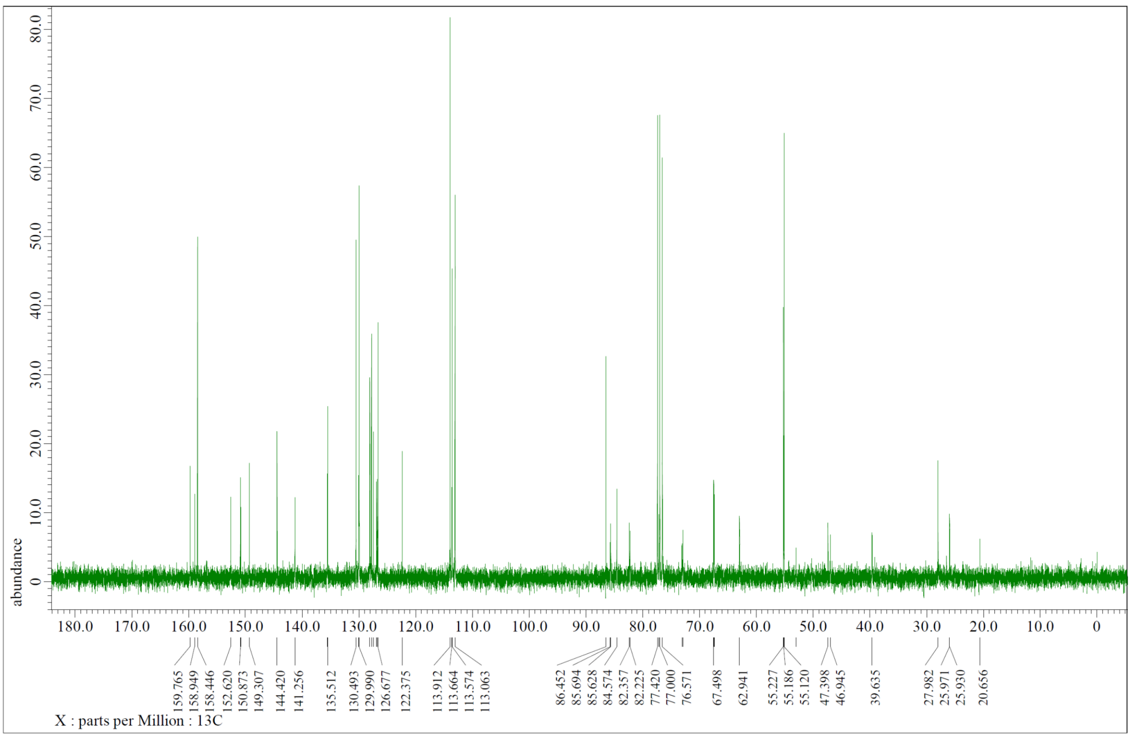

Figure S46 $^{13}C\{^{1}H\}$ NMR spectra of (*S*p)-**17** ($CDCl_3$, 75 MHz)

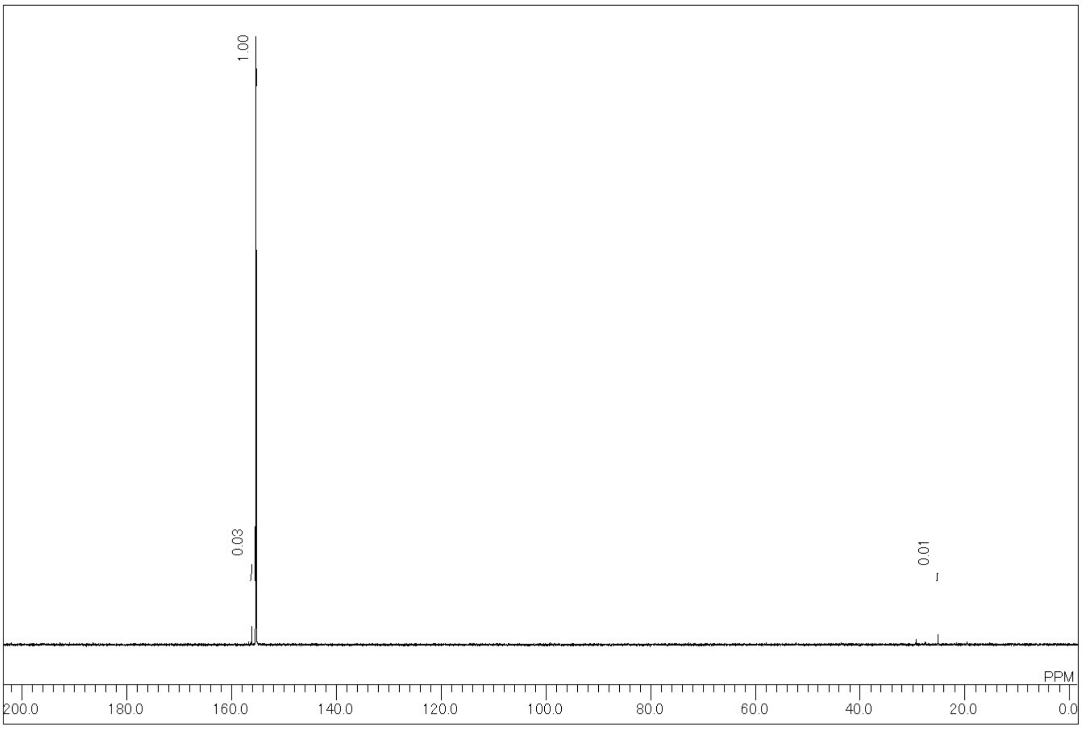

Figure S47 $^{31}P\{^1H\}$ NMR spectra of (*S*p)-**17** ($CDCl_3$, 162 MHz)

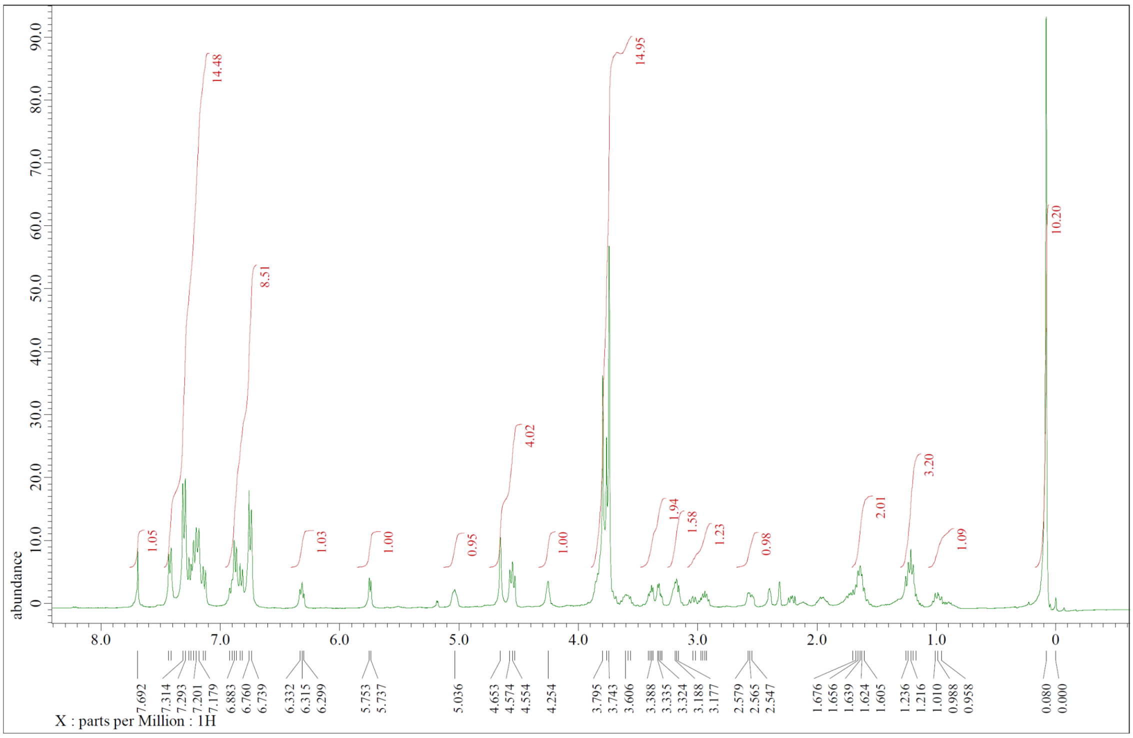

Figure S48 ^{1}H NMR spectra of (Rp)-**18** ($CDCl_3$, 400 MHz)

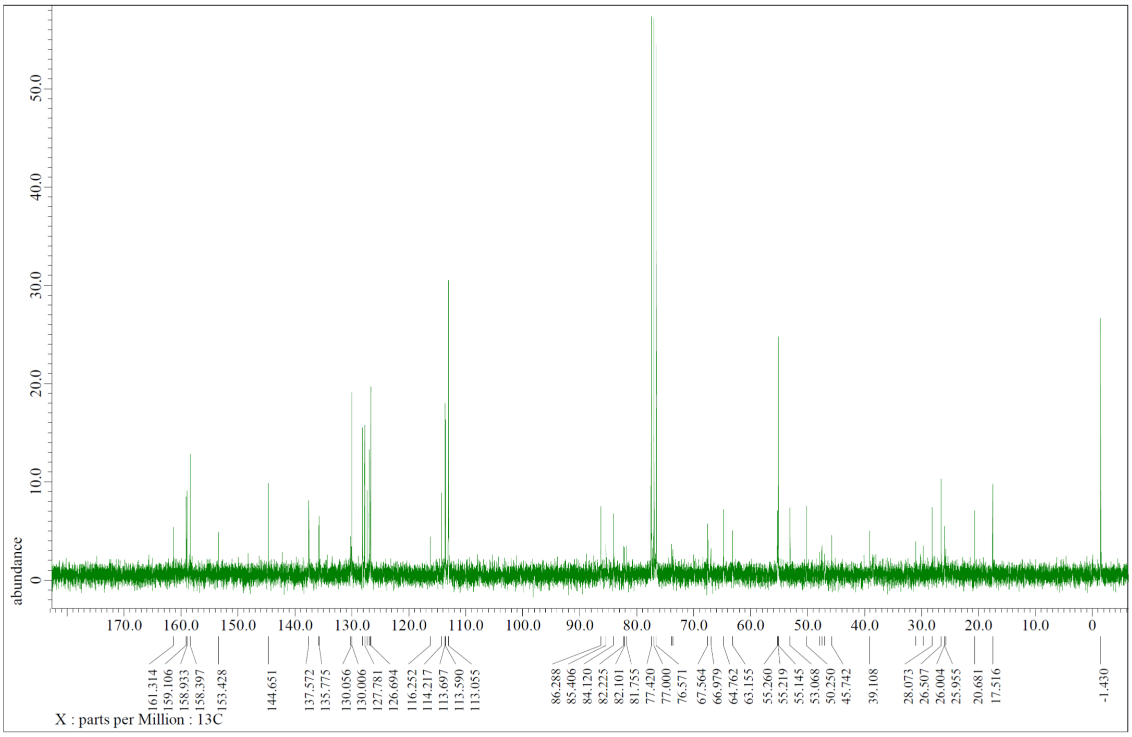

Figure S49 $^{13}C\{^{1}H\}$ NMR spectra of (Rp)-**18** ($CDCl_3$, 75 MHz)

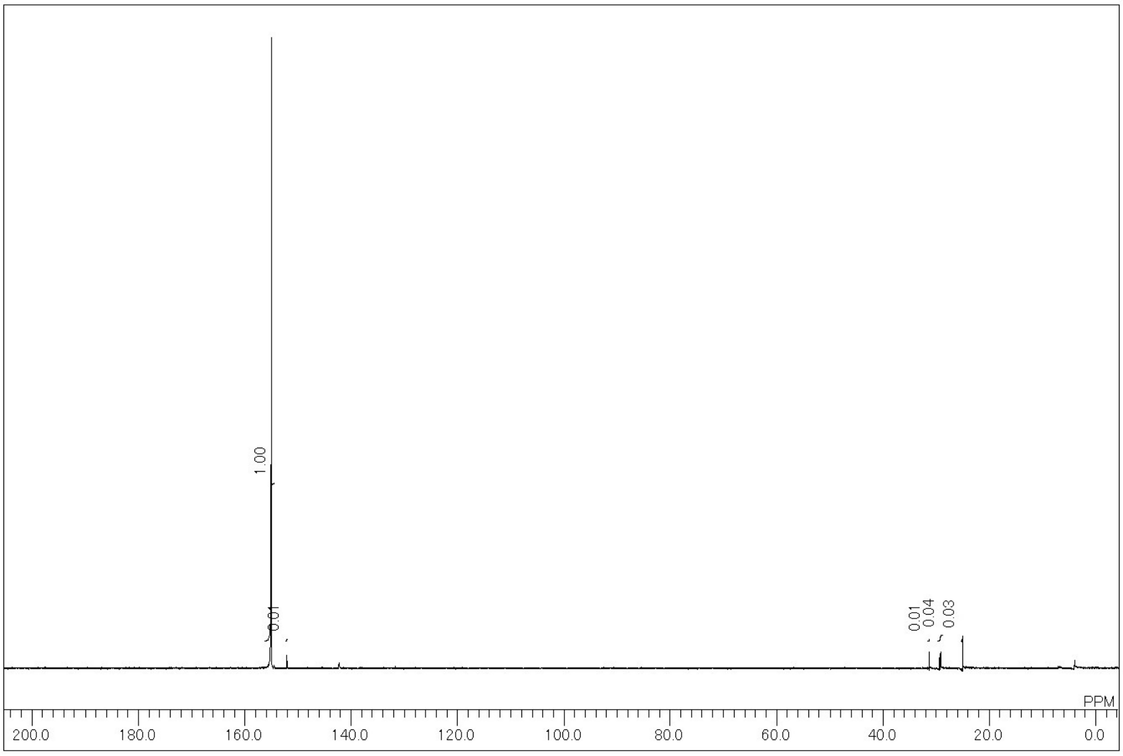

Figure S50 $^{31}P\{^{1}H\}$ NMR spectra of (*R*p)-**18** ($CDCl_3$, 162 MHz)

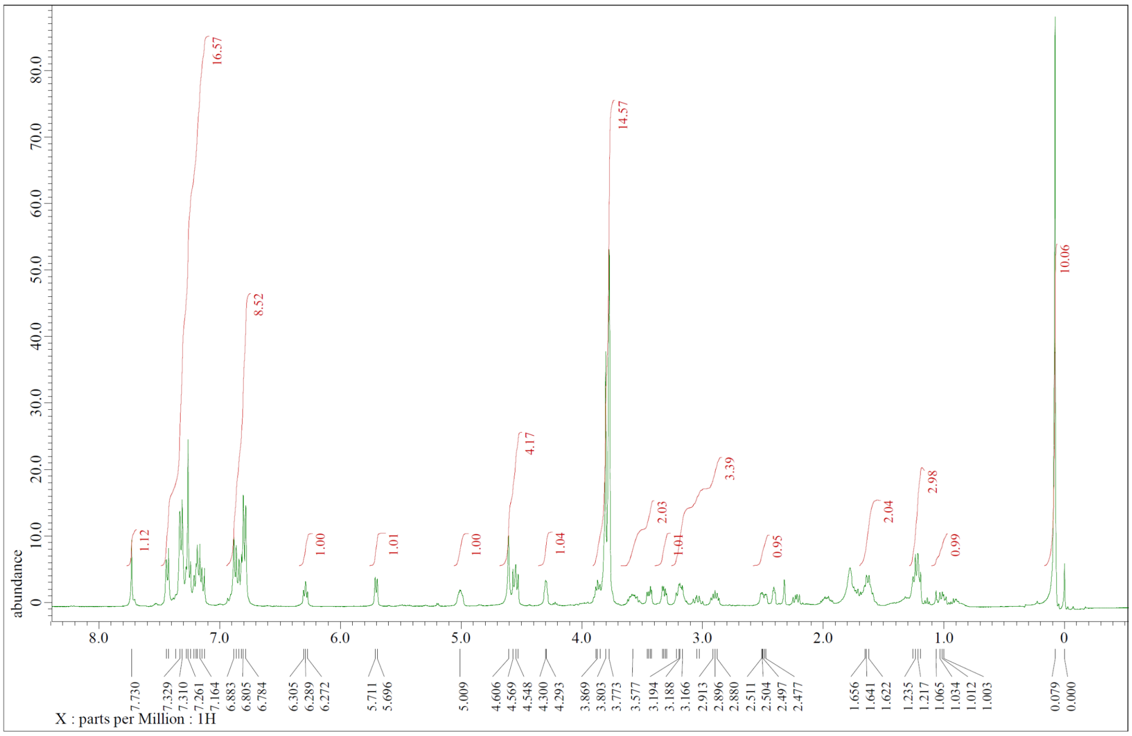

Figure S51 ^{1}H NMR spectra of (*S*p)-**18** ($CDCl_3$, 400 MHz)

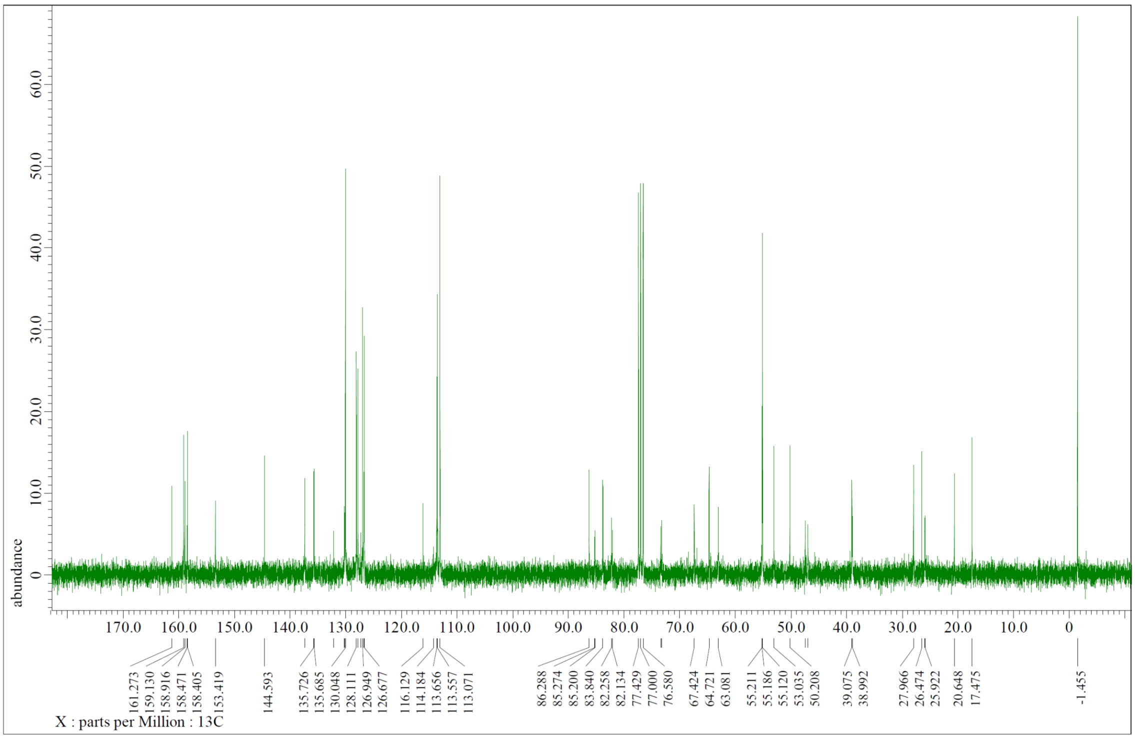

Figure S52 $^{13}C\{^{1}H\}$ NMR spectra of (*S*p)-**18** ($CDCl_3$, 75 MHz)

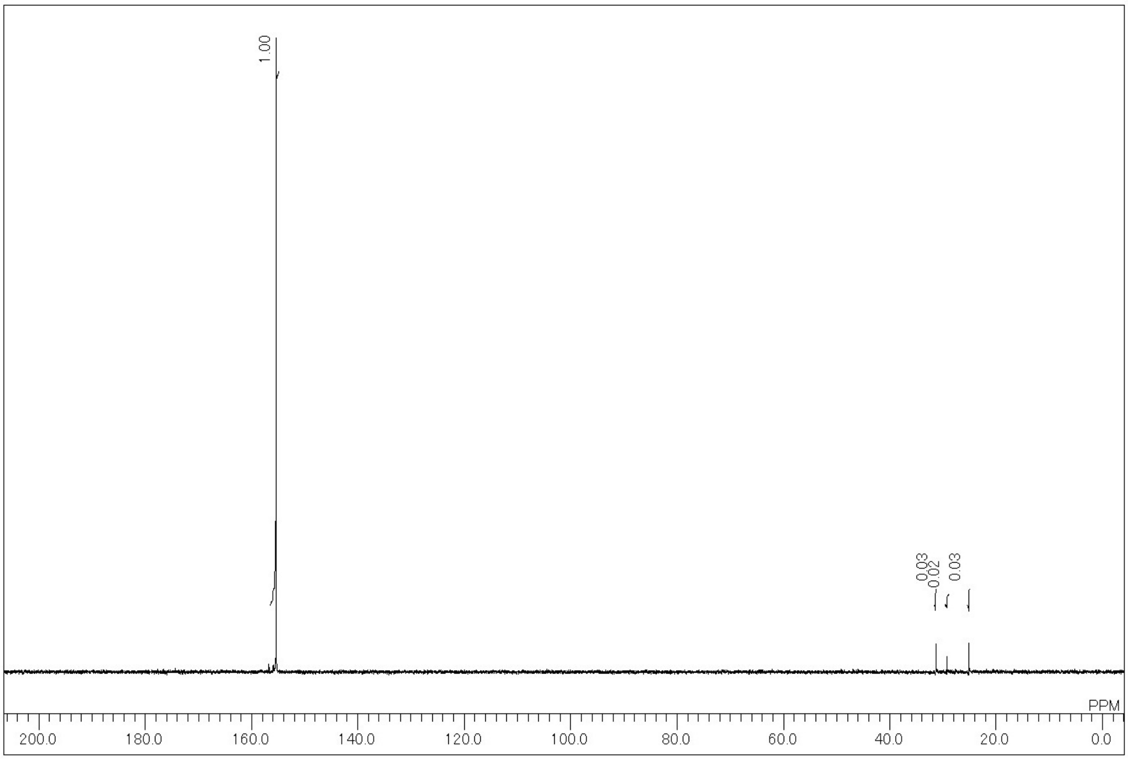

Figure S53 $^{31}P\{^{1}H\}$ NMR spectra of (*S*p)-**18** ($CDCl_3$, 162 MHz)