Supplementary Information

Synthesis and properties of DNA oligonucleotides with a zwitterionic backbone structure

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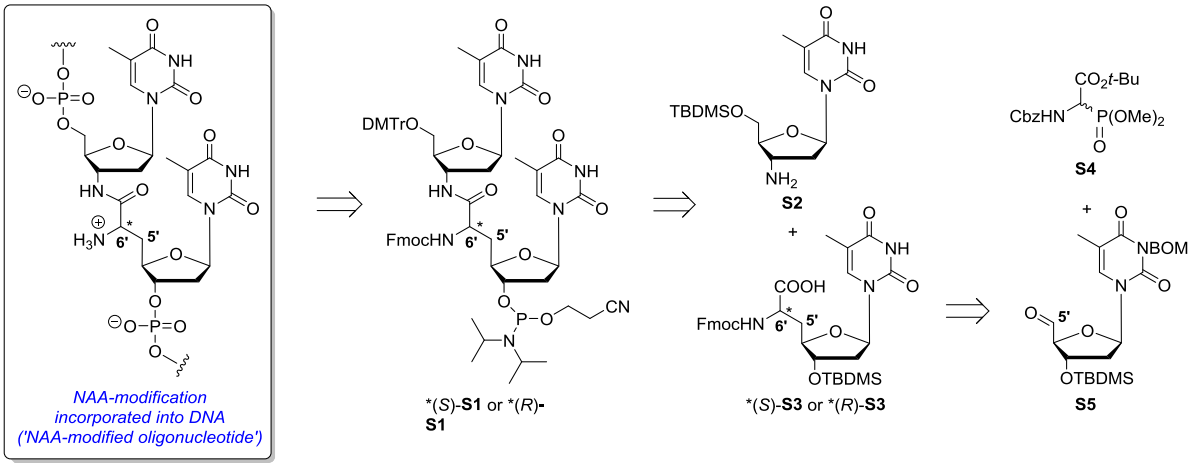
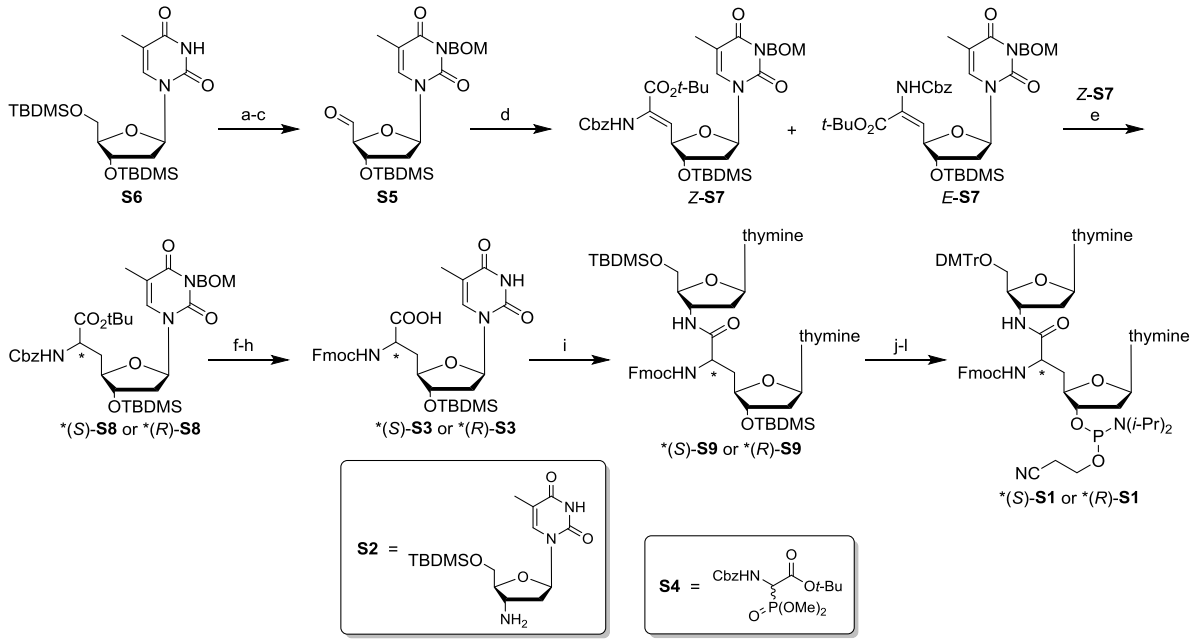
Synthesis of phosphoramidites for oligonucleotide synthesis



Scheme S1. Retrosynthetic analysis of NAA-modified oligonucleotides.

For the automated solid-phase synthesis of stereoisomerically pure NAA-modified oligonucleotides, phosphoramidite building blocks **S1** with either (6'*S*)- or (6'*R*)-configuration were required (Scheme S1). The synthesis of such building blocks is feasible by peptide coupling of protected 3'-amino-3'-deoxythymidine **S2**[S1,S2] and nucleosyl amino acids ((6'*S*)- or (6'*R*)-NAA) **S3**, followed by several protecting group manipulations. The precursors of key intermediates **S3** are accessible *via* Wittig-Horner reaction of the phosphonoglycine derivative **S4**[S3-S6] and protected thymidine-5'-aldehyde **S5**. The didehydro amino acid resulting from this reaction would then undergo stereoselective catalyst-controlled asymmetric hydrogenation as previously reported by us for analogous uridine derivatives.[S7,S8] The stereoselective synthesis of the required 'dimeric' phosphoramidites (*S*)-**S1** and (*R*)-**S1** was carried out according to this convergent retrosynthetic scheme.



Scheme S2. Synthesis of phosphoramidite building blocks **S1** for the preparation of NAA-modified oligonucleotides. Reagents and conditions. (a) BOMCl, NaH, DMF, -10 °C to 0 °C, 3 h, 96%; (b) AcCl, MeOH, -10 °C to 0 °C, 3 h, 59%; (c) IBX, MeCN, reflux, 45 min, 99%; (d) **S4**, KO*t*-Bu, THF, -78 °C to rt, 16 h, 71% *Z*-**S7**, 3% *E*-**S7**; (e) H_2 (1 bar), **cat.**, MeOH, rt, 2-7 d, 94% (*S*)-**S8** (with **cat.** = (*S*,*S*)-Me-DuPHOS-Rh, *d.r.* > 98:2), 99% (*R*)-**S8** (with **cat.** = (*R*,*R*)-Me-DuPHOS-Rh, *d.r.* > 98:2); (f) H_2 (1 bar), 10% Pd/C, *n*-$BuNH_2$, MeOH, rt, 24 h, 93% (*S*)-isomer, 92% (*R*)-isomer; (g) FmocCl, NEt_3, THF, 0 °C, 30 min, 97% (*S*)-isomer, 95% (*R*)-isomer; (h) SiO_2, toluene, reflux, 20 h, 94% (*S*)-**S3**, 90% (*R*)-**S3**; (i) **S2**, EDC, HOBt, CH_2Cl_2, rt, 16 h, 79% (*S*)-**S9**, 78% (*R*)-**S9**; (j) AcCl, MeOH, 0° C to rt, 24 h, 92% (*S*)-isomer, 91% (*R*)-isomer; (k) DMTrCl, pyridine, rt, 16 h, 75% (*S*)-isomer, 75% (*R*)-isomer; (l) 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropyl phosphordiamidite, 4,5-dicyano-imidazole, CH_2Cl_2, rt, 1 h, 76% (*S*)-**S1**, 74% (*R*)-**S1**. From intermediates (*S*)-**S8** and (*R*)-**S8** onwards, all reactions were carried out with diastereomerically pure compounds (with either (6'*S*)- or (6'*R*)-configuration) and are just summarised in one scheme in the interest of conciseness.

The synthesis of the required NAA building blocks (Scheme S2) started from thymidine which was silylated at the 5'- and 3'-hydroxy groups in quantitative yield (not displayed). To avoid side reactions in the subsequent steps, silyl ether **S6** was converted into the N^3-BOM-protected derivative in 96% yield. Selective cleavage of the 5'-*O*-silyl ether was attained using catalytic amounts of acetyl chloride in methanol[S9] in 59% yield. The resultant alcohol was then oxidised nearly quantitatively to the corresponding aldehyde **S5** using IBX in refluxing acetonitrile.[S10] Wittig-Horner reaction of aldehyde **S5** with phosphonoglycine derivative **9**[S3-S6] afforded didehydro amino acid **S7** with pronounced diastereoselectivity towards the desired *Z*-isomer.[S11] Diastereomers *Z*-**S7** and *E*-**S7** were separated by column chomatography and characterised (isolated yields: 71% *Z*-**S7**, 3% *E*-**S7**). The configurations of *Z*-**S7** and *E*-**S7** were assigned based on typical patterns of ^{1}H NMR signals according to established criteria for this specific class of compounds.[S12] The correctness of this assignment was further corroborated by NOE-based NMR experiments (see NMR spectra of *Z*-**S7**). Only pure *Z*-**S7** was then applied in an asymmetric hydrogenation in the presence of the chiral rhodium catalysts[S13] (+)-1,2-bis((2*S*,5*S*)-2,5-dimethylphospholano)-benzene-(cyclooctadiene)-rhodium(I) tetrafluoroborate ((*S*,*S*)-Me-DuPHOS-Rh) or its enantiomeric counterpart (*R*,*R*)-Me-DuPHOS-Rh.[S14-S16] In both cases, homogeneous hydrogenation proceeded smoothly furnishing the fully protected (6'*S*)-NAA (*S*)-**S8** or (6'*R*)-NAA (*R*)-**S8**, respectively, in very good yields (94 and 99%, respectively) and high diastereoselectivities (*d.r.* > 98:2 each, Scheme S2). It is firmly established that asymmetric hydrogenations of *Z*-configured didehydro amino acids catalysed by (*S*,*S*)-Me-DuPHOS-Rh give L-amino acids and that analogous reactions catalysed by (*R*,*R*)-Me-DuPHOS-Rh afford D-amino acids.[S15,S16] Thus, it was possible to direct the stereochemical outcome of the hydrogenation by the choice of either the (*S*,*S*)- or the (*R*,*R*)-catalyst. The high diastereoselectivities represent a clear proof of these reactions being catalyst-controlled, with full conversion of *Z*-**S7** to (*S*)-**S8** being reached

after 2 days when the (*S*,*S*)-Me-DuPHOS-Rh catalyst was employed (apparent 'matched' case). In contrast, the hydrogenation was completed after 7 days in the presence of the (*R*,*R*)-Me-DuPHOS-Rh catalyst (apparent 'mismatched' case). Similar reactions using uridine analogues as substrates gave comparable results,[S7,S8] and in case of the uridine-derived congeners, the stereochemical assignment was further confirmed by X-ray crystal structure analysis.[S8]

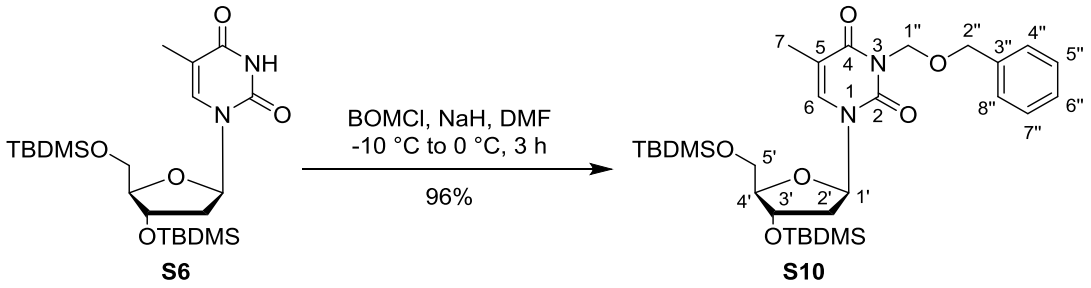
After the construction of the elongated carbon scaffold of the NAA, manipulation of the protecting groups was performed. First, the BOM- and the Cbz-Groups of **S8** were cleaved concomitantly by heterogeneous hydrogenolysis in high yields (Scheme S2). In this reaction, the presence of an excess of *n*-butylamine was essential in order to prevent the undesired methylation of the primary amino group at the 6'-position by formaldehyde, which is formed upon reductive BOM-cleavage. To finally obtain building blocks applicable to DNA synthesis, the introduction of a base-labile protecting group at the 6'-amino moiety was required. Therefore, the Fmoc group was chosen, which could easily be removed during the base-mediated cleavage of the oligonucleotides from the solid support. Treatment of the 6'-amines with Fmoc-chloride afforded the corresponding carbamates in very good yields of 95 to 97%. The selective cleavage of the *tert*-butyl ester in the presence of an acid-labile silyl ether was performed using silica in refluxing toluene affording the desired NAAs (*S*)-**S3** (94% yield) and (*R*)-**S3** (90% yield). These building blocks were then reacted with protected 3'-deoxy-3'-aminothymidine **S2**[S1,S2] under usual peptide coupling conditions. The resultant dinucleoside derivatives (*S*)-**S9** (79% yield) and (*R*)-**S9** (78% yield) were then treated with acetyl chloride in methanol, resulting in the cleavage of both silyl ethers[S9] and thus furnishing the corresponding diols in yields of 91 to 92%. Reactions of these diol intermediates with 4,4'-dimethoxytrityl (DMTr) chloride afforded the 5'-*O*-DMTr derivatives in yields of 75% for both isomers. Finally, phosphitylation of the 3'-hydroxy group gave the target compounds

(*S*)-**S1** and (*R*)-**S1** (76% and 74% yield, respectively, Scheme S2). For this reaction, 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropyl phosphordiamidite was employed under slightly acidic conditions, *i.e.*, in the presence of the activator 4,5-dicyanoimidazole. In comparison, the standard method for the introduction of this functionality using the respective chlorophosphite and a base resulted in unwanted concomitant cleavage of the Fmoc group. Experimental details of this synthetic route are given below, including synthetic intermediates **S10**-**S15** (which are not explicitly displayed in Scheme S2) and compound (*S*)-**S16** (*vide infra*).

General methods. Compounds **S2**[S1,S2] and **S4**[S3-S6] were prepared according to established procedures. All other chemicals were purchased from standard suppliers. Reactions involving oxygen and/or moisture sensitive reagents were carried out under an atmosphere of argon using anhydrous solvents. Anhydrous solvents were obtained in the following manner: THF was dried over sodium/benzophenone and distilled, CH_2Cl_2 was dried over CaH_2 and distilled, MeOH was dried over activated molecular sieves (3 Å) and degassed, MeCN was dried over P_2O_5 and distilled, pyridine was dried over CaH_2 and distilled, toluene was dried over sodium/benzophenone and distilled. The thus obtained solvents were stored over molecular sieves (4 Å, in case of MeOH and MeCN 3 Å). All other solvents were of technical quality and distilled prior to their use, and deionised water was used throughout. Column chromatography was carried out on silica gel 60 (0.040-0.063 mm, 230-400 mesh ASTM, VWR) under flash conditions except where indicated. TLC was performed on aluminium plates precoated with silica gel 60 F_{254} (VWR). Visualisation of the spots was carried out using UV light (254 nm) and/or staining under heating (H_2SO_4 staining solution: 4 g vanillin, 25 mL conc. H_2SO_4, 80 mL AcOH and 680 mL MeOH; $KMnO_4$ staining solution: 1 g $KMnO_4$, 6 g K_2CO_3 and 1.5 mL 1.25 M NaOH solution, all dissolved in 100 mL H_2O; ninhydrin staining solution: 0.3 g ninhydrin, 3 mL AcOH and 100 mL 1-butanol). 300 MHz-,

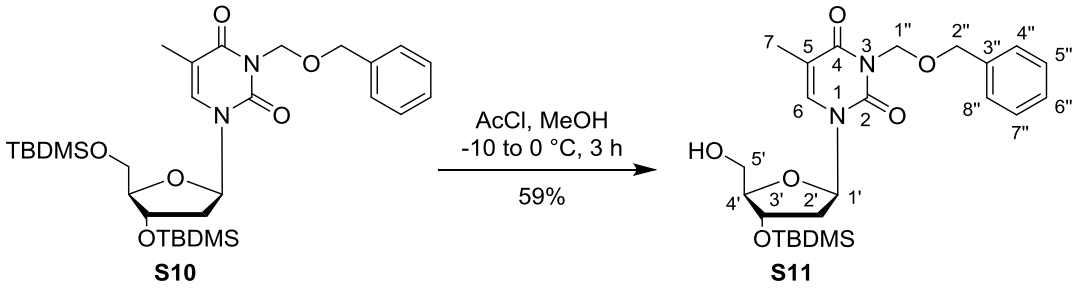
500 MHz- and 600 MHz-^{1}H and 75 MHz-, 76 MHz- and 126 MHz-^{13}C as well as 121 MHz-^{31}P NMR spectra were recorded on Varian MERCURY 300, UNITY 300, INOVA 500 and INOVA 600 spectrometers. All ^{13}C NMR spectra are H-decoupled. All spectra were recorded at room temperature except where indicated otherwise and were referenced internally to solvent reference frequencies. For the calibration of ^{31}P NMR signals, 85% phosphoric acid was used as an external standard. Chemical shifts (δ) are quoted in ppm, and coupling constants (*J*) are reported in Hz. Assignment of signals was carried out using H,H-COSY, HSQC and TOCSY spectra obtained on the spectrometers mentioned above. Mass spectra of small molecules were measured on a Finnigan LCQ ion-trap mass spectrometer or on a Bruker microTOF spectrometer. For ESI measurements in the negative mode, solutions of the compounds in pure MeOH were used whereas for measurements in the positive mode, solutions with the addition of 0.1% formic acid were used. High resolution spectra were measured on a Bruker 7 Tesla fourier transform ion cyclotron resonance (FTICR) mass spectrometer. Melting points (mp) were measured on a Büchi instrument and are not corrected. Optical rotations were recorded on a Perkin-Elmer polarimeter 241 with a Na source using a 10 cm cell. Solutions of the compounds (~ 10 mg) in $CHCl_3$ or pyridine (1 mL) were used, and concentrations are given in g/100 mL. Infrared spectroscopy (IR) was performed on a Bruker Vector 22 FTIR spectrometer using a thin film on an NaCl plate or a KBr pellet. For each compound the wavenumbers (ν) of the nine most intense absorption bands are given in cm^{-1}. UV spectroscopy of small molecules was carried out on a Perkin-Elmer Lambda 2 spectrometer. Measurements were performed with solutions of ~ 0.1 mg of the compound in 10 mL MeCN and in the range of 190-500 nm. Wavelengths of maximum absorption (λ_{max}) are reported in nm with the corresponding logarithmic molar extinction coefficient given in parenthesis (log ε, $\varepsilon/dm^3\,mol^{-1}\,cm^{-1}$).

3',5'- Bis-*O*-(*tert*-butyldimethylsilyl)-3-*N*-(benzyloxymethyl)-thymidine S10



To a suspension of NaH (461 mg, 19.2 mmol, 60% suspension in mineral oil) in DMF (30 mL), a solution of 3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-thymidine **S6** (5.00 g, 10.6 mmol) in DMF (17 mL) was slowly added at -10 °C. The resultant solution was stirred at -5 °C for 20 min and then treated dropwise with benzylchloromethylether (1.53 mL, 1.99 g, 12.8 mmol). The solution was stirred at 0 °C for another 3 h. The reaction was then diluted with EtOAc (200 mL), and water (60 mL) was slowly added. The organic layer was washed with sat. $NaHCO_3$ solution (2 x 150 mL) and brine (1 x 80 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude product was purified by column chromatography (4:1 petroleum ether-EtOAc) to give **S10** as a colorless oil (6.00 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.05 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$), 0.09 (s, 6H, $SiCH_3$), 0.87 (s, 9H, $SiC(CH_3)_3$), 0.91 (s, 9H, $SiC(CH_3)_3$), 1.89 (d, J = 0.9 Hz, 3H, 7-H), 1.96 (ddd, J = 13.1, 7.8, 6.0 Hz, 1H, 2'-H_b), 2.24 (ddd, J = 13.1, 5.8, 2.6 Hz, 1H, 2'-H_a), 3.74 (dd, J = 11.4, 2.6 Hz, 1H, 5'-H_a), 3.85 (dd, J = 11.4, 2.6 Hz, 1H, 5'-H_b), 3.91 (dd, J = 4.5, 2.6 Hz, 1H, 4'-H) 4.35-4.39 (m, 1H, 3'-H), 4.68 (s, 2H, 2"-H), 5.47 (s, 2H, 1"-H), 6.33 (dd, J = 7.8, 5.8 Hz, 1H, 1'-H), 7.23-7.43 (m, 5H, aryl-H), 7.28 (d, J = 0.9 Hz, 1H, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ -5.5, -5.4, -4.9, -4.7, 13.2, 18.0, 18.4, 25.7, 25.9, 41.4, 63.0, 70.5, 72.2, 72.2, 85.5, 87.8, 110.1, 127.6, 127.7, 128.2, 134.2, 138.0, 151.0, 163.5; HRMS (ESI^+) *m/z* calcd for $C_{30}H_{50}N_2NaO_6Si_2$ 613.3105 ($M + Na^+$), found 613.3109 ($M + Na^+$); IR (KBr) ν 2929, 1712, 1670, 1464, 1361, 1255, 1106, 836, 775; UV (MeCN) λ_{max} (log ε) 205 (4.30), 268 (3.94); TLC R_f 0.48 (4:1 petroleum ether-EtOAc); $[\alpha]_D^{20}$ +17.7 (*c* 1.0, $CHCl_3$).

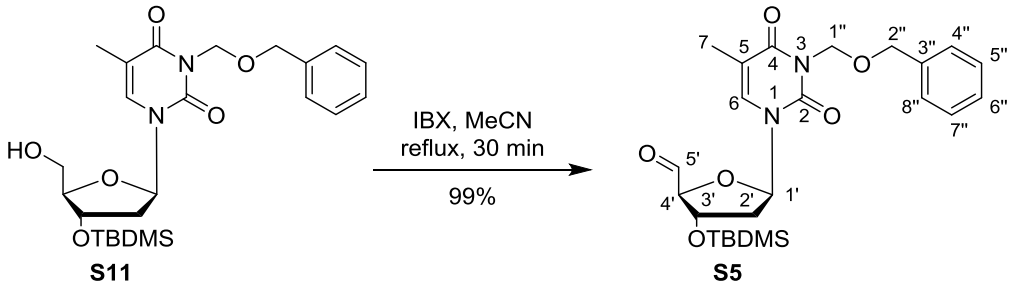
3'-*O*-(*tert*-Butyldimethylsilyl)-3-*N*-(benzyloxymethyl)-thymidine S11



S10 **S11**

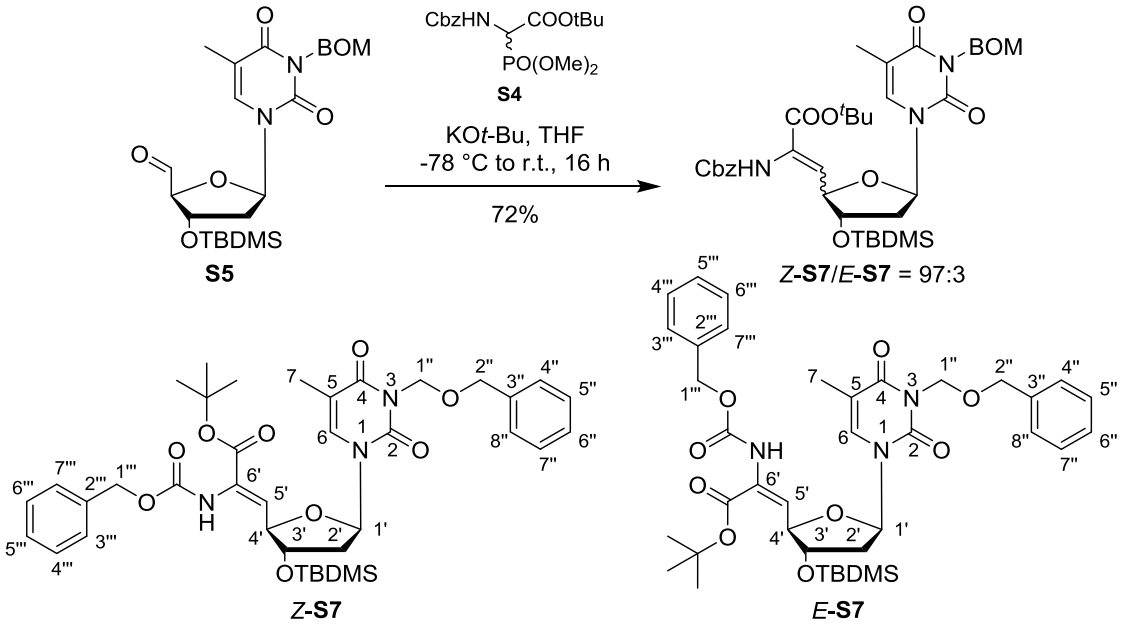
To a solution of 3',5'- bis-*O*-(*tert*-butyldimethylsilyl)-3-*N*-(benzyloxymethyl)-thymidine **S10** (6.00 g, 10.2 mmol) in MeOH (300 mL), acetyl chloride (160 mg, 145 µL, 2.03 mmol) was added dropwise at -10 °C. The solution was allowed to warm to 0 °C and stirred for 3 h at this temperature. The reaction was monitored by TLC (1:1 petroleum ether-EtOAc). It was then quenched by the addition of sat. $NaHCO_3$ solution (10 mL) and stirring for 15 min. The solution was diluted with EtOAc (300 mL), washed with sat. $NaHCO_3$ solution (2 x 100 mL) and water (1 x 100 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude product was purified by column chromatography (1:1 petroleum ether-EtOAc) to give **S11** as a colorless oil (2.82 g, 59%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.06 (s, 6H, $SiCH_3$), 0.88 (s, 9H, $SiC(CH_3)_3$), 1.88 (d, J = 1.0 Hz, 3H, 7-H), 2.15-2.31 (m, 2H, 2'-H_a, 2'-H_b), 2.62 (brs, 1H, OH), 3.68-3.76 (m, 1H, 5'-H_a), 3.84-3.93 (m, 2H, 4'-H, 5'-H_b), 4.43-4.42 (m, 1H, 3'-H), 4.67 (s, 2H, 2''-H), 5.46 (s, 2H, 1''-H), 6.14 (dd, J = 6.9, 6.7 Hz, 1H, 1'-H), 7.21-7.35 (m, 5H, aryl-H), 7.37 (d, J = 1.0 Hz, 1H, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ -4.8, -4.6, 13.3, 18.0, 25.7, 40.7, 62.0, 70.5, 71.6, 72.2, 87.2, 87.5, 110.1, 127.5, 127.5, 128.1, 135.4, 137.8, 150.8, 163.3; HRMS (ESI^+) *m/z* calcd for $C_{24}H_{36}N_2NaO_6Si$ 499.2240 ($M + Na^+$), found 499.2234 ($M + Na^+$); IR (KBr) ν 2930, 1667, 1468, 1362, 1253, 1101, 835, 776, 698; UV (MeCN) λ_{max} (log ε) 205 (4.28), 268 (3.96); TLC R_f 0.38 (1:1 petroleum ether-EtOAc); $[\alpha]_D^{20}$ +21.0 (*c* 1.1, $CHCl_3$).

3'-*O*-(*tert*-Butyldimethylsilyl)-3-*N*-(benzyloxymethyl)-thymidine-5'-aldehyde S5



To a solution of 3'-*O*-(*tert*-butyldimethylsilyl)-3-*N*-(benzyloxymethyl)-thymidine **S11** (1.00 g, 2.10 mmol) in MeCN (20 mL), 2-iodoxybenzoic acid (IBX, 1.47 g, 5.25 mmol) was added. The resultant suspension was heated under reflux for 30 min. After cooling to rt, the suspension was filtered and the residue was washed with EtOAc (3 x 10 mL). The combined filtrates were evaporated under reduced pressure to give **S5** as a colorless foam which was used in the subsequent reaction without further purification (990 mg, 99%). ^{1}H NMR (300 MHz, C_6D_6): δ -0.04 (s, 3H, $SiCH_3$), -0.03 (s, 3H, $SiCH_3$), 0.85 (s, 9H, $SiC(CH_3)_3$),1.67 (ddd, *J* = 13.6, 7.4, 5.8 Hz, 1H, 2'-H_a), 1.73 (d, *J* = 1.2 Hz, 3H, 7-H), 1.84 (ddd, *J* = 13.6, 6.5, 2.1 Hz, 1H, 2'-H_b), 4.04 (ddd, *J* = 2.1, 2.1, 2.1 Hz, 1H, 4'-H), 4.40 (ddd, *J* = 5.8, 2.1, 2.1 Hz, 1H, 3'-H), 4.63 (s, 2H, 2''-H), 5.39 (s, 2H, 1''-H), 5.83 (dd, *J* = 6.5, 6.5 Hz, 1H, 1'-H), 6.68 (d, *J* = 1.2 Hz, 1H, 6-H), 6.99 (d, *J* = 7.2 Hz, 1H, 6''-H), 7.07 (dd, *J* = 7.5, 7.5 Hz, 2H, 7''-H, 5''-H), 7.29 (d, *J* = 7.5 Hz, 2H, 4''-H, 8''-H), 9.27 (s, 1H, 5'-H); ^{13}C NMR (75 MHz, C_6D_6): δ -4.8, -4.7, 13.4, 18.2, 26.0, 39.8, 70.8, 72.4, 73.6, 90.5, 92.3, 110.1, 127.6, 127.7, 128.1, 135.6,138.9, 150.8, 163.3, 198.7; HRMS (ESI$^-$) *m/z* calcd for $C_{24}H_{33}N_2O_6Si$ 473.2113 ($M - H^+$), found 473.2108 ($M - H^+$); IR (KBr) ν 2955, 1669, 1467, 1362, 1253, 1074, 834, 775, 697; UV (MeCN) λ_{max} (log ε) 267 (3.94); TLC R_f 0.30 (1:1 petroleum ether-EtOAc); $[\alpha]_D^{20}$ +20.9 (*c* 1.0, $CHCl_3$).

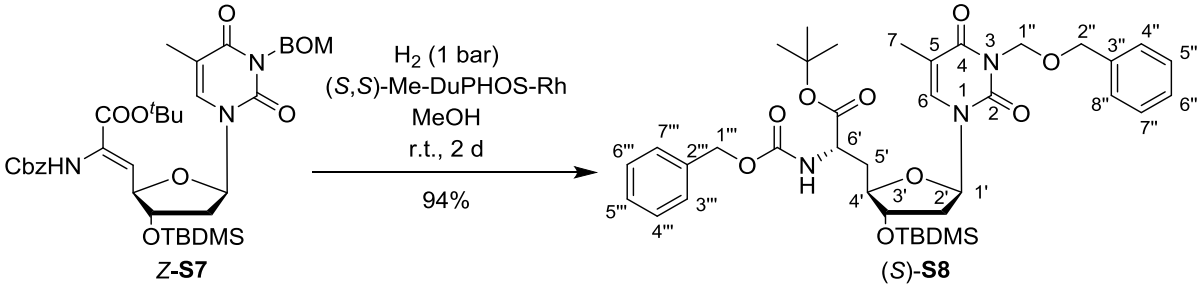
Didehydro thymidinyl amino acid esters *Z*-S7 and *E*-S7



A solution of phosphonate **S4**[S3-S6] (2.97 g, 7.96 mmol) in THF (60 mL) was slowly added to a precooled (-78 °C) solution of KO*t*-Bu (828 mg, 7.39 mmol) in THF (70 mL). After stirring for 5 min at -78 °C, a solution of 3'-*O*-(*tert*-butyldimethylsilyl)-3-*N*-(benzyloxymethyl)-thymidine-5'-aldehyde **S5** was slowly added at this temperature. The resultant solution was stirred for 16 h and slowly warmed to rt during this period. The reaction was then cooled to 0 °C, treated with MeOH (5 mL) and diluted with EtOAc (300 mL). The mixture was washed with water (2 x 100 mL) and brine (1 x 100 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude product was purified by column chromatography (4:1 petroleum ether-EtOAc) to give the desired product *Z*-**S7** as a colorless foam (3.81 g, 71%) and a minor amount of byproduct *E*-**S7** (138 mg, 3%). *Z*-**S7**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 6H, $SiCH_3$), 0.87 (s, 9H, $SiC(CH_3)_3$), 1.46 (s, 9H, *t*-Bu-CH_3), 1.91 (d, *J* = 1.2 Hz, 3H, 7-H), 2.10 (dd, *J* = 13.4, 6.7 Hz, 2'-H_a), 2.36 (ddd, *J* = 13.4, 6.3, 4.3 Hz, 1H, 2'-H_a), 4.32 (ddd, *J* = 6.3, 4.3, 4.3 Hz, 1H, 3'-H), 4.68 (s, 2H, 2''-H), 4.69 (dd, *J* = 7.8, 4.3 Hz, 1H, 4'-H), 5.08-5.15 (m, 2H, 1'''-H), 5.46 (s, 2H, 1''-H), 6.12 (d, *J* = 7.8 Hz, 1H, 5'-H), 6.18 (dd, *J* = 6.7, 6.7 Hz, 1H, 1'-H), 6.77 (brs, 1H, NH), 7.08 (d, *J* = 1.2 Hz, 1H, 6-H), 7.22-7.36 (m, 10H, aryl-

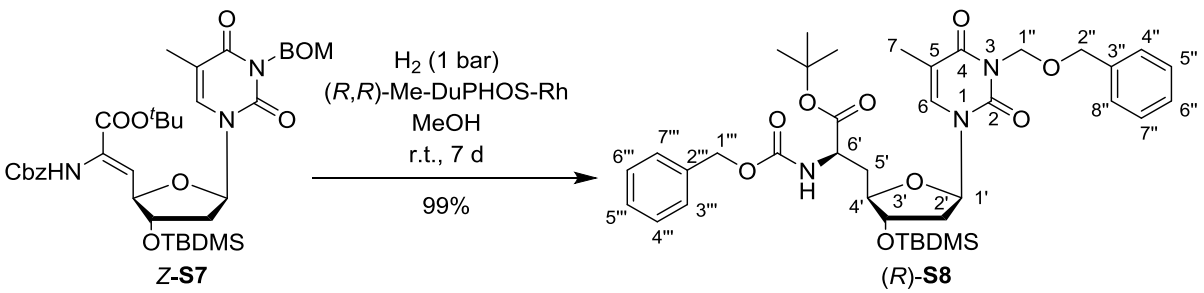
H); ^{13}C NMR (75 MHz, $CDCl_3$) δ -4.9, -4.8, 13.4, 17.9, 25.3, 25.6, 40.5, 67.6, 70.5, 72.2, 76.0, 77.2, 82.7, 86.8, 110.1, 124.7, 127.6, 127.6, 128.2, 128.2, 128.3, 128.5, 130.4, 134.0, 135.7, 137.9, 150.7, 153.5, 162.7, 163.3; HRMS (ESI$^+$) *m/z* calcd for $C_{38}H_{51}N_3NaO_9Si$ 744.3292 (M + Na^+), found 744.3296 (M + Na^+); IR (KBr) ν 2930, 1667, 1466, 1367, 1257, 1071, 837, 775, 698; UV (MeCN) λ_{max} (log ε) 191 (5.03), 205 (4.52), 263 (4.11); TLC R_f 0.51 (1:1 petroleum ether-EtOAc); $[\alpha]_D^{20}$ +51.1 (*c* 1.1, $CHCl_3$). *E*-**S7**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04 (s, 6H, $SiCH_3$), 0.87 (s, 9H, $SiC(CH_3)_3$), 1.52 (s, 9H, *t*-Bu-CH_3), 1.91 (d, *J* = 0.9 Hz, 3H, 7-H), 2.02 (ddd, *J* = 13.4, 7.7, 5.3 Hz, 1H, 2'-H_a), 2.36 (ddd, *J* = 13.4, 5.9, 2.5 Hz, 1H, 2'-H_b), 4.19 (ddd, *J* = 5.3, 2.5, 2.5 Hz, 1H, 3'-H), 4.67 (s, 2H, 2''-H), 5.09 (d, *J* = 12.6 Hz, 1H, 1'''-H_a), 5.14 (d, *J* = 12.6 Hz, 1H, 1'''-H_b), 5.38 (dd, *J* = 9.8, 2.5 Hz, 1H, 4'-H), 5.47 (s, 2H, 1''-H), 6.34 (dd, *J* = 7.7, 5.9 Hz, 1H, 1'-H), 6.76 (d, *J* = 9.8 Hz, 1H, 5'-H), 7.11 (s, 1H, Cbz-NH), 7.18-7.39 (m, 10H, aryl-H), 7.28 (s, 1H, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ -4.7, -4.7, 13.2, 18.0, 25.7, 28.0, 40.8, 67.1, 70.5, 72.1, 77.0, 83.2, 84.4, 86.2, 110.4, 120.2, 127.4, 127.6, 128.1, 128.3, 128.5, 128.7, 130.4, 134.0, 135.6, 137.9, 150.9, 153.0, 161.5, 163.34; HRMS (ESI$^+$) *m/z* calcd for $C_{38}H_{51}N_3NaO_9Si$ 744.3292 (M + Na^+), found 744.3296 (M + Na^+); IR (KBr) ν 2930, 1666, 1513, 1456, 1252, 1047, 836, 736, 698; UV (MeCN) λ_{max} (log ε) 191 (4.96), 263 (4.12); TLC R_f 0.16 (4:1 petroleum ether-EtOAc); $[\alpha]_D^{20}$ +18.4 (*c* 1.1, $CHCl_3$).

_N_-Cbz-Protected (6'_S_)-thymidinyl amino acid ester (_S_)-S8



The reaction was performed under strict exclusion of oxygen. Oxygen was removed from a solution of didehydro thymidinyl amino acid ester *Z*-**S7** (1.50 g, 2.07 mmol) in MeOH (72 mL) by a steady stream of argon over 15 min. (*S*,*S*)-Me-DuPHOS-Rh (25 mg, 41 µmol) was added and the solution was stirred under a hydrogen atmosphere (1 bar) for 2 d. The reaction mixture was then evaporated under reduced pressure. The resultant crude product was purified by column chromatography (4:1 petroleum ether-EtOAc) to give (*S*)-**S8** as a colorless oil (1.41 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04 (s, 6H, $SiCH_3$), 0.86 (s, 9H, $SiC(CH_3)_3$), 1.41 (s, 9H, *t*-Bu-CH_3), 1.96 (s, 3H, 7-H), 1.98-2.08 (m, 2H, 5'-H), 2.12-2.29 (m, 2H, 2'-H), 3.89-3.95 (m, 1H, 4'-H), 4.00-4.07 (m, 1H, 3'-H), 4.37-4.41 (m, 1H, 6'-H), 4.68 (s, 2H, 2"-H), 5.08 (s, 2H, 1'"-H), 5.47 (s, 2H, 1"-H), 5.52 (d, *J* = 6.9 Hz, 1H, NH), 6.20 (dd, *J* = 6.0, 6.0 Hz, 1H, 1'-H), 7.23-7.37 (m, 10H, aryl-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ -4.8, -4.5, 13.1, 17.9, 25.7, 28.0, 36.2, 40.3, 52.0, 66.9, 70.5, 72.2, 74.9, 82.7, 83.1, 85.7, 110.4, 127.5, 127.6, 128.0, 128.1, 128.2, 128.4, 134.3, 136.1, 137.9, 150.7, 155.3, 163.3, 170.3; HRMS (ESI$^+$) *m/z* calcd for $C_{38}H_{53}N_3NaO_9Si$ 746.3449 (M + Na^+), found 746.3453 (M + Na^+); IR (film) ν 2930, 1667, 1466, 1256, 1066, 837, 775, 736, 698; UV (MeCN) λ_{max} (log ε) 206 (4.43), 267 (3.97); TLC R_f 0.40 (7:3 petroleum ether-EtOAc); $[\alpha]_D^{20}$ +50.6 (*c* 1.0, $CHCl_3$).

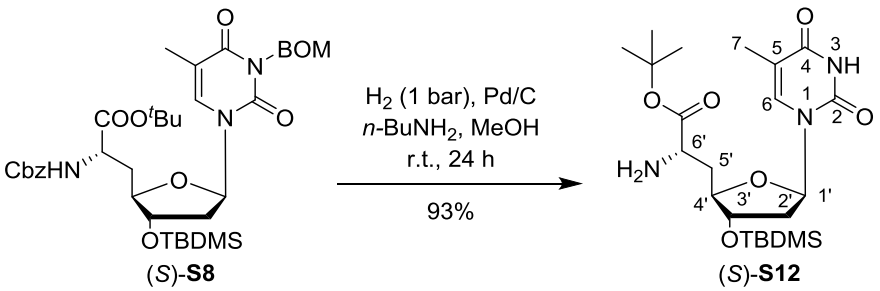
_N_-Cbz-Protected (6'_R_)-thymidinyl amino acid ester (_R_)-S8



The synthesis of (*R*)-**S8** was performed according to the protocol for the synthesis of (*S*)-**S8** with didehydro thymidinyl amino acid ester *Z*-**S7** (1.00 g, 1.39 mmol), (*R*,*R*)-Me-DuPHOS-

Rh (17 mg, 28 µmol), MeOH (48 mL) and a reaction time of 7 d to give (*R*)-**S8** as a colorless oil (990 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04 (s, 6H, $SiCH_3$), 0.86 (s, 9H, $SiC(CH_3)_3$), 1.41 (s, 9H, *t*-Bu-CH_3), 1.96 (s, 3H, 7-H), 1.98-2.08 (m, 2H, 5'-H), 2.12-2.29 (m, 2H, 2'-H), 3.89-3.95 (m, 1H, 4'-H), 4.00-4.07 (m, 1H, 3'-H), 4.37-4.41 (m, 1H, 6'-H), 4.68 (s, 2H, 2"-H), 5.08 (s, 2H, 1'"-H), 5.47 (s, 2H, 1"-H), 5.52 (d, *J* = 6.9 Hz, 1H, NH), 6.20 (dd, *J* = 6.0, 6.0 Hz, 1H, 1'-H), 7.23-7.37 (m, 10H, aryl-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ -4.8, -4.5, 13.1, 17.9, 25.7, 28.0, 36.2, 40.3, 52.0, 66.9, 70.5, 72.2, 74.9, 82.7, 83.1, 85.7, 110.4, 127.5, 127.6, 128.0, 128.1, 128.2, 128.4, 134.3, 136.1, 137.9, 150.7, 155.3, 163.3, 170.3; HRMS (ESI^+) *m/z* calcd for $C_{38}H_{53}N_3NaO_9Si$ 746.3449 ($M + Na^+$), found 746.3452 ($M + Na^+$); IR (film) ν 2930, 1667, 1466, 1256, 1066, 837, 775, 736, 698; UV (MeCN) λ_{max} (log ε) 206 (4.43), 267 (3.97); TLC R_f 0.40 (7:3 petroleum ether-EtOAc); $[\alpha]_D^{20}$ +50.6 (*c* 1.0, $CHCl_3$).

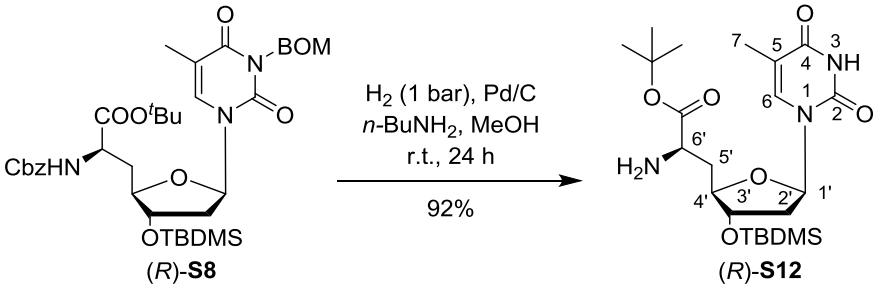
N-Deprotected (6'*S*)-thymidinyl amino acid ester (*S*)-S12



To a solution of *N*-Cbz-protected (6'*S*)-thymidinyl amino acid ester (*S*)-**S8** (1.20 g, 1.65 mmol) in MeOH (50 mL), Pd (10% on charcoal, 36 mg, 0.34 µmol Pd) and *n*-butylamine (3.5 mL, 2.6 g, 35 mmol) were added. The resultant suspension was stirred under a hydrogen atmosphere (1 bar) at rt for 24 h. It was then filtered through a celite pad and the pad was washed with MeOH (5 x 10 mL). The combined filtrates were evaporated under reduced pressure. The resultant crude product was purified by column chromatography (94:6 CH_2Cl_2-MeOH) to give (*S*)-**S12** as a pale yellowish solid (719 mg, 93%). ^{1}H NMR (300 MHz,

$CDCl_3$): δ 0.05 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$), 0.86 (s, 9H, $SiC(CH_3)_3$), 1.43 (s, 9H, *t*-Bu-CH_3), 1.75-1.85 (m, 1H, 2'-H_a), 1.92 (s, 3H, 7-H), 2.03-2.14 (m, 2H, 5'-H), 2.19-2.28 (m, 1H, 2'-H_b), 3.52 (dd, *J* = 6.2, 6.2 Hz, 1H, 6'-H), 3.89 (ddd, *J* = 8.8, 4.4, 4.4 Hz, 1H, 4'-H), 4.08 (m, 1H, 3'-H), 6.16 (dd, *J* = 6.5, 6.5 Hz, 1H, 1'-H), 7.16 (s, 1H, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ -4.9, -4.6, 12.6, 17.9, 25.6, 28.0, 38.1, 40.4, 53.1, 75.0, 81.4, 83.9, 84.6, 111.1, 135.6, 150.2, 163.7, 174.2; HRMS (ESI^+) *m/z* calcd for $C_{22}H_{39}N_3NaO_6Si$ 470.2686 ($M + Na^+$), found 470.2680 ($M + Na^+$); IR (KBr) ν 2958, 1697, 1471, 1367, 1255, 1155, 1069, 835, 609; UV (MeCN) λ_{max} (log ε) 265 (3.92); mp 78 °C; TLC R_f 0.36 (15:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +59.2 (*c* 1.0, $CHCl_3$).

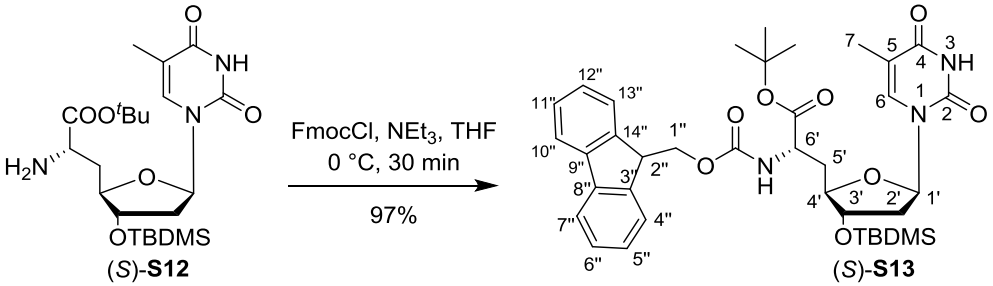
_N_-Deprotected (6'_R_)-thymidinyl amino acid ester (_R_)-S12



The synthesis of (*R*)-**S12** was performed according to the protocol for the synthesis of (*S*)-**S12** with *N*-Cbz-protected (6'*R*)-thymidinyl amino acid ester (*R*)-**S8** (1.26 g, 1.74 mmol), Pd (10% on charcoal, 36 mg, 0.34 μmol Pd), *n*-butylamine (3.5 mL, 2.6 g, 35 mmol) and MeOH (50 mL) to give (*R*)-**S12** as a pale yellowish solid (750 mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.07 (s, 6H, $SiCH_3$), 0.88 (s, 9H,$SiC(CH_3)_3$), 1.46 (s, 9H, *t*-Bu-CH_3), 1.82-1.90 (m, 1H, 2'-H_a), 1.91 (s, 3H, 7-H), 2.01-2.10 (m, 2H, 5'-H), 2.25 (ddd, *J* = 10.8, 6.4, 4.3 Hz, 1H, 2'-H_b), 3.51 (dd, *J* = 7.7, 4.1 Hz, 6'-H), 3.94-4.03 (m, 1H, 4'-H), 4.10 (ddd, *J* = 6.1, 4.3, 4.3 Hz, 1H, 3'-H), 6.19 (dd, *J* = 6.4, 6.4 Hz, 1H, 1'-H), 7.20 (s, 1H, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ -4.8, -4.7, 12.6, 17.9, 25.7, 28.0, 37.8, 40.3, 52.8, 75.1, 81.4, 83.6, 84.9, 111.0, 135.3, 150.2, 163.8, 174.9; HRMS (ESI^+) *m/z* calcd for $C_{22}H_{39}N_3NaO_6Si$ 470.2686

(M + Na^+), found 470.2680 (M + Na^+); IR (KBr) ν 2930, 1695, 1471, 1368, 1159, 1033, 837, 778, 602; UV (MeCN) λ_{max} (log ε) 265 (3.90); mp 114 °C; TLC R_f 0.36 (15:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +44.6 (*c* 1.0, $CHCl_3$).

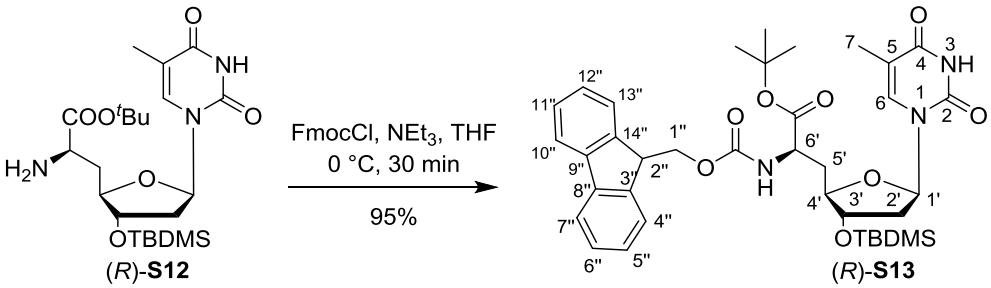
_N_-Fmoc-Protected (6'*S*)-thymidinyl amino acid ester (*S*)-S13



To a solution of *N*-deprotected (6'*S*)-thymidinyl amino acid ester (*S*)-**S12** (600 mg, 1.28 mmol) and NEt_3 (0.44 mL, 0.32 g, 3.2 mmol) in THF (10 mL), Fmoc-chloride (413 mg, 1.60 mmol) was added at 0 °C. After stirring at 0 °C for 30 min, the reaction was quenched with water (1 mL) and the mixture was diluted with EtOAc (20 mL). The organic layer was washed with water (2 x 10 mL) and brine (1 x 10 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude product was purified by column chromatography (7:3 petroleum ether-EtOAc) to give (*S*)-**S13** as a colorless foam (855 mg, 97%). ^{1}H NMR (300 MHz, C_6D_6, 70 °C): δ 0.00 (s, 3H, $SiCH_3$), 0.01 (s, 3H, $SiCH_3$), 0.90 (s, 9H, $SiC(CH_3)_3$), 1.31 (s, 9H, *t*-Bu-CH_3), 1.86 (d, *J* = 0.9 Hz, 3H, 7-H), 1.94-2.00 (m, 3H, 2'-H_a, 2'-H_b, 5'-H_a), 2.15 (ddd, *J* = 14.1, 6.8, 3.4 Hz, 1H, 5'-H_b), 3.89-3.96 (m, 1H, 4'-H), 4.00 (ddd, *J* = 6.8, 4.5, 4.5 Hz, 1H, 3'-H), 4.08 (dd, *J* = 6.8, 6.8 Hz, 1H, 2"-H), 4.37 (d, *J* = 6.8 Hz, 2H, 1"-H), 4.54 (m, 1H, 6'-H), 5.48 (d, *J* = 7.4 Hz, 1H, Fmoc-NH), 6.00 (dd, *J* = 6.1, 6.1 Hz, 1H, 1'-H), 6.92 (s, 1H, 6-H), 7.10-7.24 (m, 4H, 5"-H, 6"-H, 11"-H, 12"-H), 7.44-7.50 (m, 2H, 7"-H, 10"-H), 7.55-7.60 (m, 2H, 4"-H, 13"-H), 8.56 (brs, 1H, 3-NH); ^{13}C NMR (75 MHz, C_6D_6, 70 °C): δ -4.7, -4.5, 12.3, 18.1, 25.9, 27.9, 36.5, 40.1, 47.9, 52.8, 67.3, 75.7, 82.1, 83.6, 86.3, 111.0, 120.3, 125.4, 127.3, 128.3, 136.0, 141.9, 144.5, 144.6, 150.3, 155.9, 163.2, 170.9; HRMS

(ESI$^+$) *m/z* calcd for $C_{37}H_{49}N_3NaO_8Si$ 714.3187 (M + Na^+), found 714.3170 (M + Na^+); IR (KBr) ν 2954, 1712, 1470, 1368, 1253, 1156, 837, 778, 740; UV (MeCN) λ_{max} (log ε) 206 (4.70), 265 (4.39), 287 (3.76), 299 (3.73); mp 84 °C; TLC R_f 0.56 (15:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +42.1 (*c* 1.1, $CHCl_3$).

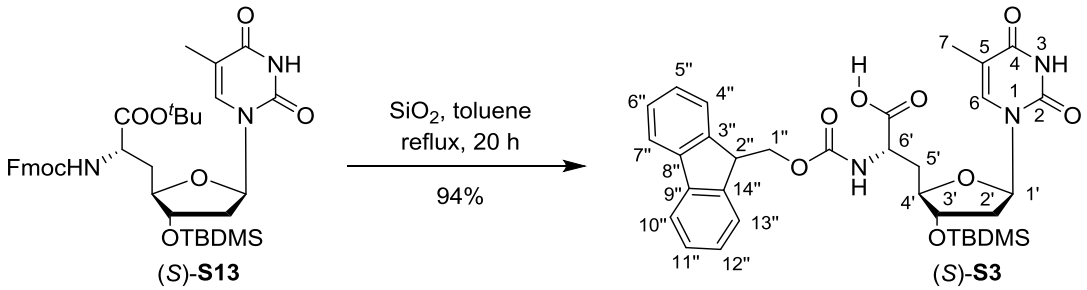
_N_-Fmoc-Protected (6'*R*)-thymidinyl amino acid ester (*R*)-S13



The synthesis of (*R*)-**S13** was performed according to the protocol for the synthesis of (*S*)-**S13** with *N*-deprotected (6'*R*)-thymidinyl amino acid ester (*R*)-**S12** (570 mg, 1.22 mmol), Fmoc-chloride (393 mg, 1.52 mmol), NEt_3 (0.42 mL, 0.31 g, 3.0 mmol) and THF (10 mL) to give (*R*)-**S13** as a colorless foam (800 mg, 95%). ^{1}H NMR (300 MHz, C_6D_6, 70 °C): δ 0.01 (s, 3H, $SiCH_3$), 0.03 (s, 3H, $SiCH_3$), 0.90 (s, 9H, $SiC(CH_3)_3$), 1.35 (s, 9H, *t*-Bu-CH_3), 1.73 (d, *J* = 1.2 Hz, 3H, 7-H), 1.96 (dd, *J* = 12.6, 6.1 Hz, 2H, 2'-H), 2.10 (dd, *J* = 5.7, 5.7 Hz, 2H, 5'-H), 3.87-3.95 (m, 1H, 4'-H), 4.04-4.13 (m, 2H, 3'-H, 2"-H), 4.31 (dd, *J* = 10.6, 7.0 Hz, 1H, 1"-H_a), 4.40 (dd, *J* = 10.6, 7.0 Hz, 1H, 1"-H_b), 4.56 (ddd, *J* = 8.5, 5.7, 5.7 Hz, 1H, 6'-H), 5.69 (d, *J* = 8.5 Hz, 1H, Fmoc-NH), 5.76 (dd, *J* = 6.1, 6.1 Hz, 1H, 1'-H), 6.62 (d, *J* = 1.2 Hz, 1H, 6-H), 7.12-7.25 (m, 4H, 5"-H, 6"-H, 11"-H, 12"-H), 7.45 (d, *J* = 7.3 Hz, 1H, 7"-H), 7.51 (d, *J* = 7.3 Hz, 1H, 10"-H), 7.56 (d, *J* = 7.2 Hz, 2H, 4"-H, 13"-H), 9.20 (brs, 1H, 3-NH); ^{13}C NMR (75 MHz, C_6D_6, 70 °C): δ -4.7, -4.6, 12.4, 18.1, 25.9, 28.0, 35.4, 40.0, 47.8, 53.5, 67.3, 75.7, 81.9, 83.6, 87.3, 110.9, 120.1, 120.3, 125.4, 125.6, 127.4, 127.9, 128.1, 128.4, 136.1, 141.8, 144.5, 144.6, 150.4, 156.2, 163.5, 171.1; HRMS (ESI$^+$) *m/z* calcd for $C_{37}H_{49}N_3NaO_8Si$ 714.3187 (M + Na^+), found 714.3180 (M + Na^+); IR (KBr) ν 2954, 1714, 1471, 1368, 1155,

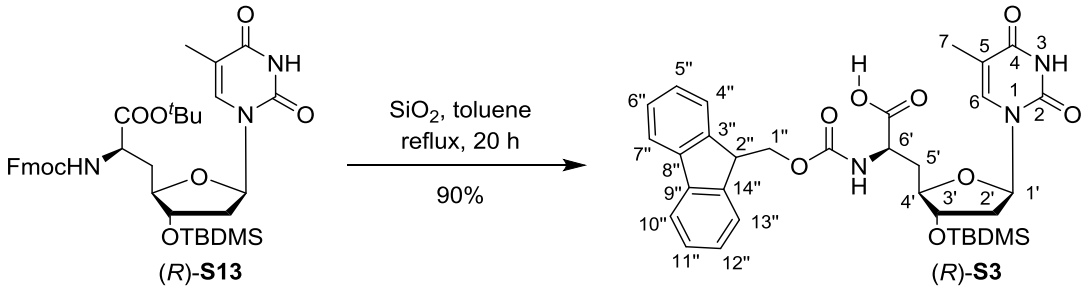
1051, 837, 778, 740; UV (MeCN) λ_{max} (log ε) 206 (4.73), 265 (4.43), 287 (3.81), 299 (3.78); mp 84 °C; TLC R_f 0.56 (15:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +47.9 (*c* 1.0, $CHCl_3$).

_N_-Fmoc-Protected (6'*S*)-thymidinyl amino acid (*S*)-S3



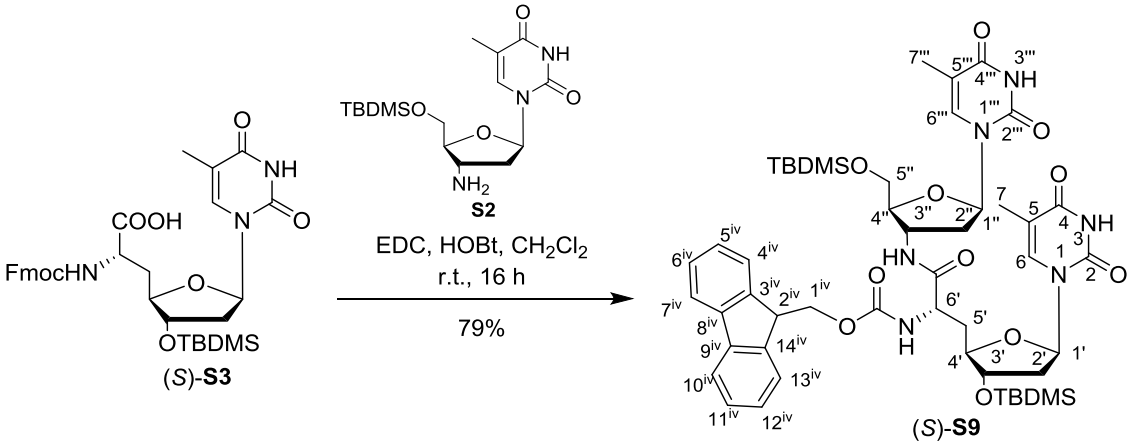
To a solution of *N*-Fmoc-protected (6'*S*)-thymidinyl amino acid ester (*S*)-**S13** (820 mg, 1.19 mmol) in anhydrous toluene (25 mL), silica (1.77 g) was added and the resultant suspension was heated under reflux for 20 h. After cooling to rt, the suspension was filtered and the silica residue was washed with a CH_2Cl_2/MeOH mixture (1:1, 5 x 5 mL). The combined filtrates were evaporated under reduced pressure to give (*S*)-**S3** as a dark yellowish solid (710 mg, 94%). ^{1}H NMR (300 MHz, CD_3OD, 50 °C): δ 0.08 (s, 6H, $SiCH_3$), 0.88 (s, 9H, $SiC(CH_3)_3$), 1.84 (s, 3H, 7-H), 1.97-2.11 (m, 1H, 5'-H_a), 2.14 (dd, *J* = 6.8, 5.1 Hz, 2H, 2'-H), 2.18-2.31 (m, 1H, 5'-H_b), 3.93-4.04 (m, 1H, 4'-H), 4.18 (dd, *J* = 6.7, 6.7 Hz, 1H, 2''-H), 4.22-4.28 (m, 1H, 3'-H), 4.23-4.28 (m, 3H, 6'-H, 1''-H), 6.13 (dd, *J* = 6.8, 6.8 Hz, 1H, 1'-H), 7.26 (m, 2H, 6''-H, 11''-H), 7.32-7.38 (m, 2H, 5''-H, 12''-H), 7.42 (s, 1H, 6-H), 7.61 (d, *J* = 6.8 Hz, 1H, 10''-H), 7.62 (d, *J* = 7.3 Hz, 1H, 7''-H), 7.80 (d, *J* = 7.2 Hz, 2H, 4''-H, 13''-H). ^{13}C NMR (75 MHz, CD_3OD, 50 °C): δ -4.6, -4.5, 12.3, 18.7, 26.2, 36.7, 40.7, 48.2, 53.3, 68.0, 76.6, 85.5, 86.6, 111.9, 120.9, 126.1, 126.2, 128.1, 128.1, 128.8, 137.8, 142.6, 145.3, 145.3, 152.2, 158.2, 166.2, 175.4; HRMS (ESI$^-$) *m/z* calcd for $C_{33}H_{40}N_3O_8Si$ 634.2590 (M - H^+), found 634.2590 (M - H^+); IR (KBr) ν 2954, 1708, 1513, 1450, 1252, 1052, 836, 778, 739; UV (MeCN) λ_{max} (log ε) 206 (4.70), 265 (4.41), 287 (3.79), 299 (3.74); mp 119 °C; TLC R_f 0.19 (12:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +47.0 (*c* 1.1, $CHCl_3$).

***N*-Fmoc-Protected (6'*R*)-thymidinyl amino acid (*R*)-S3**



The synthesis of (*R*)-**S3** was performed according to the protocol for the synthesis of (*S*)-**S3** with *N*-Fmoc-protected (6'*R*)-thymidinyl amino acid ester (*R*)-**S13** (800 mg, 1.16 mmol), silica (1.75 g) and anhydrous toluene (25 mL) to give (*R*)-**S3** as a dark yellowish solid (660 mg, 90%). ^{1}H NMR (300 MHz, CD_3OD, 50 °C): δ 0.09 (s, 6H, $SiCH_3$), 0.89 (s, 9H, $SiC(CH_3)_3$), 1.83 (s, 3H, 7-H), 1.99-2.25 (m, 2H, 5'-H_a, 5'-H_b), 2.18 (dd, *J* = 6.7, 4.7 Hz, 2H, 2'-H), 3.92 (ddd, *J* = 9.7, 3.5, 3.5 Hz, 1H, 4'-H), 4.17 (dd, *J* = 6.9, 6.9 Hz, 1H, 2"-H), 4.23-4.29 (m, 1H, 3'-H), 4.29-4.38 (m, 3H, 6'-H, 1"-H), 6.19 (dd, *J* = 6.7, 6.7 Hz, 1H, 1'-H), 7.25 (dd, *J* = 7.4, 7.4 Hz, 2H, 6"-H, 11"-H), 7.34 (dd, *J* = 7.4, 7.4 Hz, 2H, 5"-H, 12"-H), 7.40 (s, 1H, 6-H), 7.61 (m, 2H, 7"-H, 10"-H), 7.70 (d, *J* = 7.5 Hz, 2H, 4"-H, 13"-H); ^{13}C NMR (75 MHz, CD_3OD, 50 °C): δ -4.6, -4.5, 12.3, 18.8, 26.3, 36.5, 40.6, 48.2, 53.2, 68.1, 76.8, 85.1, 86.8, 112.0, 120.9, 126.2, 126.2, 128.1, 128.1, 128.7, 137.7, 142.6, 145.2, 145.3, 152.2, 158.4, 166.2, 176.0; HRMS (ESI$^-$) *m/z* calcd for $C_{33}H_{40}N_3O_8Si$ 634.2590 (M - H^+), found 634.2590 (M - H^+); IR (KBr) ν 2954, 1707, 1528, 1450, 1253, 1047, 835, 778, 739; UV (MeCN) λ_{max} (log ε) 206 (4.65), 265 (4.35), 287 (3.73), 299 (3.70); mp 119 °C; TLC R_f 0.19 (12:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +33.3 (*c* 1.0, $CHCl_3$).

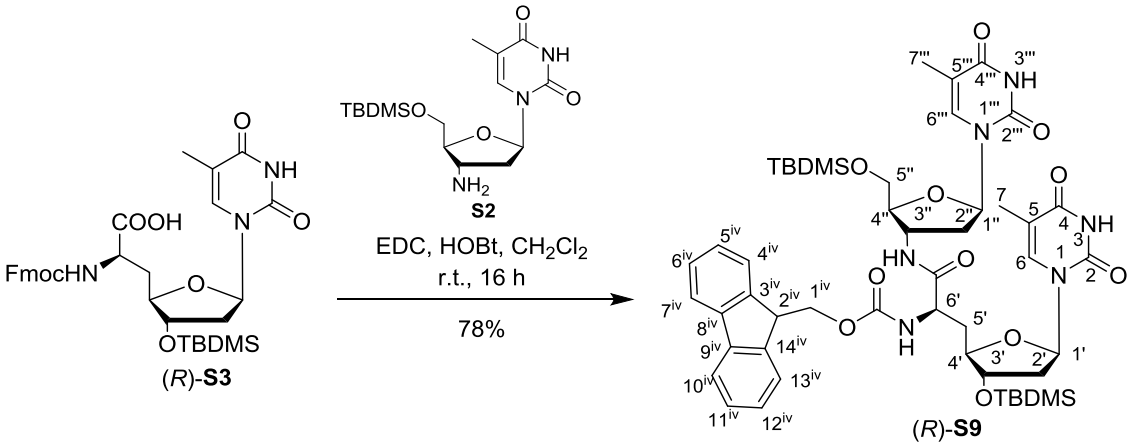
Bis-*O*-TBDMS-protected (6'*S*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*S*)-S9



To a solution of *N*-Fmoc-protected (6'*S*)-thymidinyl amino acid (*S*)-**S3** (190 mg, 0.299 mmol) in CH_2Cl_2 (3 mL), 1-hydroxybenzotriazole (HOBt, 47 mg, 0.35 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 68 mg, 0.35 mmol) were added and the resultant solution was stirred at rt for 45 min. Subsequently, 5'-*O*-(*tert*-butyldimethylsilyl)-3'-deoxy-3'-aminothymidine **S2**[S1,S2] (117 mg, 0.329 mmol) was added and the reaction mixture was stirred at rt for 16 h. The solution was then diluted with EtOAc (50 mL) and washed with sat. NH_4Cl solution (1 x 30 mL), water (1 x 30 mL) and brine (1 x 30 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude product was purified by column chromatography (1:2 petroleum ether-EtOAc) to give (*S*)-**S9** as a yellowish solid (230 mg, 79%). ^{1}H NMR (300 MHz, C_6D_6, 70 °C): δ 0.01-0.12 (m, 12H, $SiCH_3$), 0.92 (s, 18H, $SiC(CH_3)_3$), 1.96 (d, *J* = 0.9 Hz, 3H, 7'''-H), 1.98 (s, 3H, 7-H), 2.03-2.24 (m, 4H, 2'-H_2, 5'-H_a, 2''-H_a), 2.29-2.39 (m, 1H, 5'-H_b), 2.43-2.55 (m, 1H, 2''-H_b), 3.92 (s, 2H, 5''-H), 3.97-4.04 (m, 1H, 4'-H), 4.10-4.19 (m, 2H, 3'-H, 2^{iv}-H), 4.20-4.26 (m, 1H, 4''-H), 4.12 (d, *J* = 7.0 Hz, 2H, 1^{iv}-H), 4.56-4.65 (m, 1H, 3''-H), 4.81 (dd, *J* = 13.3, 7.1 Hz, 1H, 6'-H), 6.06 (dd, *J* = 6.7, 6.7 Hz, 1H, 1'-H), 6.33 (d, *J* = 7.1 Hz, 1H, 6'-NH), 6.45 (dd, *J* = 7.8, 5.7 Hz, 1H, 1''-H), 6.97 (s, 1H, 6-H), 7.16-7.23 (m, 4H, aryl-H), 7.54 (d, *J* = 0.9 Hz, 1H, 6'''-H), 7.49-7.62 (m, 4H, aryl-H), 8.10 (d, *J* = 5.8 Hz, 1H, 3''-NH), 10.42 (brs, 2H, 3-NH, 3'''-NH); ^{13}C NMR

(75 MHz, C_6D_6, 70 °C): δ -5.3, -5.3, -4.6, -4.5, 12.5, 12.7, 18.2, 18.6, 26.0, 26.2, 37.0, 37.9, 39.7, 47.9, 51.8, 64.6, 67.5, 76.1, 83.7, 85.7, 86.6, 87.2, 111.3, 111.5, 120.2, 125.5, 125.6, 127.4, 127.9, 135.3, 137.0, 141.8, 141.9, 144.6, 144.6, 151.1, 151.5, 156.4, 164.3, 164.4, 171.7; HRMS (ESI^+) *m/z* calcd for $C_{49}H_{68}N_6NaO_{11}Si_2$ 995.4382 ($M + Na^+$), found 995.4372 ($M + Na^+$); IR (KBr) ν 2954,1692, 1470, 1362, 1126, 835, 779, 740, 557; UV (MeCN) λ_{max} (log ε) 205 (4.79), 265 (4.54), 299 (3.75); mp 140 °C; TLC R_f 0.40 (12:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +9.5 (*c* 1.1, $CHCl_3$).

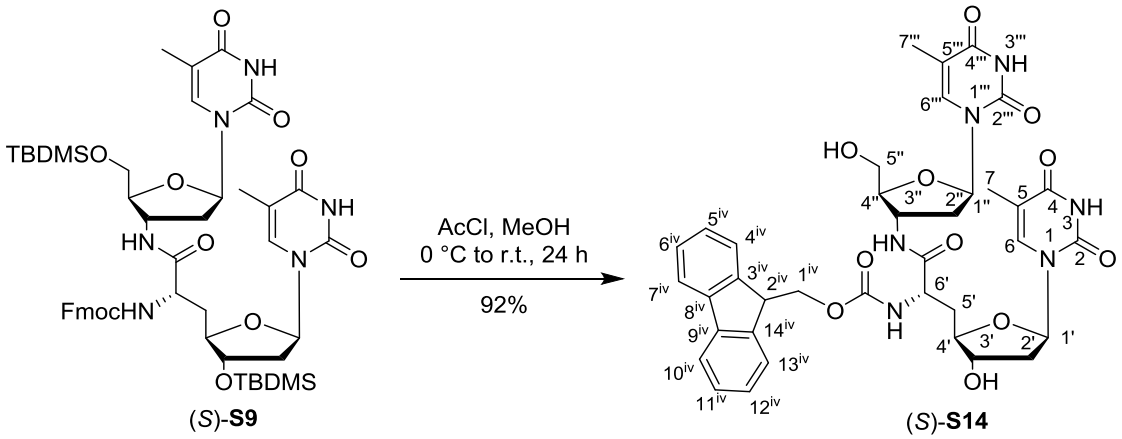
Bis-*O*-TBDMS-protected (6'*R*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*R*)-S9



The synthesis of (*R*)-**S9** was performed according to the protocol for the synthesis of (*S*)-**S9** with *N*-Fmoc-protected (6'*R*)-thymidinyl amino acid (*R*)-**S3** (150 mg, 0.236 mmol), 5'-*O*-(*tert*-butyldimethylsilyl)-3'-deoxy-3'-aminothymidine **S2**[S1,S2] (92 mg, 0.26 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 54 mg, 0.27 mmol), 1-hydroxybenzotriazole (HOBt, 37 mg, 0.27 mmol) and CH_2Cl_2 (3 mL) to give (*R*)-**S9** as a yellowish solid (180 mg, 78%). ^1H NMR (300 MHz, C_6D_6, 70 °C): δ 0.02 (s, 3H, $SiCH_3$), 0.04 (s, 3H, $SiCH_3$), 0.07 (s, 3H, $SiCH_3$), 0.08 (s, 3H, $SiCH_3$), 0.88 (s, 9H, $SiC(CH_3)_3$), 0.94 (s, 9H, $SiC(CH_3)_3$), 1.79 (d, *J* = 1.0 Hz, 3H, 7'''-H), 1.84-1.97 (m, 2H, 2'-H_a, 5'-H_a), 1.87 (d, *J* = 1.2 Hz, 3H, 7-H), 2.03-2.18 (m, 1H, 2''-H_a), 2.32 (ddd, *J* = 14.2, 11.2, 2.9 Hz, 1H, 5'-H_b), 2.48 (ddd, *J* = 6.3, 5.3,

1.4 Hz, 1H, 2''-H_b), 2.66 (ddd, *J* = 13.3, 7.3, 6.1 Hz, 1H, 2'-H_b), 3.72 (dd, *J* = 11.4, 2.0 Hz, 1H, 5''-H_a), 3.81 (dd, *J* = 11.4, 2.0 Hz, 1H, 5''-H_b), 4.06 (dd, *J* = 4.2, 2.0 Hz, 1H, 4''-H), 4.13-4.21 (m, 2H, 3'-H, 2^{iv}-H), 4.25 (ddd, *J* = 5.7, 2.4, 2.4 Hz, 1H, 4'-H), 4.45 (dd, *J* = 10.9, 7.2 Hz, 1H, 1^{iv}-H_a), 4.52 (dd, *J* = 10.9, 7.2 Hz, 1H, 1^{iv}-H_b), 4.63-4.74 (m, 1H, 3''-H), 4.90 (dd, *J* = 8.8, 2.9 Hz, 6'-H), 5.54 (dd, *J* = 7.3, 7.3 Hz, 1H, 1'-H), 5.91 (d, *J* = 8.8 Hz, 1H, 6'-NH), 6.44 (d, *J* = 1.0 Hz, 1H, 6'''-H), 6.61 (dd, *J* = 8.9, 5.3 Hz, 1''-H), 7.16-7.25 (m, 4H, aryl-H), 7.48 (d, *J* = 1.2 Hz, 1H, 6-H), 7.52-7.65 (m, 4H, aryl-H), 7.98 (d, *J* = 7.1 Hz, 1H, 3''-NH), 10.09 (brs, 1H, 3-NH), 10.35 (brs, 1H, 3'''-NH); ^{13}C NMR (75 MHz, C_6D_6, 70 °C): δ -5.3, -4.6, 12.3, 12.5, 18.2, 18.5, 26.0, 26.1, 37.0, 38.0, 38.1, 47.9, 51.6, 64.7, 67.7, 76.6, 84.5, 85.2, 87.51, 87.51, 110.5, 111.7, 120.1, 125.6, 125.7, 127.3, 127.9, 128.2, 134.8, 139.7, 141.8, 141.9, 144.6, 144.8, 150.9, 151.7, 157.3, 163.5, 164.5, 172.4; HRMS (ESI^+) *m/z* calcd for $C_{49}H_{68}N_6NaO_{11}Si_2$ 995.4382 ($M + Na^+$), found 995.4378 ($M + Na^+$); IR (KBr) ν 2929, 1692, 1470, 1259, 1125, 1074, 835, 778, 739; UV (MeCN) λ_{max} (log ε) 205 (4.80), 265 (4.54), 299 (3.74); mp 127 °C; TLC R_f 0.40 (12:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ -45.1 (*c* 1.0, $CHCl_3$).

Bis-*O*-deprotected (6'*S*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*S*)-S14

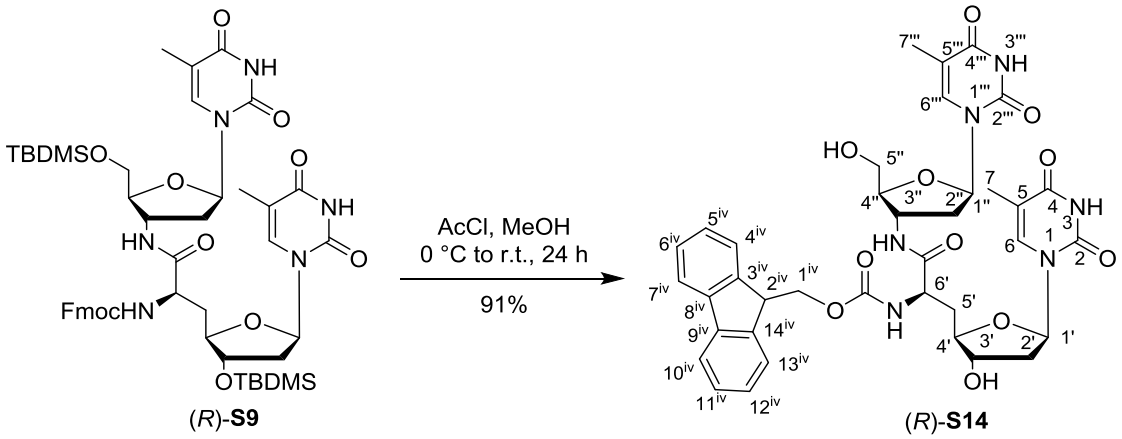


(*S*)-**S9** (*S*)-**S14**

To a solution of bis-*O*-TBDMS-protected (6'*S*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*S*)-**S9** (240 mg, 0.247 mmol) in MeOH (5 mL), acetyl chloride

(4.4 µL, 4.8 mg, 62 µmol, solution in MeOH (67 mM)) was added dropwise at 0 °C. The resultant solution was stirred at 0 °C for 30 min and then allowed to warm up to rt. After stirring at rt for 24 h, a viscous precipitate had formed. The suspension was treated with sat. $NaHCO_3$ solution (one drop) and pyridine (3 mL), thus furnishing a clear solution. To this solution, silica was added (~ one third of the volume of the solution) and the mixture was evaporated under reduced pressure. The resultant crude product (adsorbed on silica) was purified by column chromatography (9:1 CH_2Cl_2-MeOH) to give (*S*)-**S14** as a colorless solid (167 mg, 92%). ^{1}H NMR (300 MHz, pyridine-d_5, 50 °C): δ 1.87 (d, *J* = 0.9 Hz, 3H, 7'''-H), 2.01 (s, 3H, 7-H), 2.29-2.46 (m, 2H, 5'-H), 2.56 (ddd, *J* = 9.8, 6.7, 3.9 Hz, 1H, 2'-H_a), 2.62-2.70 (m, 2H, 2''-H), 2.69-2.79 (m, 1H, 2'-H_b), 4.17 (dd, *J* = 12.0, 2.8 Hz, 1H, 5''-H_a), 4.24 (dd, *J* = 12.0, 2.8 Hz, 1H, 5''-H_b), 4.31 (dd, *J* = 6.5, 6.5 Hz, 1H, 2^{iv}-H), 4.39 (ddd, *J* = 5.9, 2.8, 2.8 Hz, 1H, 4''-H), 4.47-4.61 (m, 3H, 3'-H, 4'-H, 1^{iv}-H), 5.06 (ddd, *J* = 8.3, 6.4, 6.4 Hz, 1H, 6'-H), 5.17 (ddd, *J* = 13.8, 6.9, 6.9 Hz, 1H, 3''-H), 6.70 (dd, *J* = 6.1, 6.1 Hz, 1H, 1''-H), 6.86 (dd, *J* = 6.7, 6.7 Hz, 1H, 1'-H), 7.28 (dd, *J* = 7.5, 7.5 Hz, 2H, 6^{iv}-H, 11^{iv}-H), 7.40 (dd, *J* = 7.5, 7.5 Hz, 2H, 5^{iv}-H, 12^{iv}-H), 7.56 (d, *J* = 0.9 Hz, 1H, 6-H), 7.69 (d, *J* = 7.5 Hz, 2H, 4^{iv}-H, 13^{iv}-H), 7.83 (d, *J* = 7.5 Hz, 2H, 7^{iv}-H, 10^{iv}-H), 8.07 (d, *J* = 1.1 Hz, 1H, 6'''-H), 8.89 (d, *J* = 8.3 Hz, 1H, 6'-NH), 9.73 (d, *J* = 6.9 Hz, 1H, 3''-NH), 12.87 (brs, 1H, 3'''-NH), 12.95 (brs, 1H, 3-NH); ^{13}C NMR (75 MHz, pyridine-d_5, 50 °C): δ 12.5, 12.6, 37.7, 38.4, 39.9, 47.9, 50.3, 53.6, 62.1, 66.8, 74.8, 84.6, 85.1, 85.5, 86.3, 110.4, 111.2, 120.4, 125.6, 125.6, 127.5, 128.1, 136.0, 136.4, 141.8, 144.6, 144.7, 150.1, 151.7, 151.8, 164.9, 164.9, 172.6; HRMS (ESI$^+$) *m/z* calcd for $C_{37}H_{40}N_6NaO_{11}$ 767.2653 (M + Na^+), found 767.2640 (M + Na^+); IR (KBr) ν 3493, 1693, 1530, 1472, 1274, 1057, 891, 738, 565; UV (MeCN) λ_{max} (log ε) 205 (4.74), 265 (4.48), 299 (3.76); mp 168 °C; TLC R_f 0.21 (9:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +18.4 (*c* 1.0, $CHCl_3$).

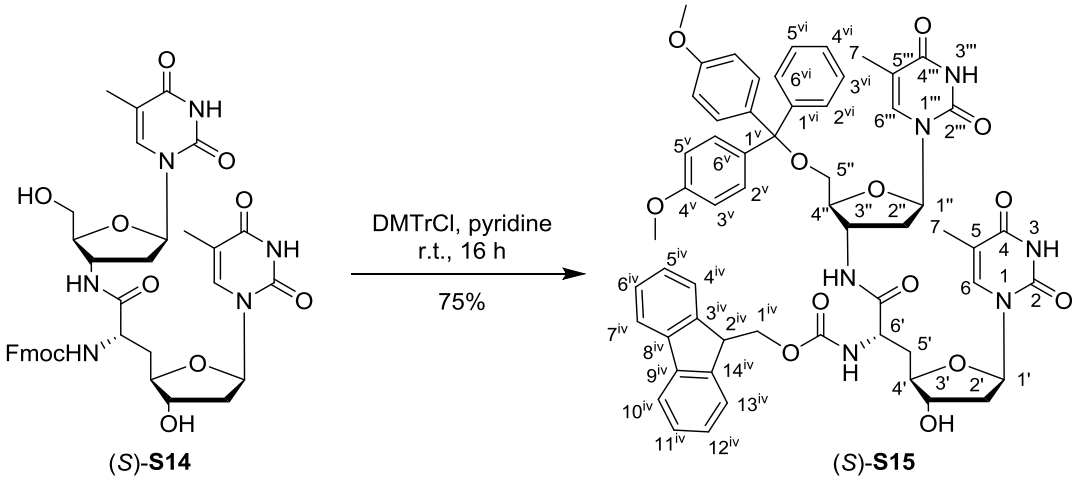
Bis-*O*-deprotected (6'*R*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*R*)-S14



The synthesis of (*R*)-**S14** was performed according to the protocol for the synthesis of (*S*)-**S14** with bis-*O*-TBDMS-protected (6'*R*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*R*)-**S9** (153 mg, 0.157 mmol), acetyl chloride (2.8 µL, 3.1 mg, 39 µmol, solution in MeOH (67 mM)) and MeOH (3.2 mL). For the workup of this reaction, no addition of pyridine was required as no precipitation occurred. The crude product (adsorbed on silica) was purified by column chromatography (9:1 CH_2Cl_2-MeOH) to give (*R*)-**S14** as a colorless solid (106 mg, 90%). ^{1}H NMR (300 MHz, pyridine-d_5, 50 °C): δ 1.87 (d, *J* = 1.0 Hz, 3H, 7'''-H), 2.01 (s, 3H, 7-H), 2.51-2.77 (m, 6H, 2'-H, 5'-H, 2''-H), 4.13 (dd, *J* = 12.2, 2.6 Hz, 1H, 5''-H_a), 4.20 (dd, *J* = 12.2, 2.6 Hz, 1H, 5''-H_b), 4.29 (dd, *J* = 7.1, 7.1 Hz, 1H, 2^{iv}-H), 4.36 (ddd, *J* = 5.8, 2.8, 2.8 Hz, 1H, 4''-H), 4.48-4.66 (m, 4H, 3'-H, 4'-H, 1^{iv}-H), 5.09 (ddd, *J* = 8.1, 5.9, 5.9 Hz, 1H, 6'-H), 5.13-5.21 (m, 1H, 3''-H), 6.66-6.78 (m, 2H, 1'-H, 1''-H), 7.29 (dd, *J* = 7.4, 7.4 Hz, 2H, 6^{iv}-H, 11^{iv}-H), 7.40 (dd, *J* = 7.4, 7.4 Hz, 2H, 5^{iv}-H, 12^{iv}-H), 7.56 (s, 1H, 6-H), 7.62-7.74 (m, 2H, 4^{iv}-H, 13^{iv}-H), 7.83 (d, *J* = 7.4 Hz, 2H, 7^{iv}-H, 10^{iv}-H), 8.08 (d, *J* = 1.2 Hz, 1H, 6'''-H), 8.73-8.82 (m, 1H, 6'-NH), 9.33 (d, *J* = 6.8 Hz, 1H, 3''-NH); ^{13}C NMR (75 MHz, pyridine-d_5, 50 °C): δ 12.6, 37.4, 38.5, 40.1, 47.8, 50.5, 53.9, 62.2, 66.9, 74.7, 83.9, 85.1, 86.1, 86.2, 110.6, 111.0, 120.4, 125.6, 125.7, 127.5, 128.1, 136.4, 136.9, 141.7, 144.6, 144.7, 150.1, 151.7, 151.8, 164.9, 164.9, 172.6; HRMS (ESI$^+$) *m/z* calcd for $C_{37}H_{40}N_6NaO_{11}$

767.2653 (M + Na^+), found 767.2640 (M + Na^+); IR (KBr) ν 3419, 3064, 1689, 1532, 1472, 1271, 1089, 741, 557; UV (MeCN) λ_{max} (log ε) 205 (4.60), 265 (4.35), 299 (3.56); mp 163 °C; TLC R_f 0.21 (9:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +38.5 (*c* 1.1, $CHCl_3$).

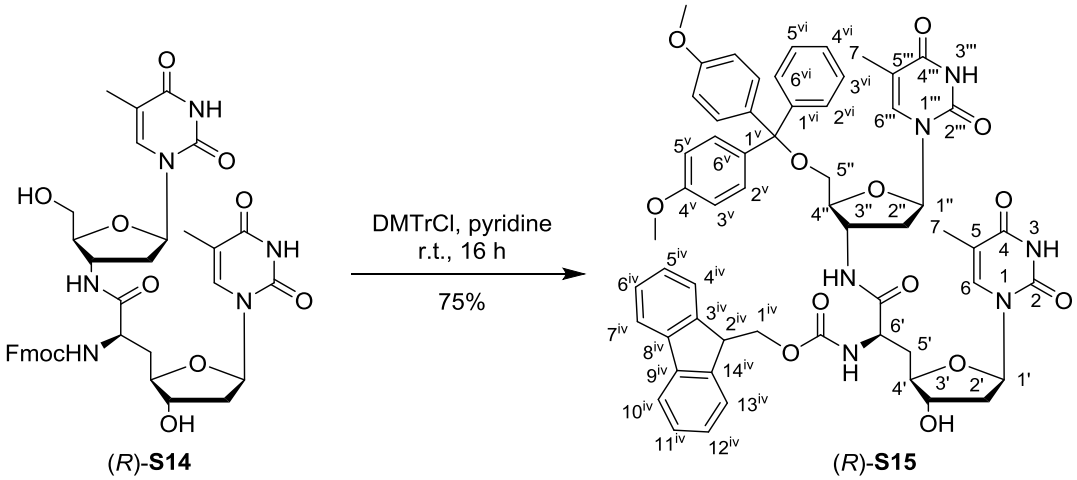
_O_-DMTr-Protected (6'_S_)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (_S_)-S15



Bis-*O*-deprotected (6'*S*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*S*)-**S14** (160 mg, 0.215 mmol) was coevaporated with pyridine (3 x 1 mL) and then dissolved in pyridine (2 mL). 4,4'-Dimethoxytrityl chloride (87 mg, 0.29 mmol) was added and the reaction mixture was stirred at rt for 16 h. The solvent was then evaporated under reduced pressure and the resultant residue was dissolved in EtOAc (30 mL). This solution was washed with sat. $NaHCO_3$ solution (1 x 20 mL), water (1 x 20 mL) and brine (1 x 20 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude product was purified by column chromatography (17:1 CH_2Cl_2-MeOH + 1% pyridine) to give (*S*)-**S15** as a colorless solid (169 mg, 75%). ^{1}H NMR (600 MHz, pyridine-d_5, 50 °C): δ 1.73 (s, 3H, 7-H), 2.02 (s, 3H, 7'''-H), 2.31-2.43 (m, 2H, 2'-H_a, 2''-H), 2.51-2.58 (m, 1H, 2'-H_b), 2.62-2.74 (m, 3H, 5'-H, 2''-H), 3.64-3.79 (m, 2H, 5''-H), 3.72 (s, 6H, OCH_3), 4.28-4.33 (m, 1H, 2^{iv}-H), 4.43-4.51 (m, 1H, 4''-H), 4.51-4.59 (m, 2H, 3'-H, 4'-H), 4.61-4.68 (m, 2H, 1^{iv}-H), 5.00-5.05 (m, 1H, 6'-H),

5.16-5.24 (m, 1H, 3''-H), 6.73-6.79 (m, 1H, 1''-H), 6.79-6.84 (m, 1H, 1'-H), 7.02 (d, J = 8.0 Hz, 4H, 3^{v}-H, 5^{v}-H), 7.24-7.34 (m, 3H, 6^{iv}-H, 11^{iv}-H, 4^{vi}-H), 7.35-7.47 (m, 4H, 5^{iv}-H, 12^{iv}-H, 3^{vi}-H, 5^{vi}-H), 7.56 (s, 1H, 6'''-H), 7.63 (d, J = 8.0 Hz, 4H, 2^{v}-H, 6^{v}-H), 7.67-7.71 (m, 2H, 2^{vi}-H, 6^{vi}-H), 7.78 (d, J = 7.8 Hz, 2H, 4^{iv}-H, 13^{iv}-H), 7.79 (s, 1H, 6-H), 7.83 (d, J = 7.4 Hz, 2H, 7^{iv}-H, 10^{iv}-H), 8.89 (brs, 1H, 6'-NH), 9.66 (brs, 1H, 3''-NH), 12.93 (brs, 2H, 3-NH, 3'''-NH); ^{13}C NMR (75 MHz, pyridine-d_5, 50 °C): δ 12.5, 12.7, 37.7, 38.5, 40.1, 48.0, 51.0, 53.6, 55.4, 64.4, 66.9, 74.9, 84.6, 85.1, 85.5, 87.3, 110.9, 111.2, 113.9, 120.4, 120.5, 125.6, 125.7, 127.4, 127.5, 128.1, 128.4, 128.9, 130.7, 130.8, 135.7, 135.8, 136.5, 136.5, 141.7, 144.6, 144.7, 145.7, 151.6, 151.7, 157.1, 159.2, 159.3, 164.8, 164.8, 172.4; HRMS (ESI^+) *m/z* calcd for $C_{58}H_{58}N_6NaO_{13}$ 1069.3960 ($M + Na^+$), found 1069.3949 ($M + Na^+$); IR (KBr) ν 1688, 1509, 1466, 1251, 1177, 1034, 829, 760, 585; UV (MeCN) λ_{max} (log ε) 200 (5.12), 238 (4.40), 265 (4.55), 299 (3.76); mp 135 °C; TLC R_f 0.18 (17:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +24.6 (*c* 1.0, $CHCl_3$).

_O_-DMTr-Protected (6'_R_)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (_R_)-S15

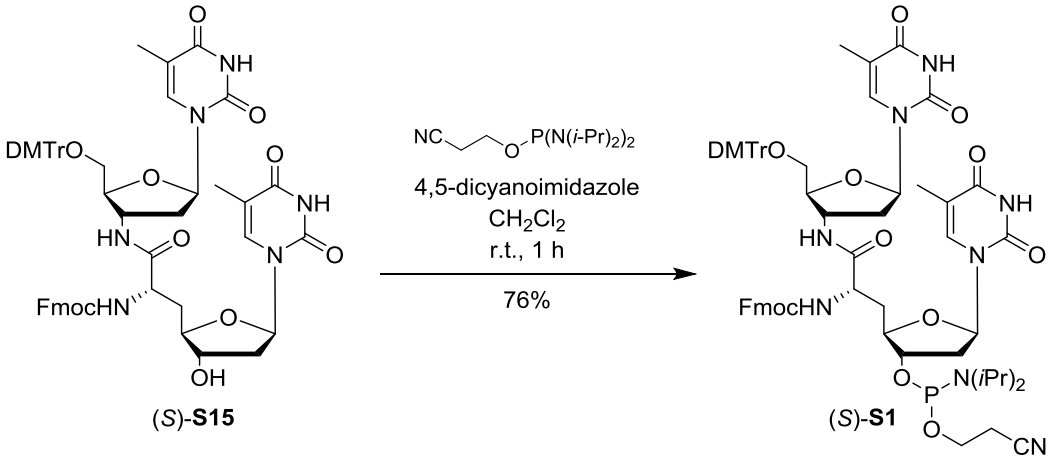


(*R*)-**S14** (*R*)-**S15**

The synthesis of (*R*)-**S15** was performed according to the protocol for the synthesis of (*S*)-**S15** with bis-*O*-deprotected (6'*R*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide

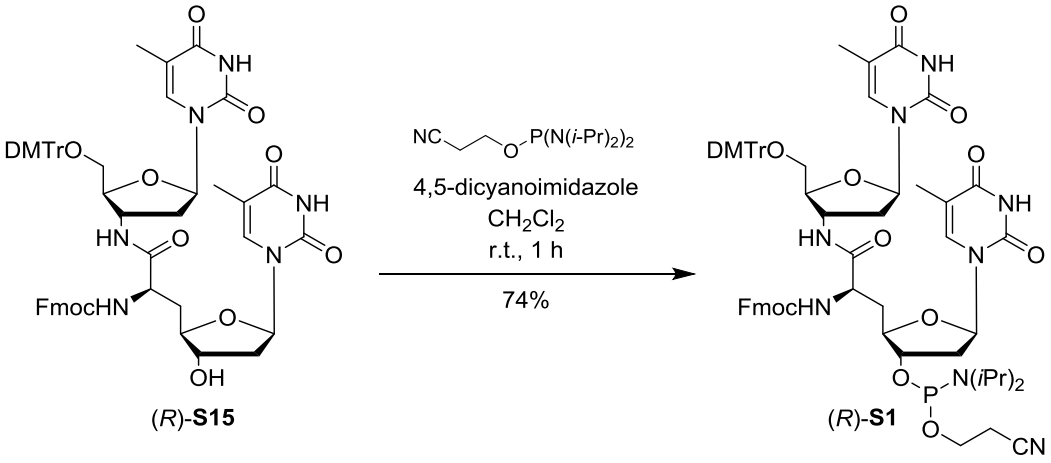
(*R*)-**S14** (230 mg, 0.309 mmol), 4,4'-dimethoxytrityl chloride (125 mg, 0.370 mmol) and pyridine (3 mL) to give (*R*)-**S15** as a colorless solid (241 mg, 75%). ^{1}H NMR (600 MHz, pyridine-d_5, 50 °C): δ 1.73 (s, 3H, 7-H), 2.01 (s, 3H, 7'''-H), 2.53-2.79 (m, 6H, 2'-H, 5'-H, 2''-H), 3.64-3.75 (m, 2H, 5''-H), 3.69 (s, 6H, OCH_3), 4.29 (dd, J = 6.9, 6.9 Hz, 1H, 2^{iv}-H), 4.44-4.46 (m, 1H, 4''-H), 4.48-4.56 (m, 2H, 3'-H, 4'-H), 4.59-4.63 (m, 2H, 1^{iv}-H), 5.04-5.10 (m, 1H, 6'-H), 5.19-5.27 (m, 1H, 3''-H), 6.71 (dd, J = 6.2, 6.2 Hz, 1H, 1'-H), 6.78 (dd, J = 6.5, 6.5 Hz, 1H, 1''-H), 7.00 (d, J = 8.8 Hz, 4H, 3^{v}-H, 5^{v}-H), 7.23-7.32 (m, 3H, 6^{iv}-H, 11^{iv}-H, 4^{vi}-H,), 7.36-7.43 (m, 4H, 5^{iv}-H, 12^{iv}-H, 3^{vi}-H, 5^{vi}-H), 7.58 (s, 1H, 6'''-H), 7.61 (d, J = 8.8 Hz, 4H, 2^{v}-H, 6^{v}-H), 7.64-7.71 (m, 2H, 2^{vi}-H, 6^{vi}-H), 7.75 (d, J = 7.5 Hz, 2H, 7^{iv}-H, 10^{iv}-H), 7.80 (s, 1H, 6-H), 7.83 (d, J = 7.6 Hz, 2H, 4^{iv}-H, 13^{iv}-H), 8.72 (brs, 1H, 6'-NH), 9.32 (brs, 1H, 3''-NH), 12.89 (brs, 2H, 3-NH, 3'''-NH); ^{13}C NMR (75 MHz, pyridine-d_5, 50 °C): δ 12.5, 12.8, 37.7, 38.6, 40.2, 48.0, 51.0, 54.0, 55.4, 64.3, 67.0, 74.8, 84.1, 85.1, 86.2, 87.3, 108.6, 111.0, 113.9, 114.0, 120.3, 120.4, 125.7, 127.3, 127.5, 128.1, 128.4, 128.9, 130.7, 130.8, 135.8, 136.4, 136.5, 141.7, 144.6, 144.7, 145.6, 151.6, 151.7, 157.2, 159.2, 159.2, 164.7, 164.8, 173.1; HRMS (ESI$^+$) *m/z* calcd for $C_{58}H_{58}N_6NaO_{13}$ 1069.3960 (M + Na^+), found 1069.3931 (M + Na^+); IR (KBr) ν 1681, 1507, 1436, 1247, 1175, 1066, 1029, 741, 702; UV (MeCN) λ_{max} (log ε) 198 (64.31), 257 (18.05), 263 (18.05), 300 (2.29); mp 172 °C; TLC R_f 0.18 (17:1 CH_2Cl_2-MeOH); $[\alpha]_D^{20}$ +9.3 (*c* 1.0, $CHCl_3$).

(6'*S*)-NAA-modified thymidine-thymidine β-cyanoethyl-*N*,*N*-diisopropylphosphor-amidite building block (*S*)-S1



O-DMTr-Protected (6'*S*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*S*)-**S15** (308 mg, 0.294 mmol) was coevaporated with pyridine (1 x 3 mL), toluene (1 x 3 mL) and MeCN (1 x 3 mL) and then dissolved in CH_2Cl_2 (3 mL). To this solution, 4,5-dicyanoimidazole (35 mg, 0.29 mmol) and a solution of 2-cyanoethyl *N*,*N*,*N'*,*N'*-tetraisopropyl phosphordiamidite (106 mg, 0.353 mmol) in CH_2Cl_2 (0.82 mL) were added. After stirring at rt for 1 h, the reaction mixture was diluted with CH_2Cl_2 (60 mL) and washed with sat. $NaHCO_3$ solution (1 x 30 mL). The aqueous layer was re-extracted with CH_2Cl_2 (1 x 15 mL). The combined organics were dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude product was purified by column chromatography (15:1 CH_2Cl_2-MeOH + 0.5% pyridine). Fractions containing the product were pooled and evaporated under reduced pressure. The thus obtained material was dissolved in CH_2Cl_2 (6 mL). This solution was slowly added to hexanes (60 mL) at -20 °C. The resultant fine precipitate was filtered off and dried under reduced pressure to give (*S*)-**S1** as a colorless powder (280 mg, 76%). ^{31}P NMR (121 MHz, pyridine-d_5): δ 149.01, 149.45; HRMS (ESI^+) *m/z* calcd for $C_{67}H_{75}N_8NaO_{14}P$ 1269.5033 ($M + Na^+$), found 1269.5033 ($M + Na^+$); TLC R_f 0.45 (EtOAc + 0.7% NEt_3, two spots).

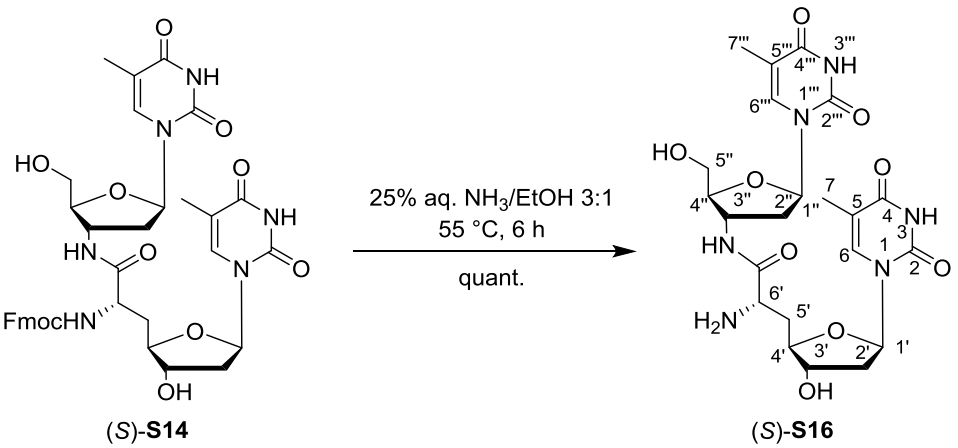
(6'*R*)-NAA-modified thymidine-thymidine β-cyanoethyl-*N*,*N*-diisopropylphosphor-amidite building block (*R*)-S1



(*R*)-**S15** (*R*)-**S1**

The synthesis of (*R*)-**S1** was performed according to the protocol for the synthesis of (*S*)-**S1** with *O*-DMTr-protected (6'*R*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*R*)-**S15** (230 mg, 0.220 mmol), 2-cyanoethyl *N*,*N*,*N'*,*N'*-tetraisopropyl phosphordiamidite (79 mg, 0.26 mmol), 4,5-dicyanoimidazole (26 mg, 0.22 mmol) and CH_2Cl_2 (2.3 mL) to give (*R*)-**S1** as a colourless powder (204 mg, 74%). ^{31}P NMR (121 MHz, pyridine-d_5): δ 147.34, 147.47; HRMS (ESI$^+$) *m/z* calcd for $C_{67}H_{75}N_8NaO_{14}P$ 1269.5033 (M + Na^+), found 1269.5032 (M + Na^+); TLC R_f 0.45 (EtOAc + 0.7% NEt_3, two spots).

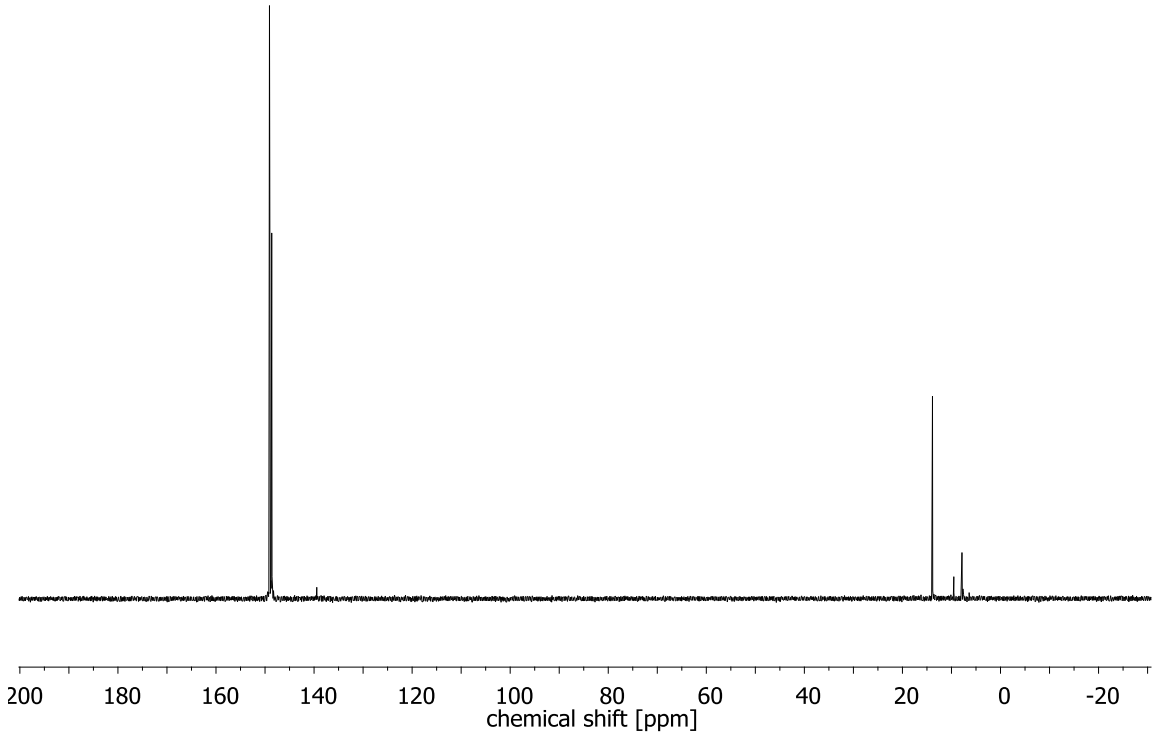
Fully deprotected (6'*S*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*S*)-S16



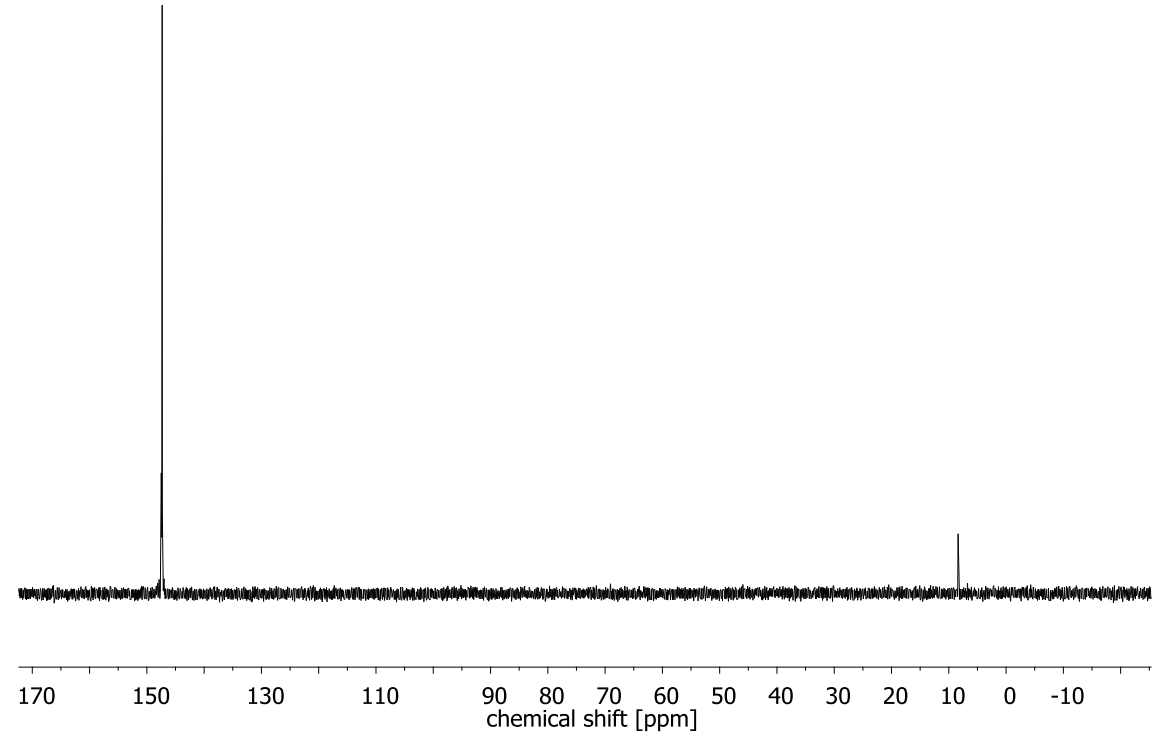
(*S*)-**S14** (*S*)-**S16**

A suspension of bis-*O*-deprotected (6'*S*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*S*)-**S14** (30 mg, 40 µmol) in a mixture of aq. 25% NH_3 and EtOH (3:1, 2 mL) was stirred at 55 °C in a closed reaction vessel for 6 h. The reaction mixture was then evaporated under reduced pressure. The resultant crude product was purified by reverse phase column chromatography (RP-silica, 1:1 MeCN-H_2O) to give (*S*)-**S16** as a colorless powder (21 mg, quant.). ^{1}H NMR (600 MHz, pyridine-d_5, 35 °C): δ 1.86 (d, *J* = 1.0 Hz, 3H, 7-H), 2.00 (d, *J* = 1.0, 3H, 7'''-H), 2.42-2.50 (m, 1H, 2''-H_a), 2.53 (dd, *J* = 13.6, 6,8 Hz, 1H, 2'-H_a), 2.56 (ddd, *J* = 13.6, 6.8, 4.8 Hz, 1H, 2'-H_b), 2.68 (dd, *J* = 6.5, 6.5 Hz, 2H, 5'-H), 2.70-2.76 (m, 1H, 2''-H_b),4.14 (dd, *J* = 12.1, 2.8 Hz, 1H, 5''-H_a), 4.21 (m, 1H, 3'-H), 4.22 (dd, *J* = 12.1, 2.8 Hz, 1H, 5''-H_b), 4.36 (dd, *J* = 6.5, 2.9 Hz, 1H, 4'-H), 4.50 (ddd, *J* = 8.9, 4.6 Hz, 1H, 3''-H), 4.63 (ddd, *J* = 6.7, 4.6 Hz, 1H, 4''-H), 5.14 (m, 1H, 6'-H), 6.74 (dd, *J* = 6.2, 6.2 Hz, 1H, 1''-H), 6.80 (dd, *J* = 6.8, 6.8 Hz, 1H, 1'-H), 7.62 (s, 1H, 6-H), 8.09 (s, 1H, 6'''-H), 9.32 (brs, 1H, NH); ^{13}C NMR (75 MHz, pyridine-d_5, 35 °C): δ 12.6, 12.7, 39.0, 38.4, 39.8, 49.9, 54.0, 61.9, 74.6, 84.9, 85.0, 85.1, 86.1, 110.4, 111.1, 136.2, 136.5, 150.2, 151.7, 164.9, 164.9, 174.9; HRMS (ESI$^+$) *m/z* calcd for $C_{22}H_{31}N_6O_9$ 523.2147 (M + H^+), found 523.2138 (M + H^+); IR (KBr) ν 1652, 1471, 1268, 1089, 1041, 966, 825, 764, 733; UV (MeCN) λ_{max} (log ε) 197 (2.45), 264 (1.09); mp 118 °C; TLC R_f 0.60 (5:2:1 *i*-PrOH-H_2O-AcOH); $[\alpha]_D^{20}$ +39.0 (*c* 1.0, pyridine).

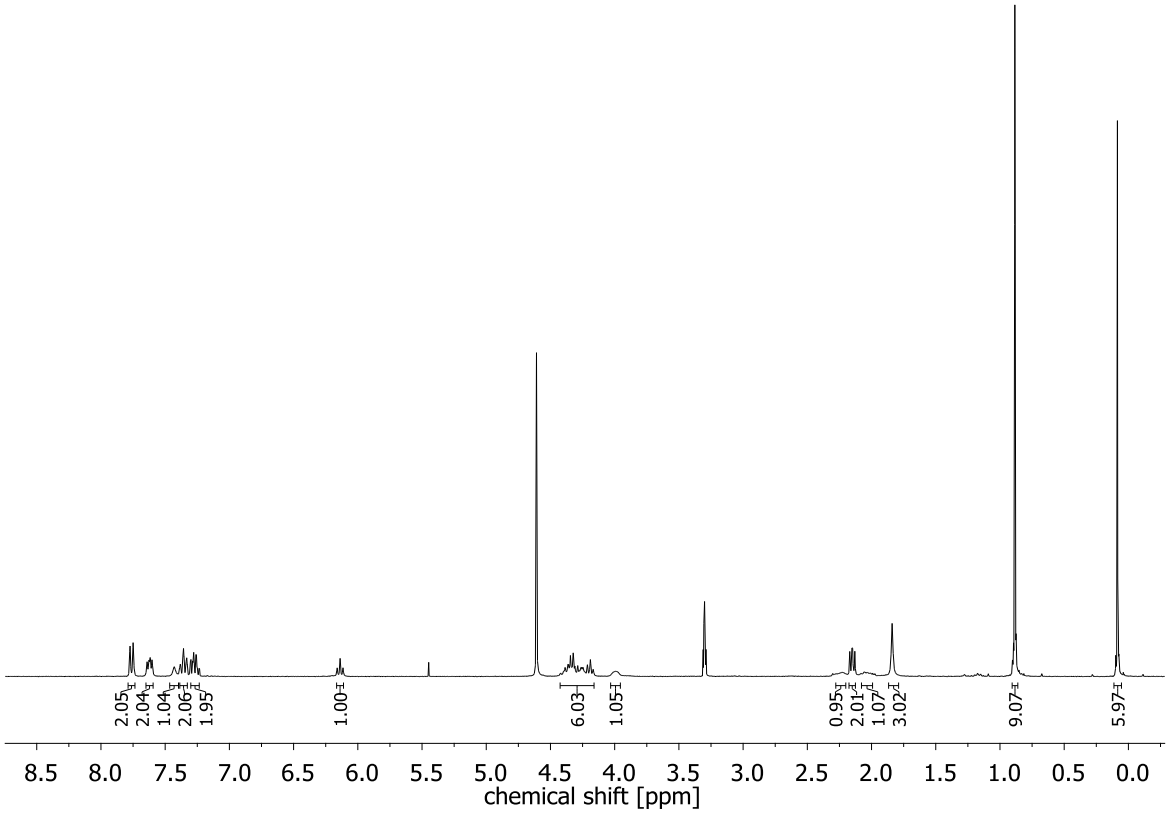
^{1}H, ^{13}C and ^{31}P NMR spectra of synthesised compounds



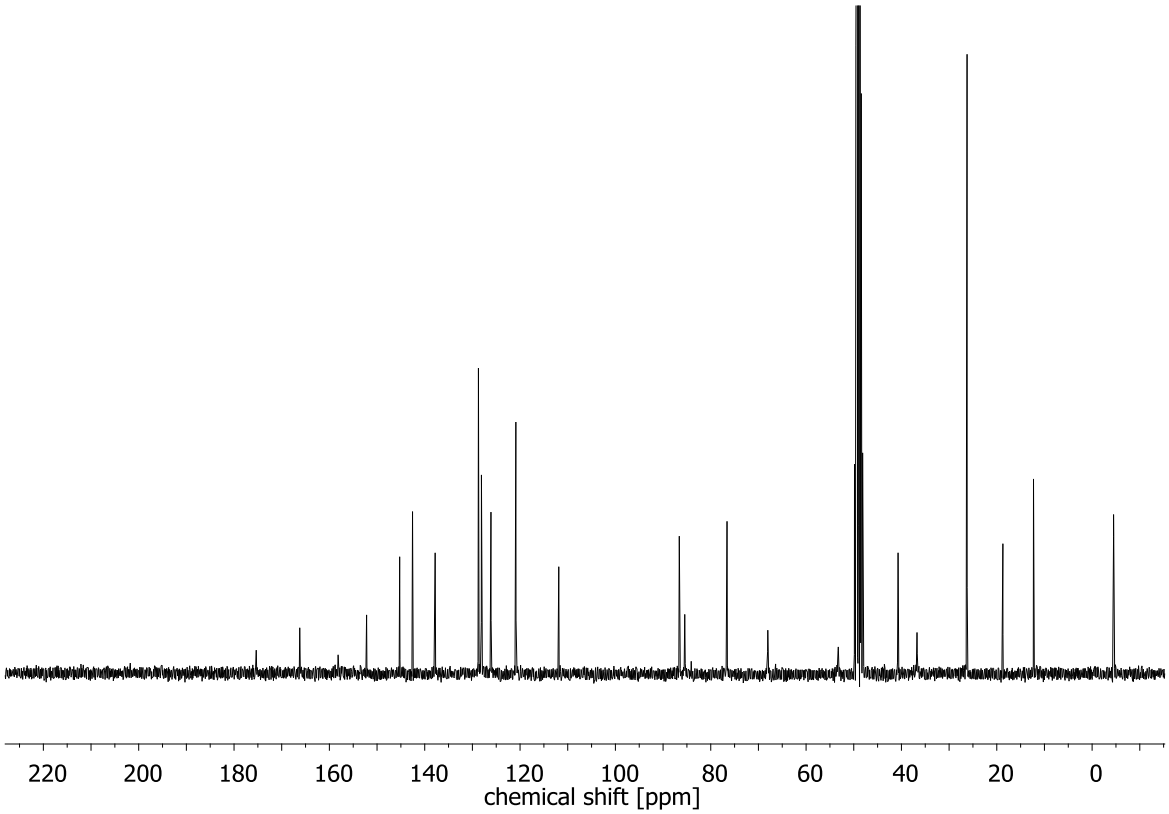
^{31}P NMR spectrum of (*S*)-**S1** (121 MHz, pyridine-d_5)



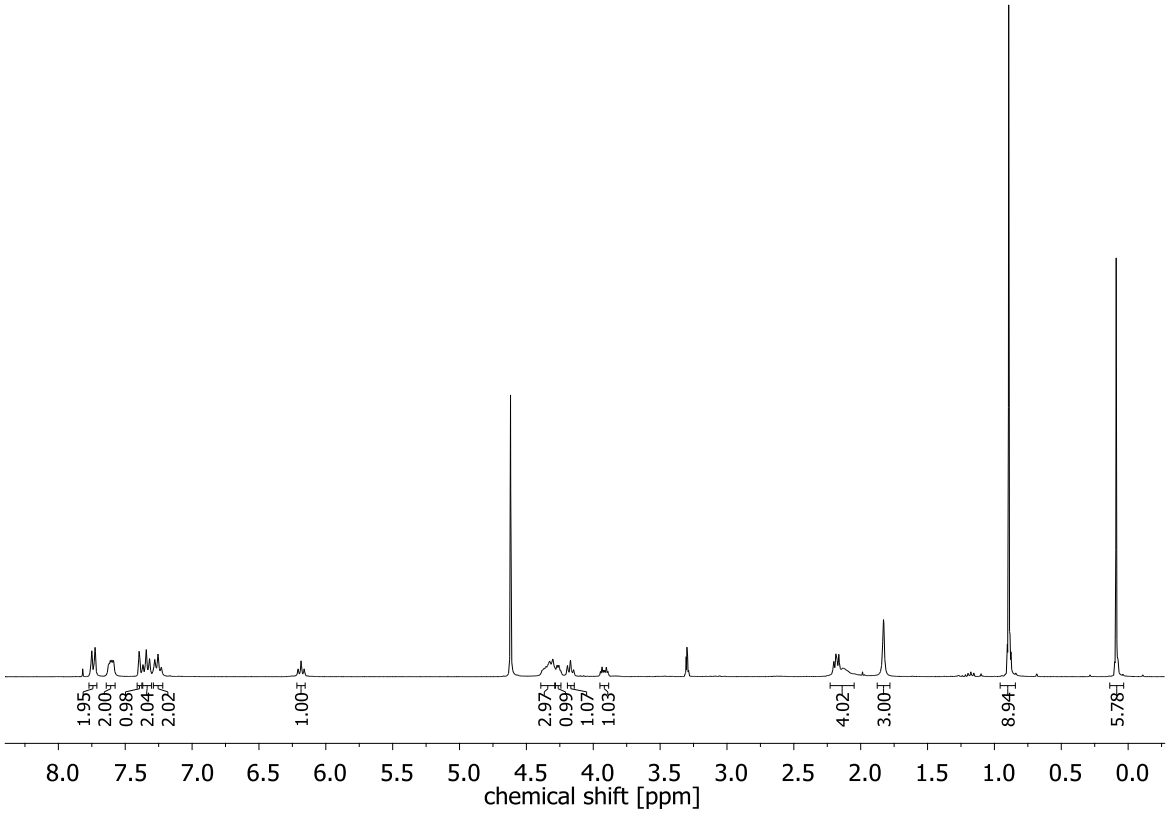
^{31}P NMR spectrum of (*R*)-**S1** (121 MHz, pyridine-d_5)



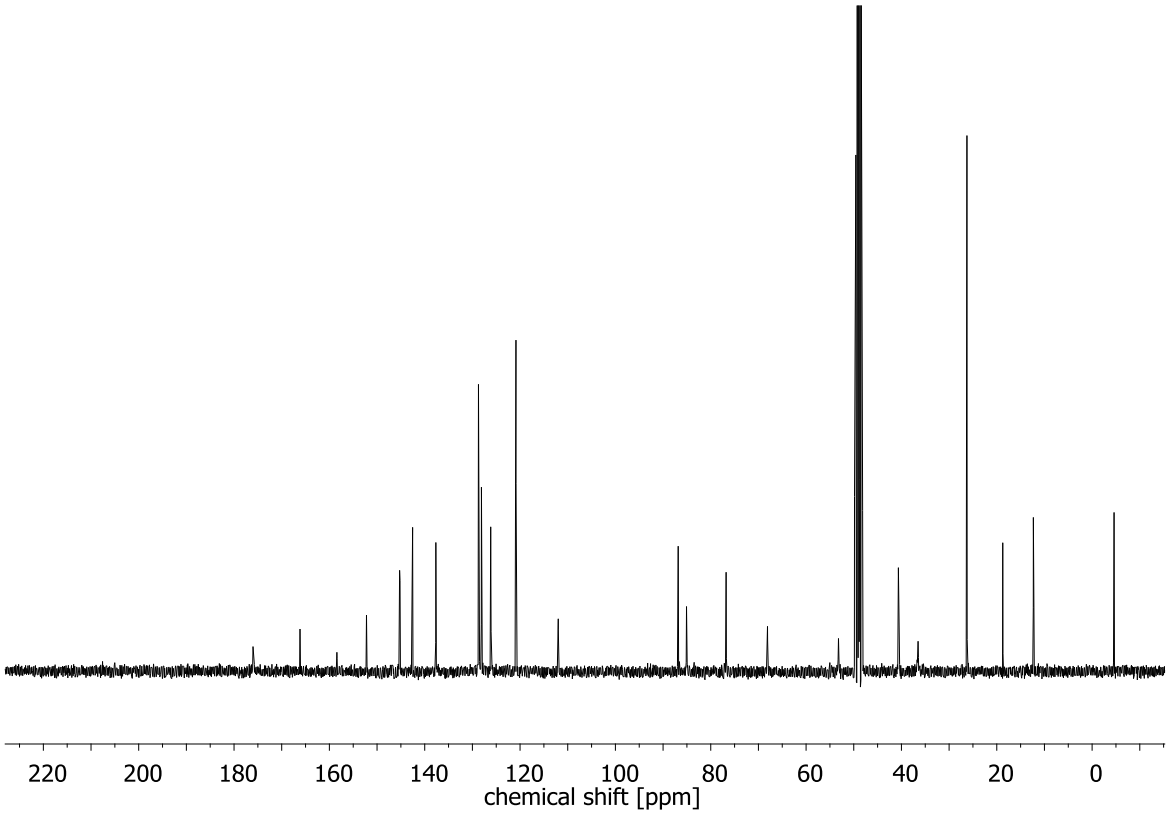
^{1}H NMR spectrum of (*S*)-**S3** (300 MHz, CD_3OD, 50 °C)



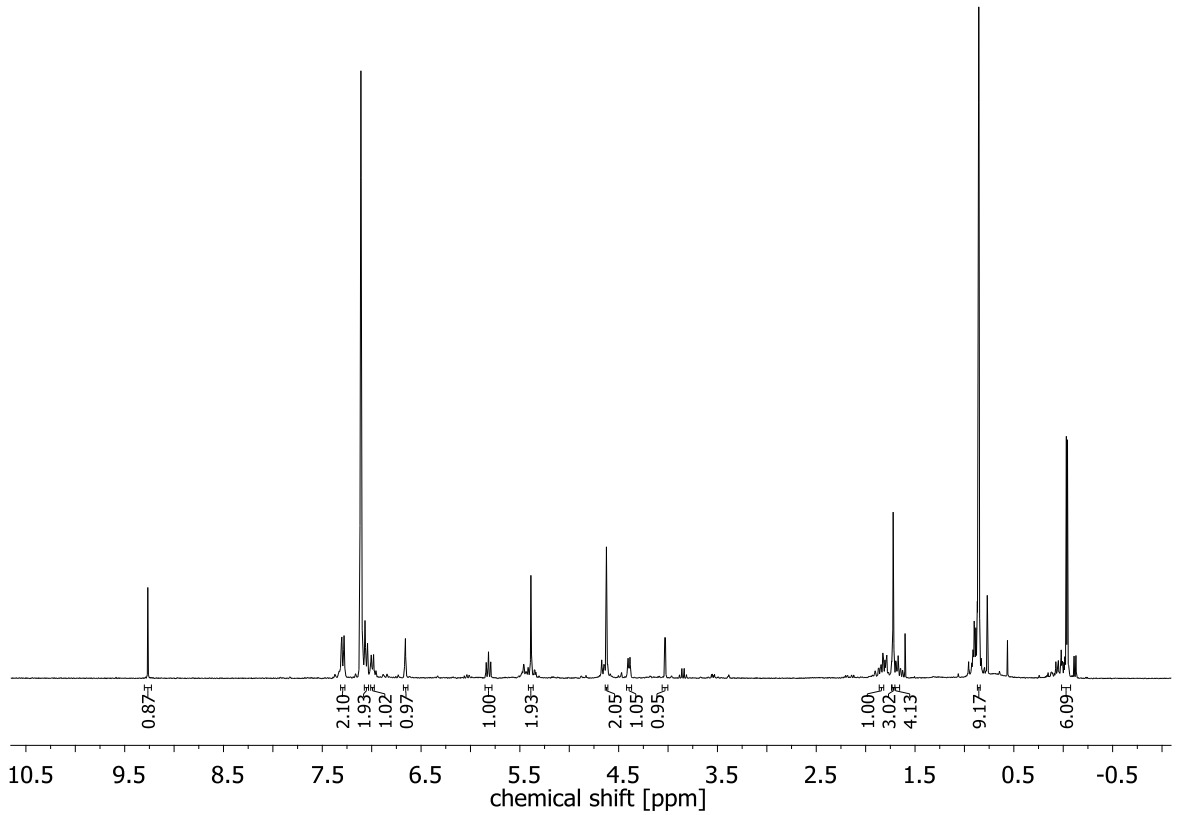
^{13}C NMR spectrum of (*S*)-**S3** (75 MHz, CD_3OD, 50 °C)



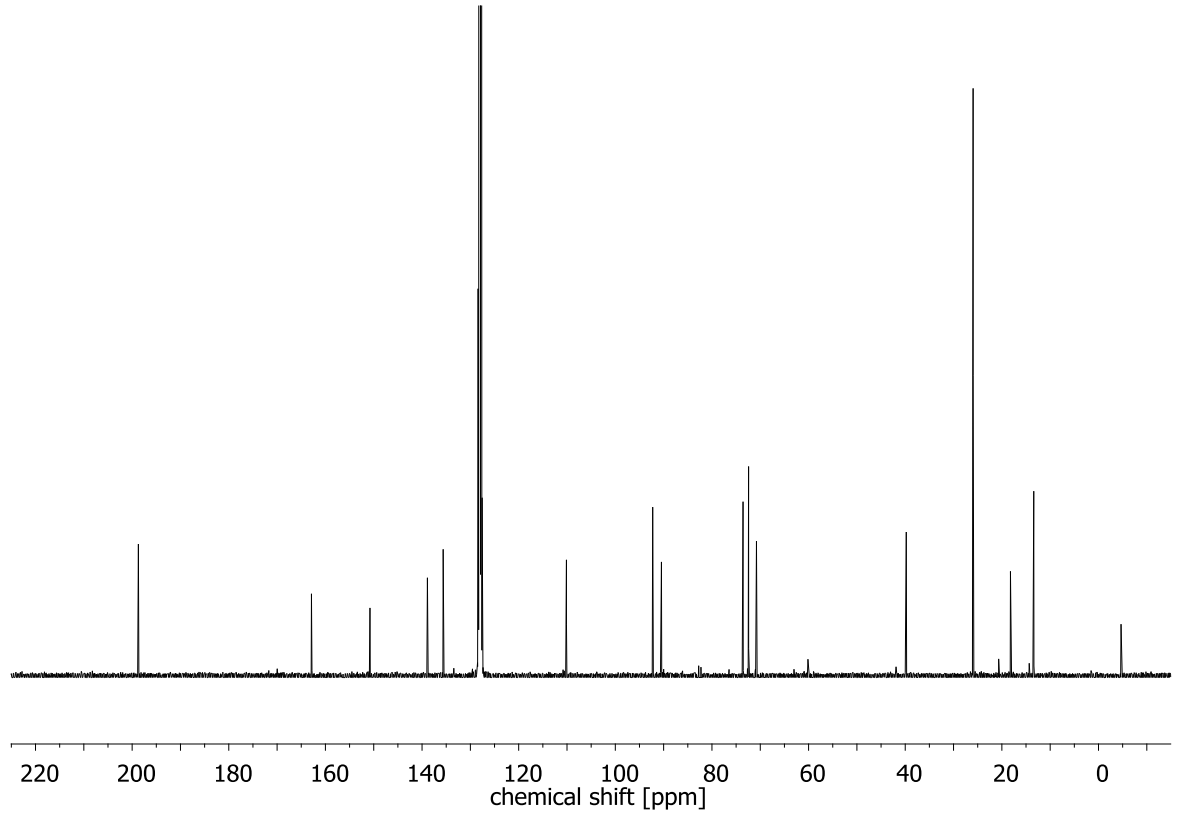
^{1}H NMR spectrum of (*R*)-**S3** (300 MHz, CD_3OD, 50 °C)



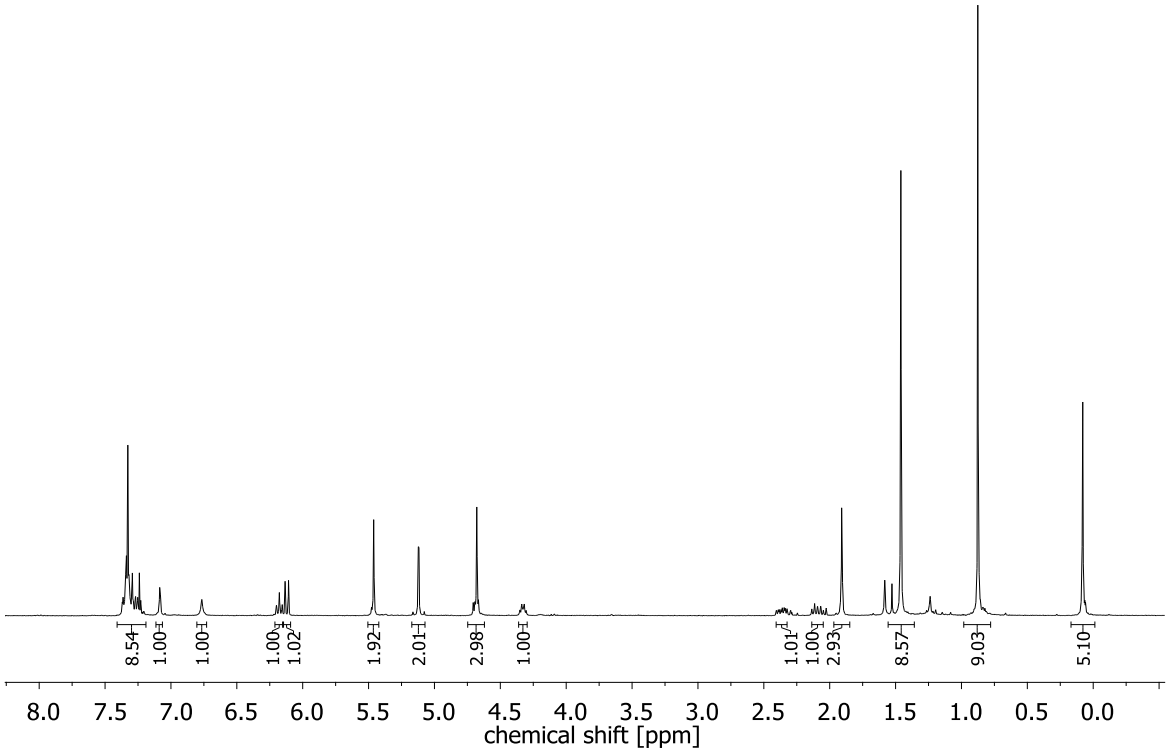
^{13}C NMR spectrum of (*R*)-**S3** (75 MHz, CD_3OD, 50 °C)



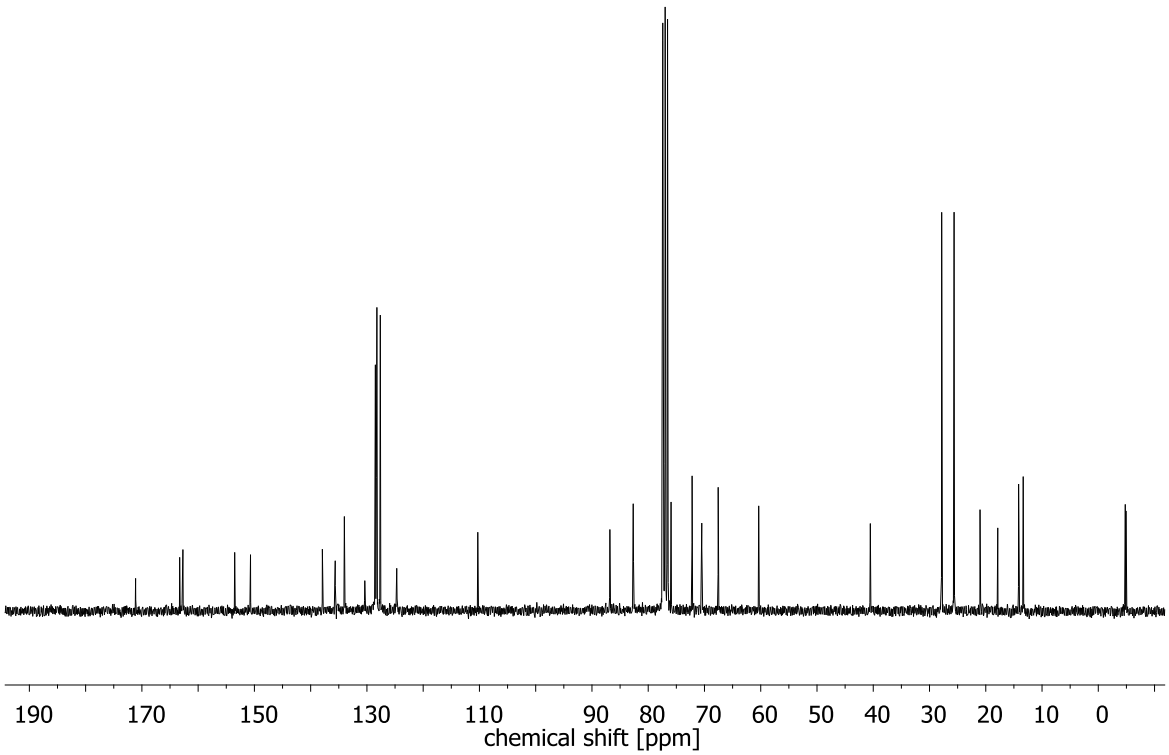
^{1}H NMR spectrum of **S5** (300 MHz, C_6D_6)



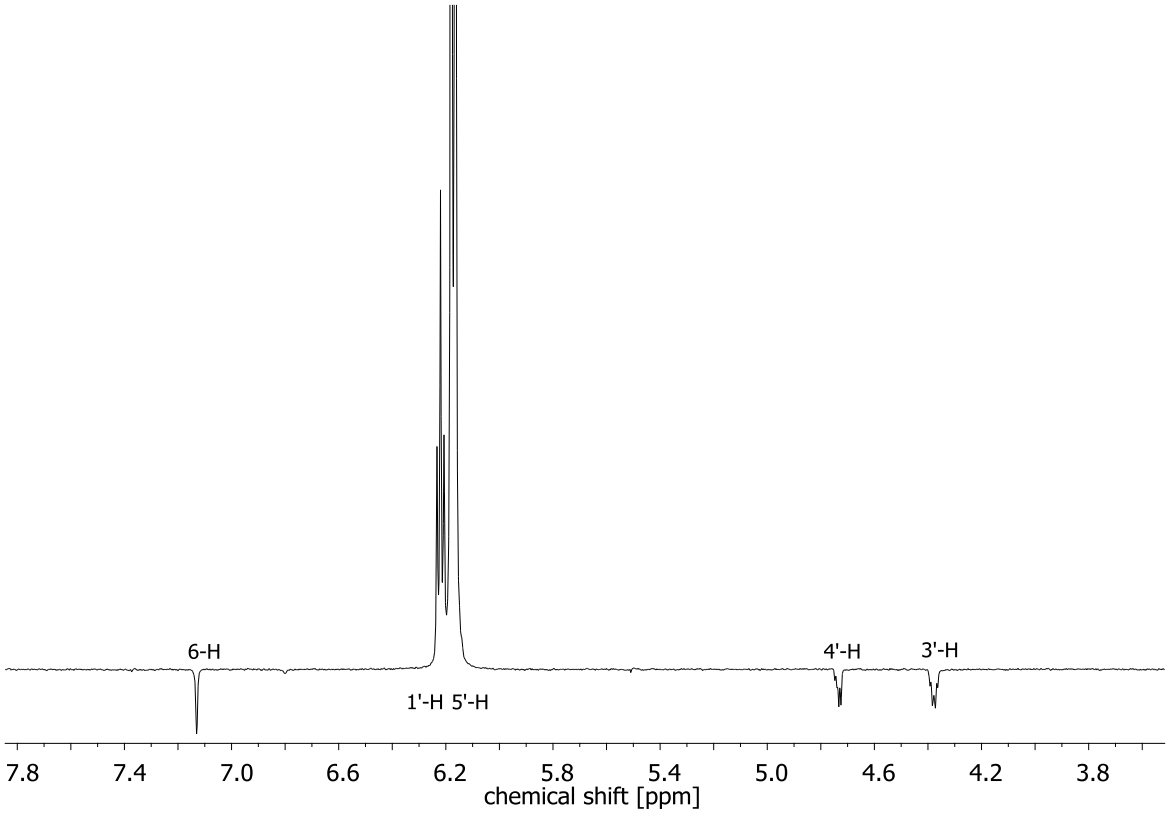
^{13}C NMR spectrum of **S5** (75 MHz, C_6D_6)



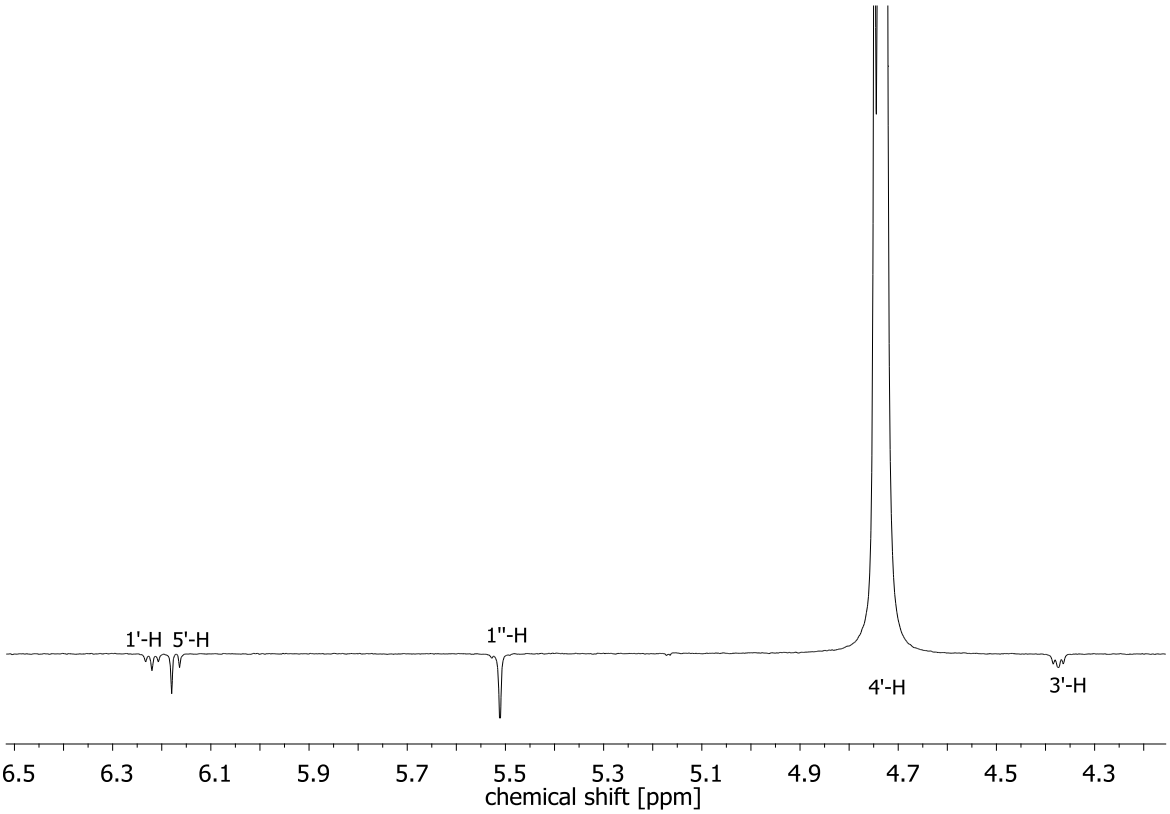
^{1}H NMR spectrum of *Z*-**S7** (300 MHz, $CDCl_3$)



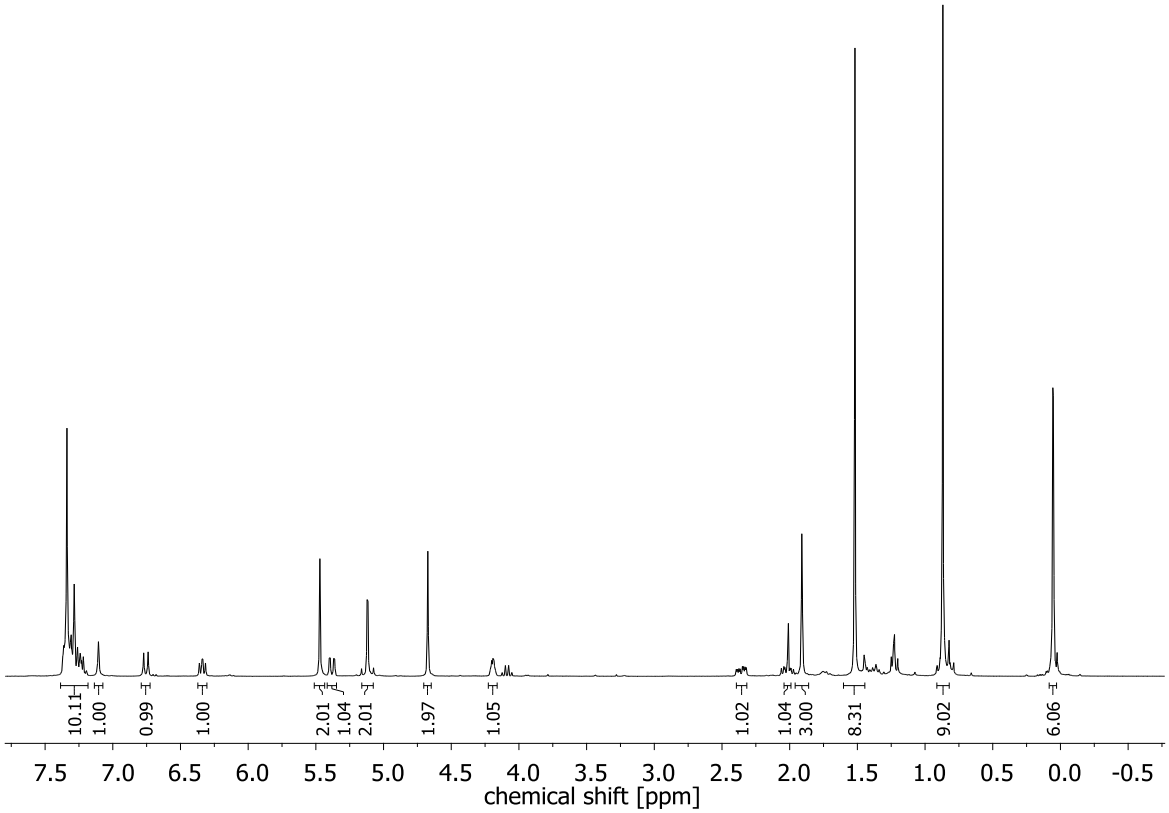
^{13}C NMR spectrum of *Z*-**S7** (75 MHz, $CDCl_3$)



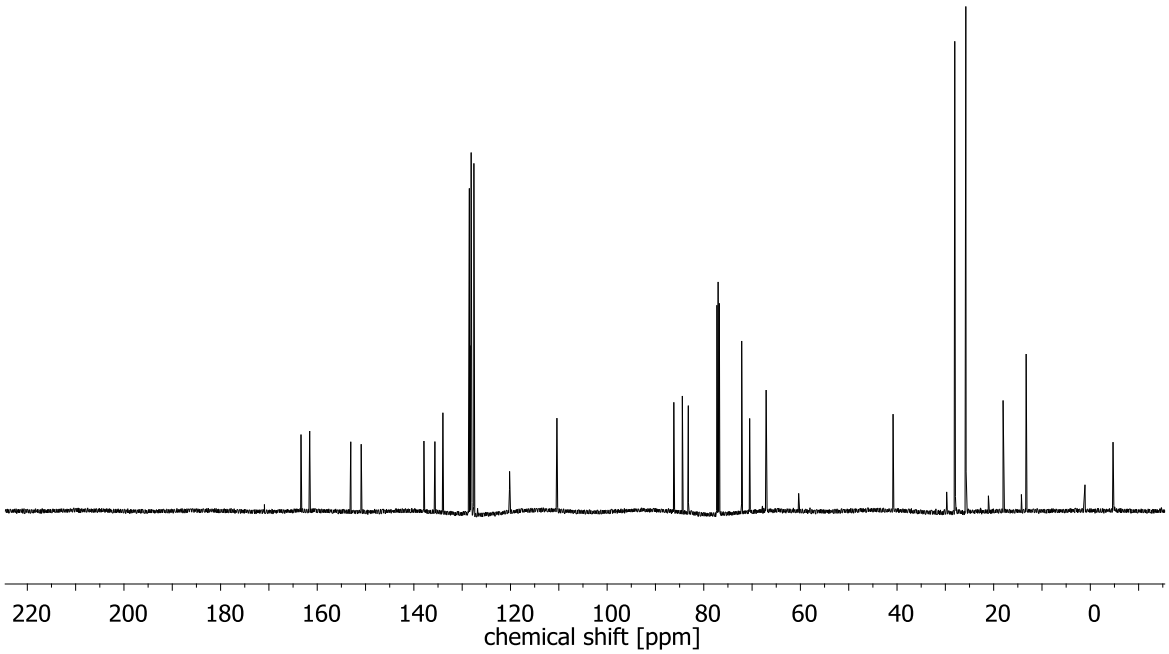
^{1}H NOE NMR spectrum of *Z*-**S7** (300 MHz, $CDCl_3$)



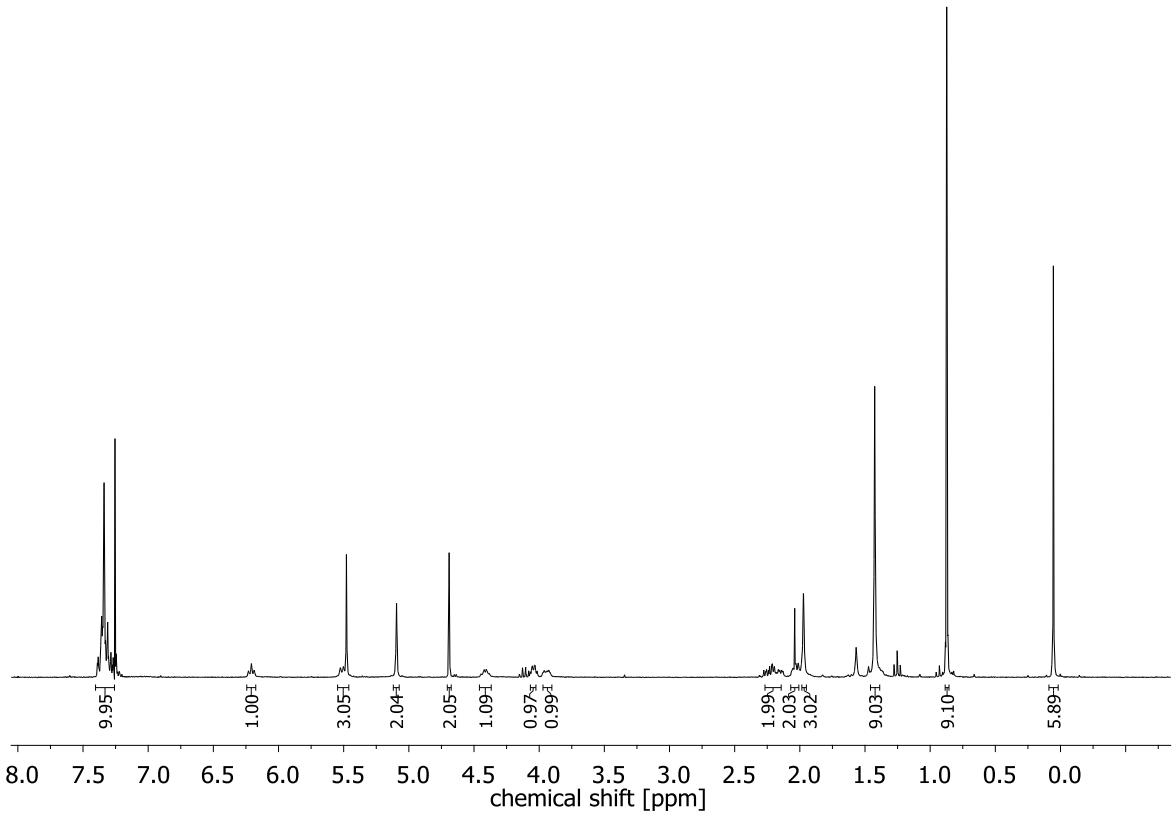
^{1}H NOE NMR spectrum of *Z*-**S7** (300 MHz, $CDCl_3$)



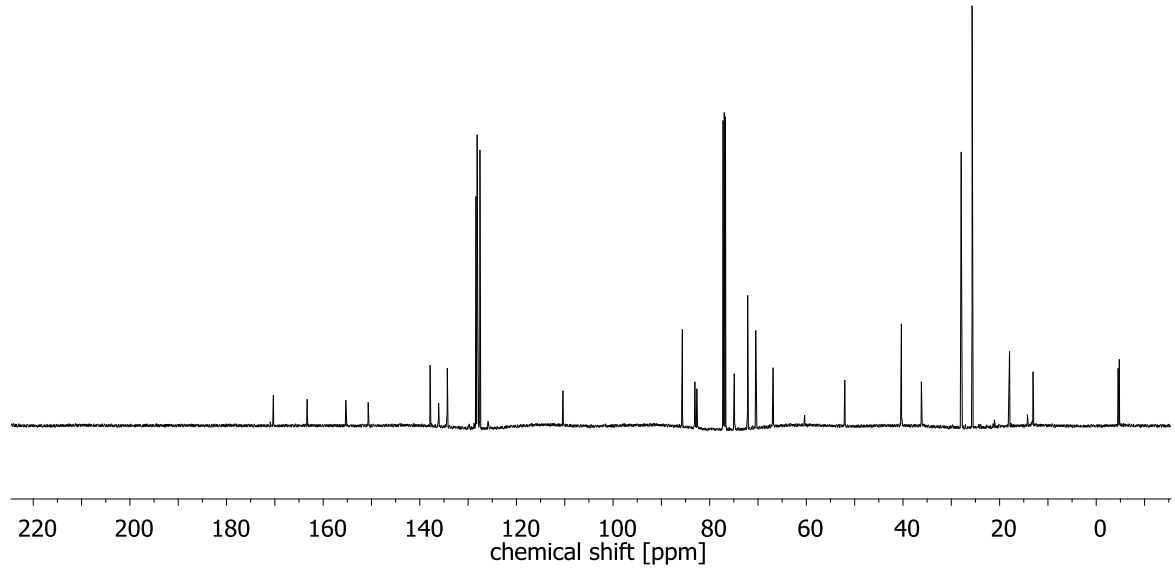
^{1}H NMR spectrum of *E*-**S7** (300 MHz, $CDCl_3$)



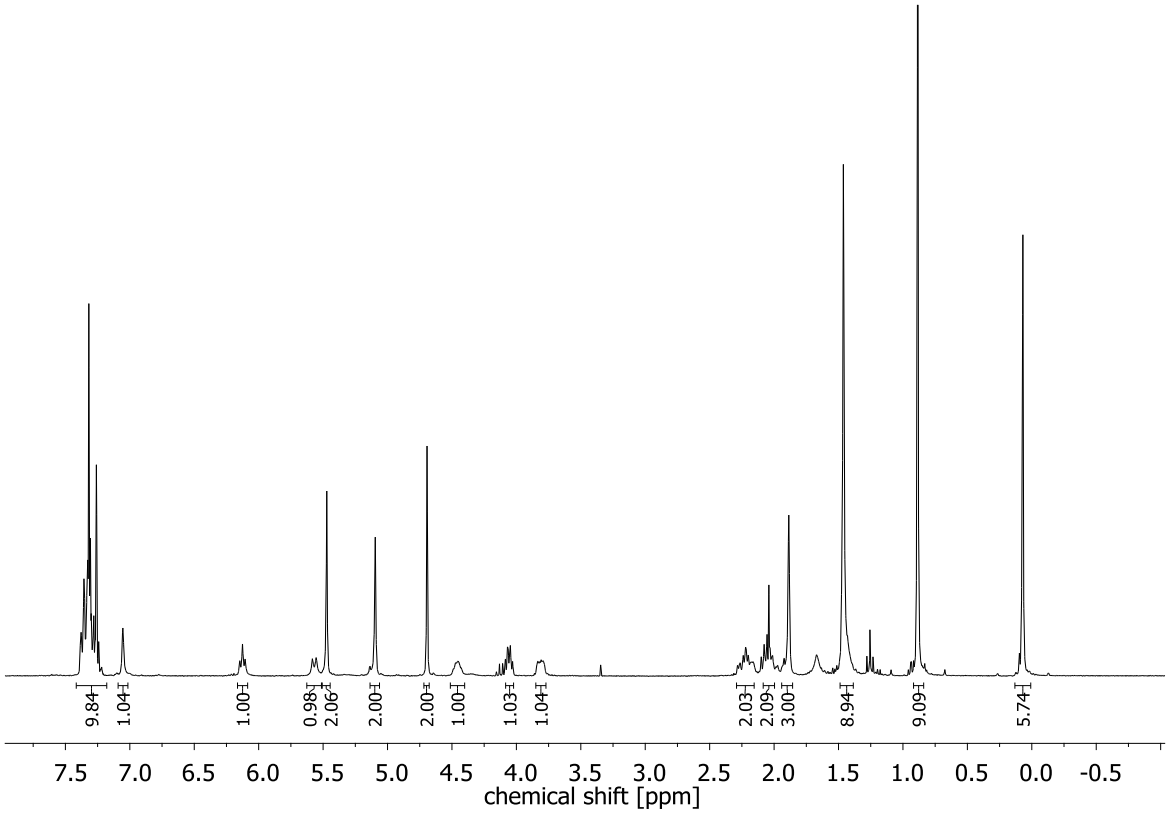
^{13}C NMR spectrum of *E*-**S7** (75 MHz, $CDCl_3$)



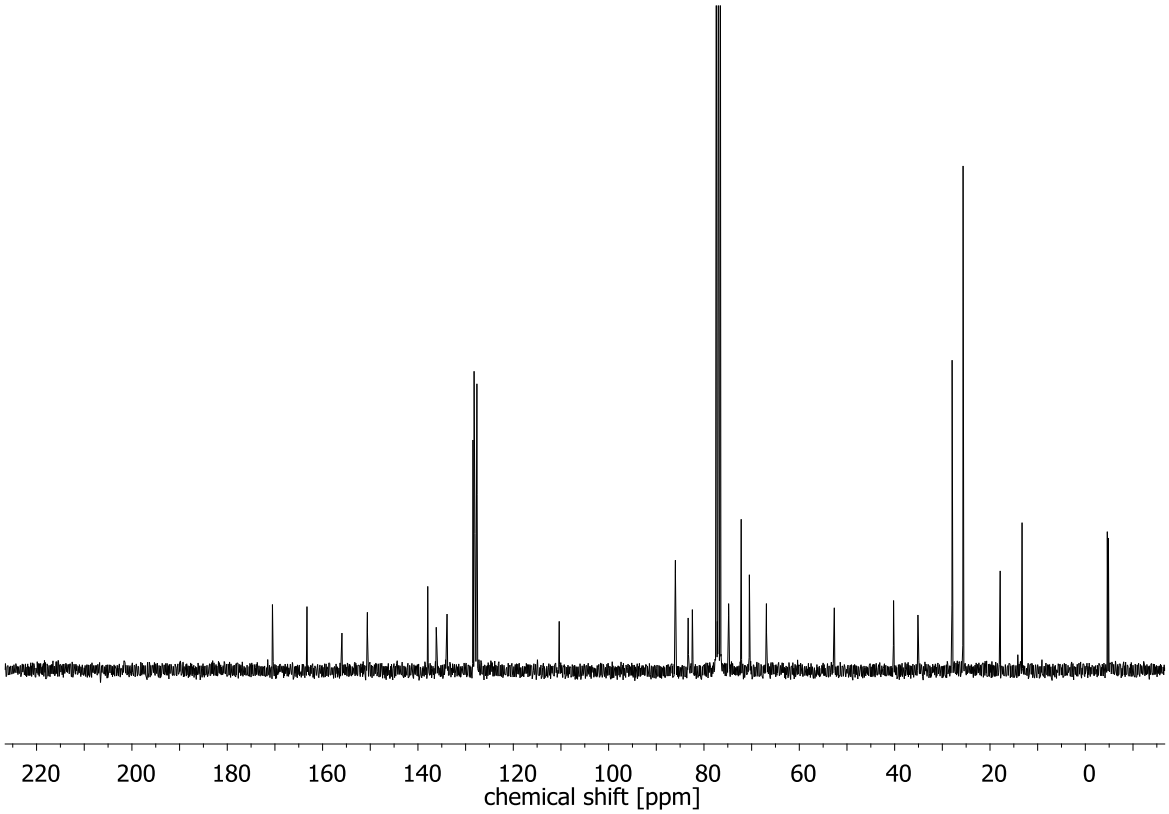
^{1}H NMR spectrum of (*S*)-**S8** (300 MHz, $CDCl_3$)



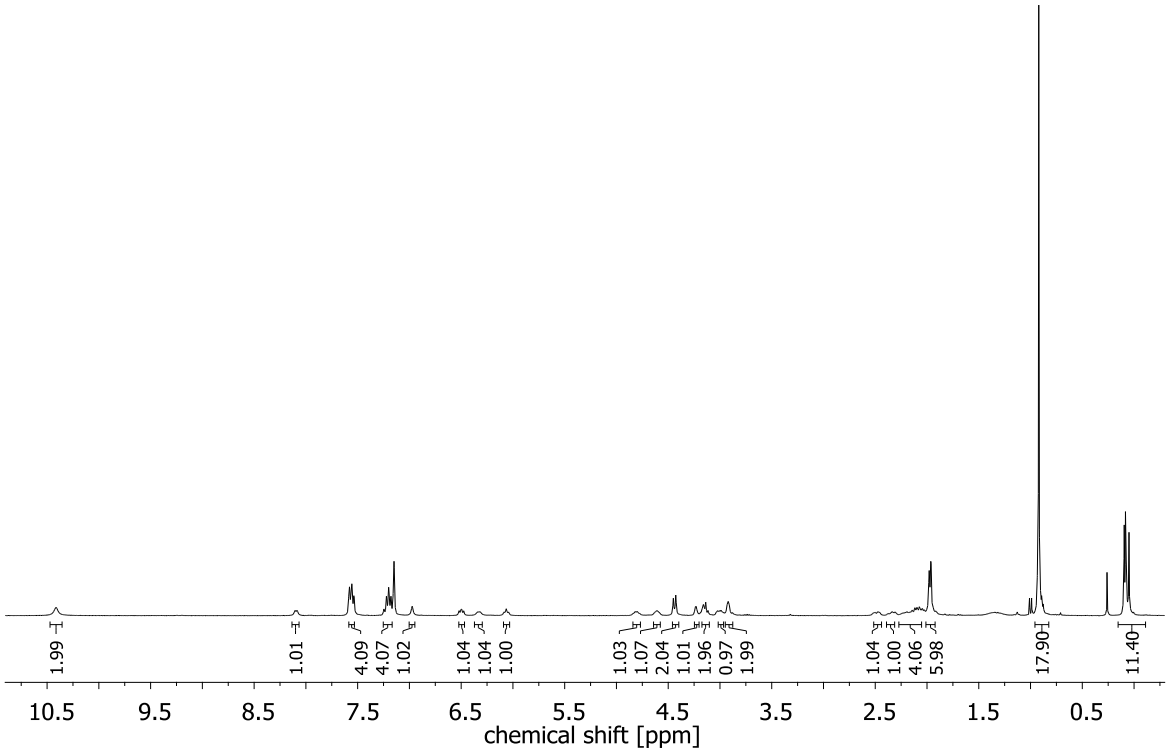
^{13}C NMR spectrum of (*S*)-**S8** (75 MHz, $CDCl_3$)



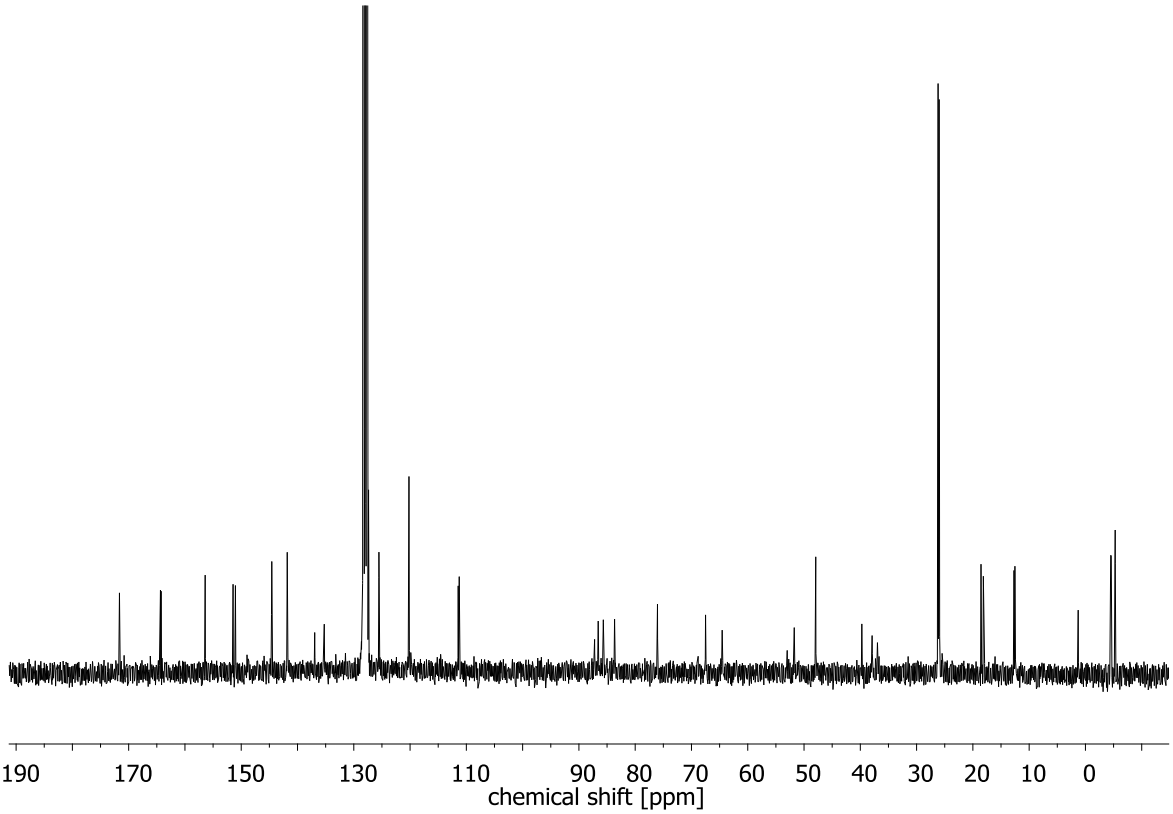
^{1}H NMR spectrum of (*R*)-**S8** (300 MHz, $CDCl_3$)



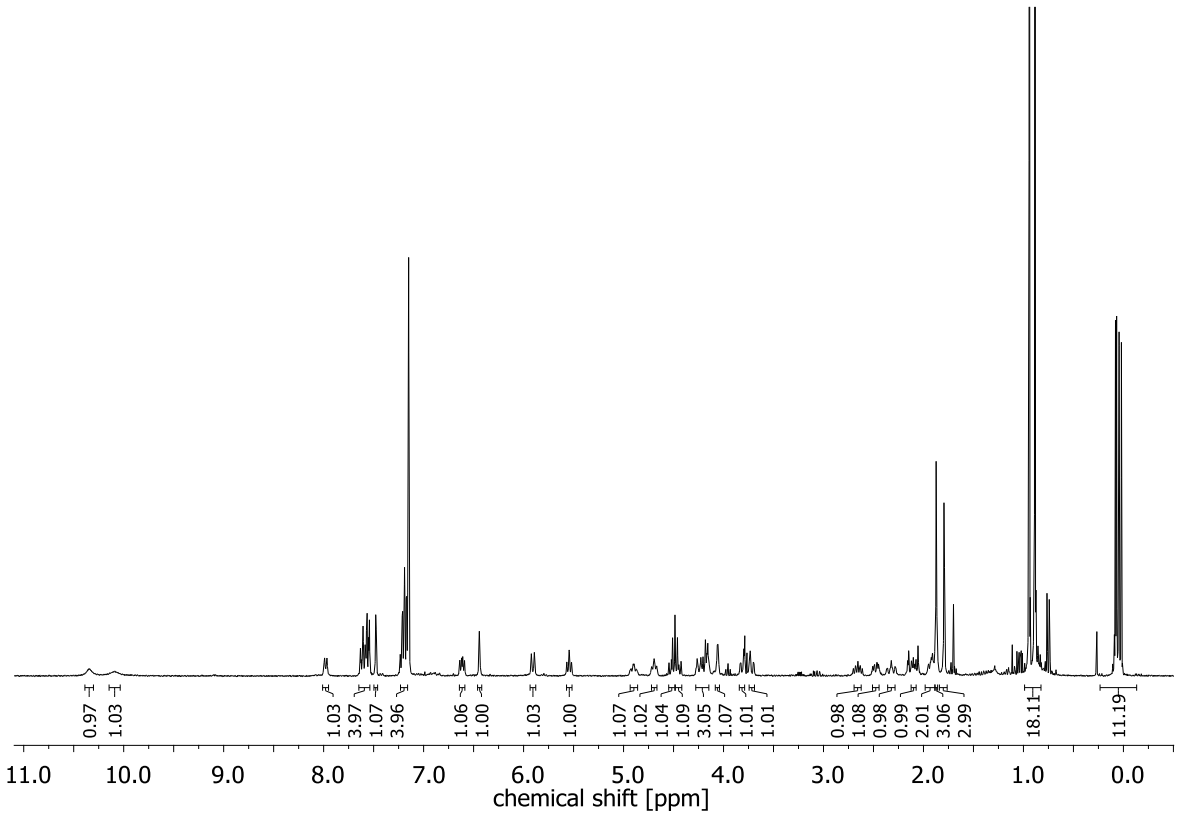
^{13}C NMR spectrum of (*R*)-**S8** (75 MHz, $CDCl_3$)



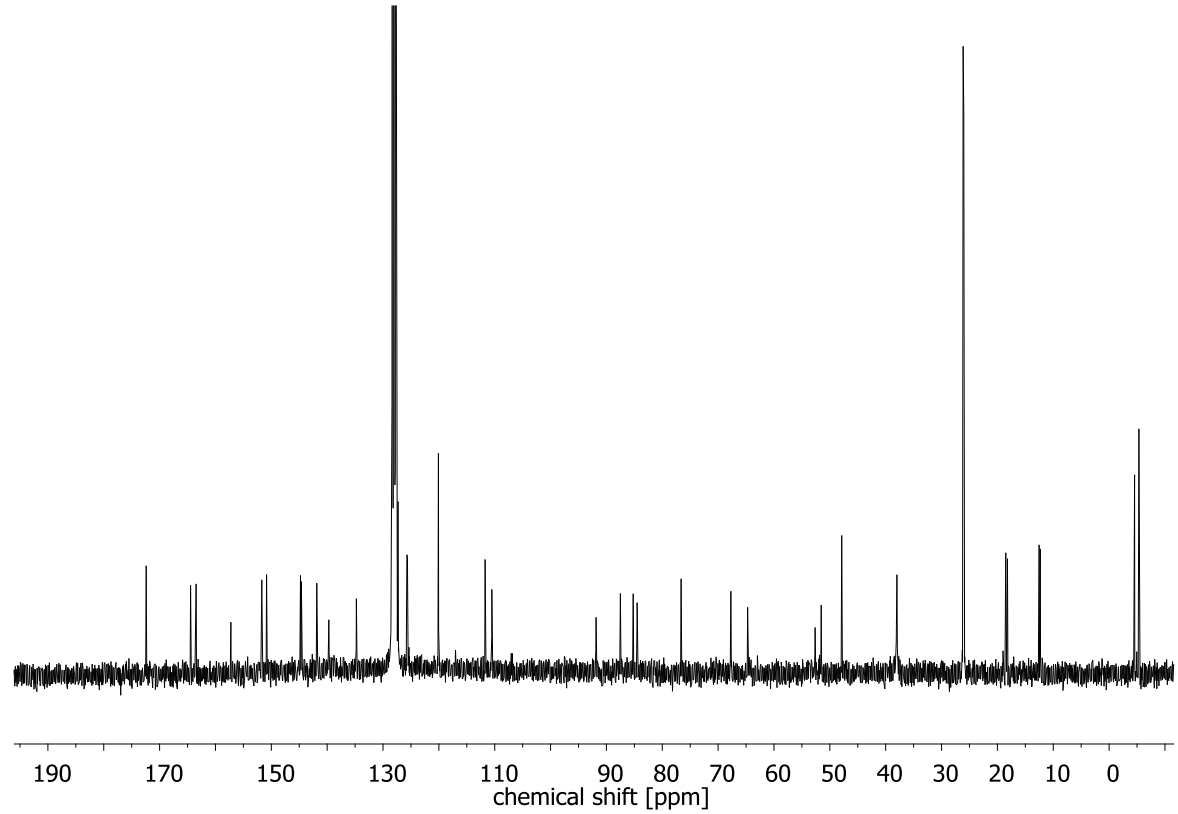
^{1}H NMR spectrum of (*S*)-**S9** (300 MHz, C_6D_6, 70 °C)



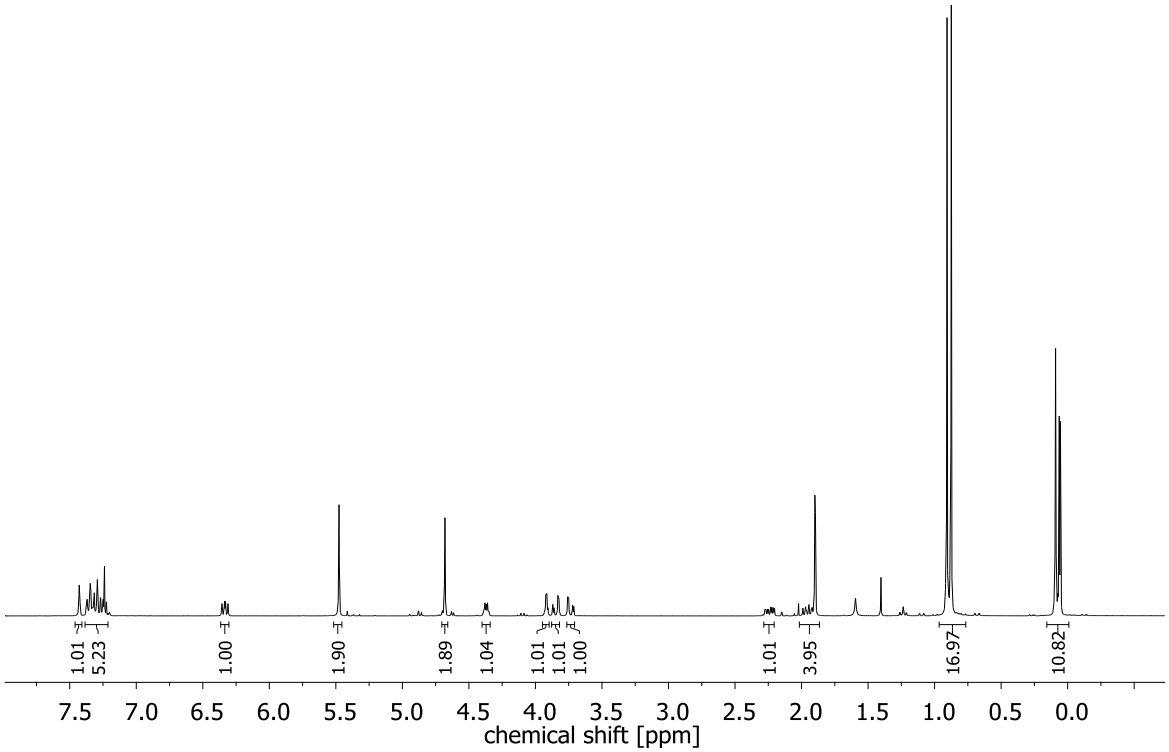
^{13}C NMR spectrum of (*S*)-**S9** (75 MHz, C_6D_6, 70 °C)



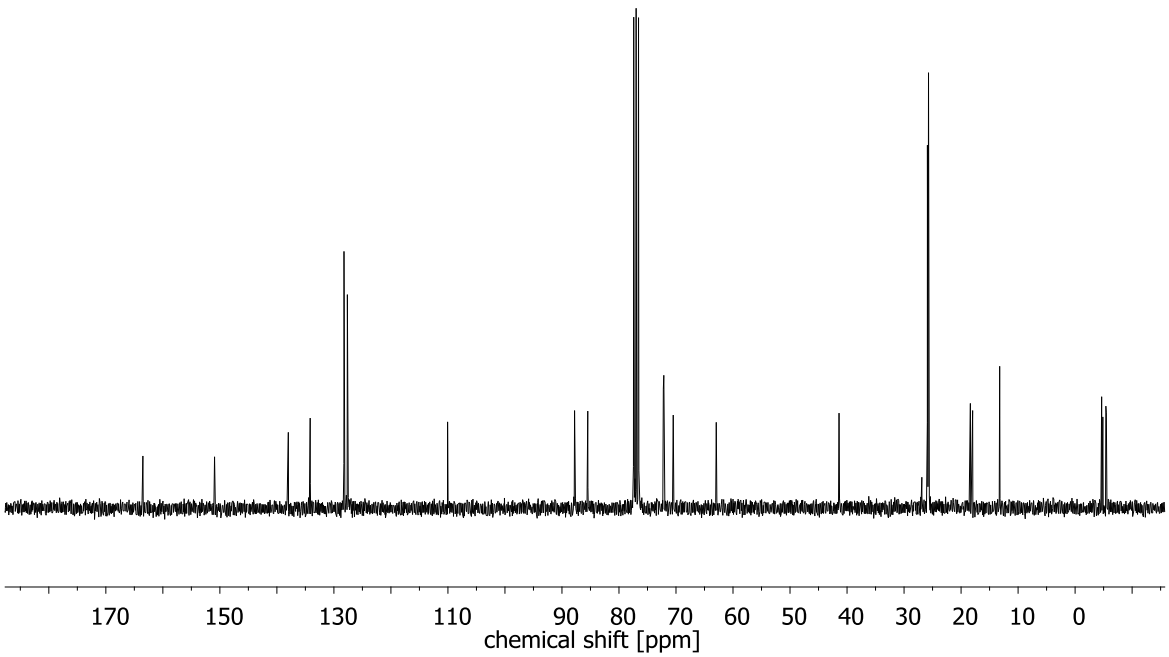
^{1}H NMR spectrum of (*R*)-**S9** (300 MHz, C_6D_6, 70 °C)



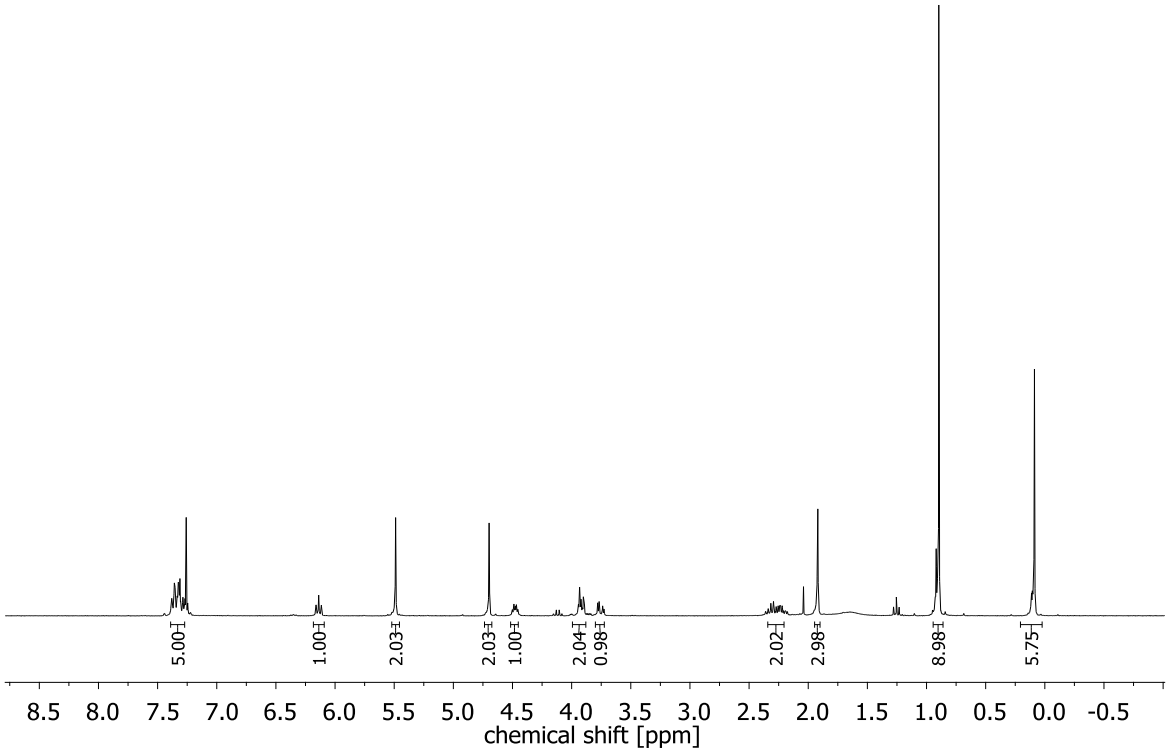
^{13}C NMR spectrum of (*R*)-**S9** (75 MHz, C_6D_6, 70 °C)



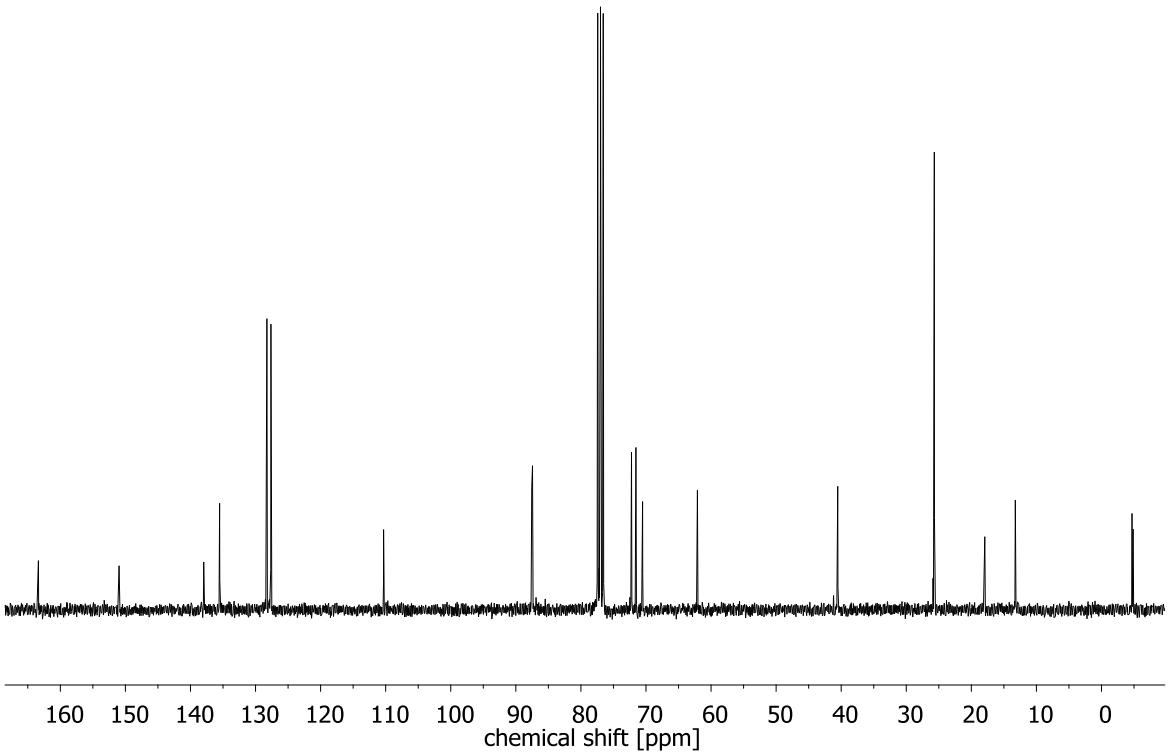
^{1}H NMR spectrum of **S10** (300 MHz, $CDCl_3$)



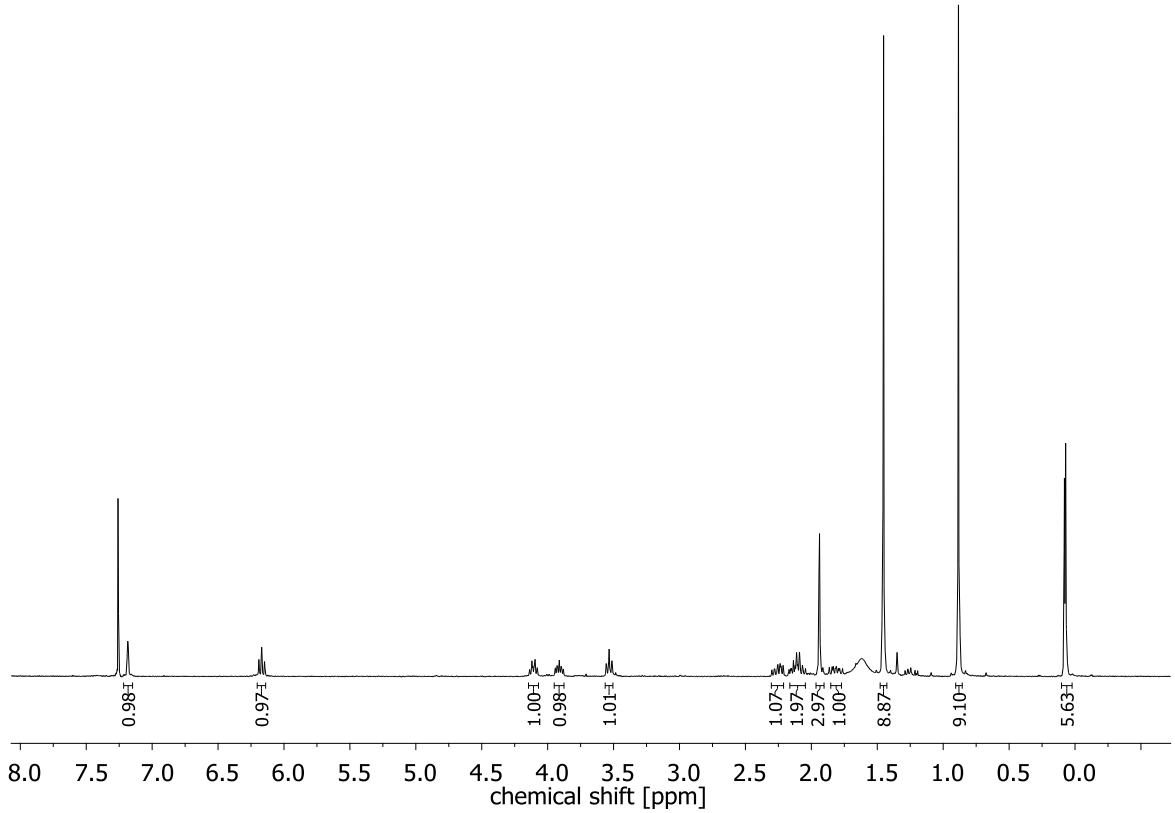
^{13}C NMR spectrum of **S10** (75 MHz, $CDCl_3$)



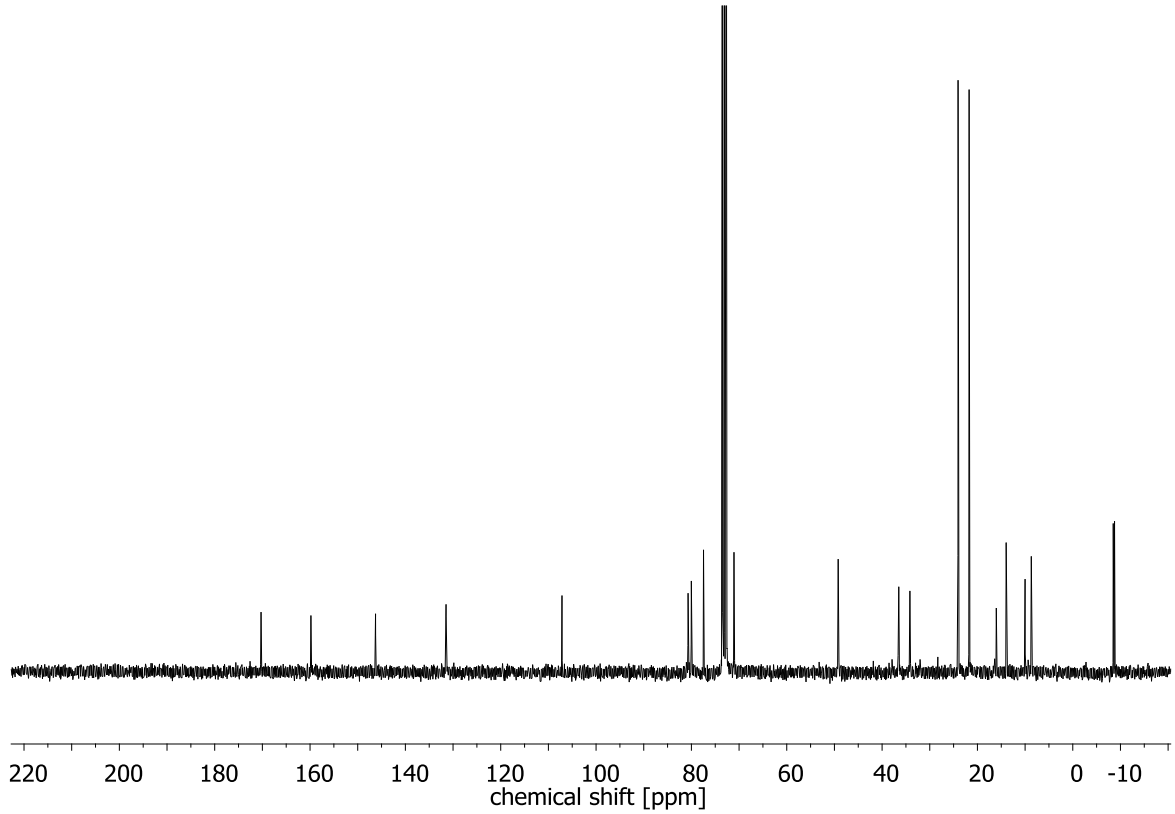
^{1}H NMR spectrum of **S11** (300 MHz, $CDCl_3$)



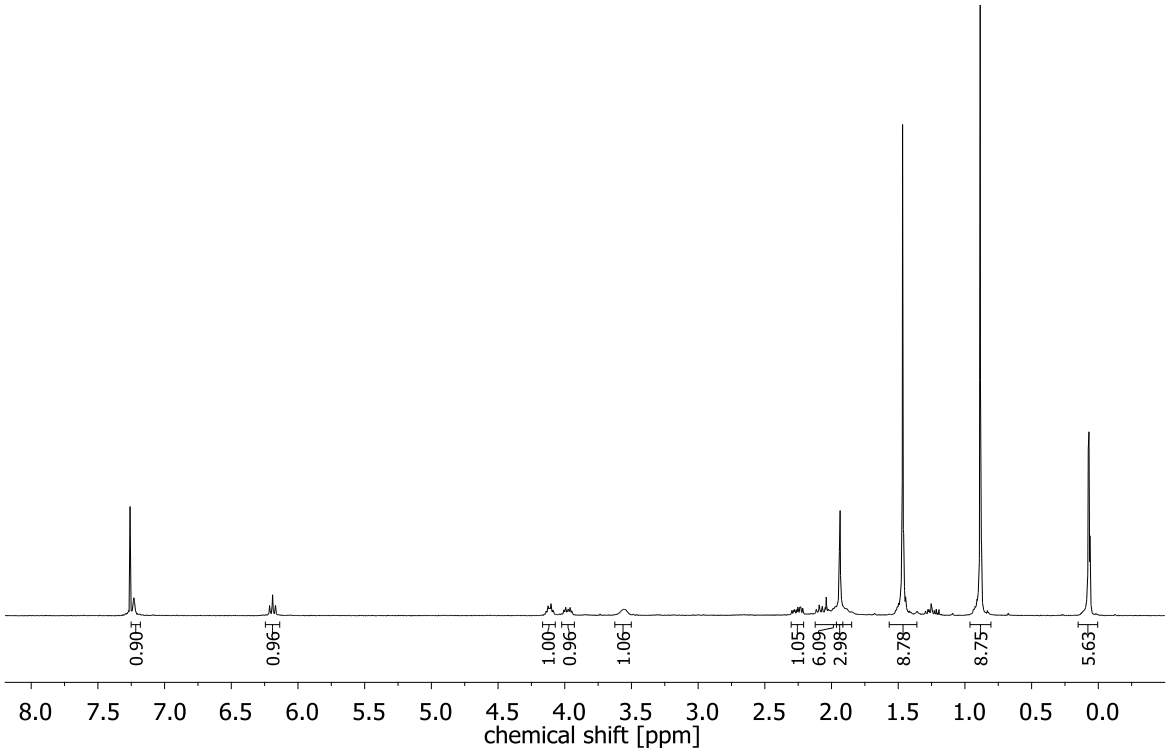
^{13}C NMR spectrum of **S11** (75 MHz, $CDCl_3$)



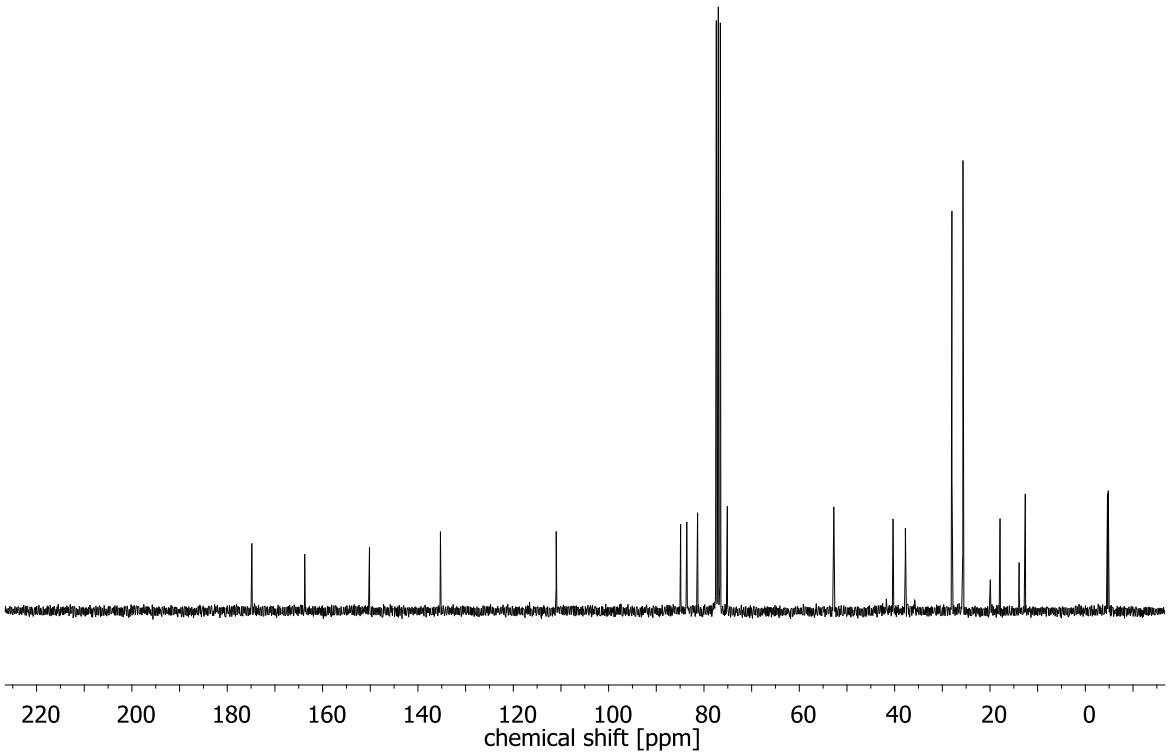
^{1}H NMR spectrum of (*S*)-**S12** (300 MHz, $CDCl_3$)



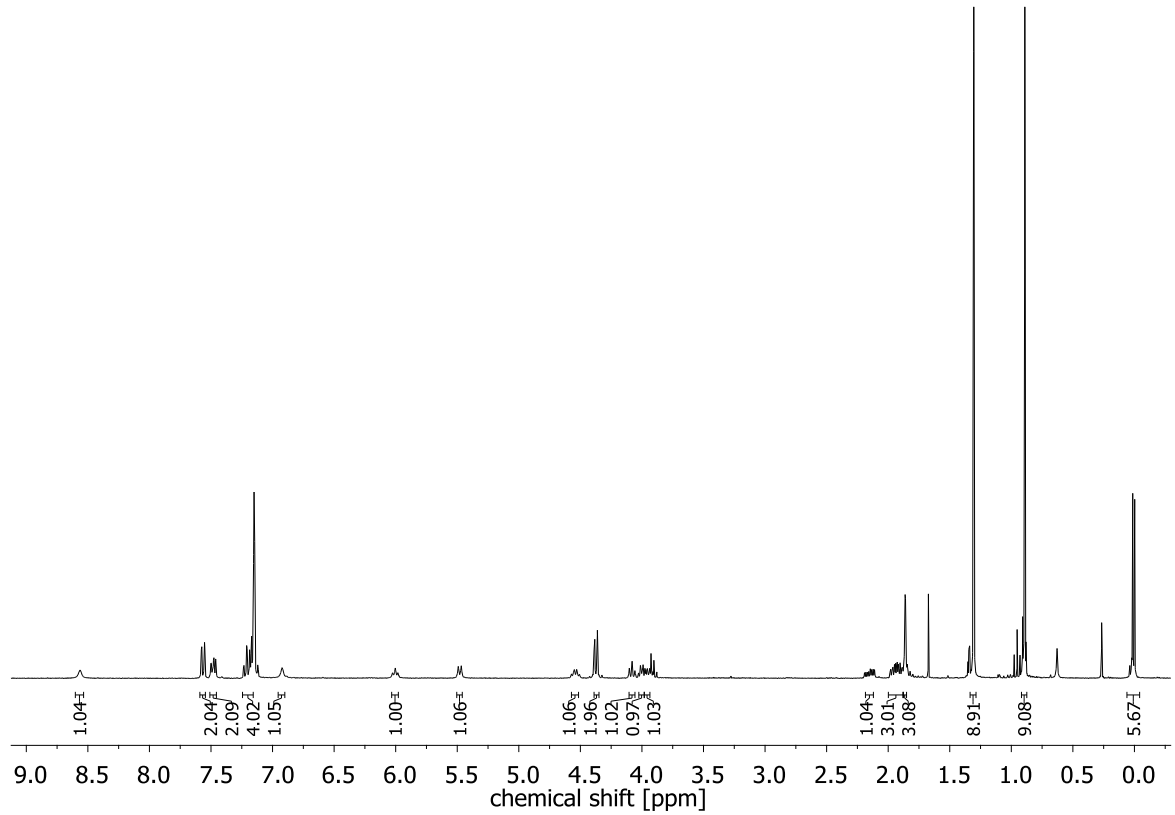
^{13}C NMR spectrum of (*S*)-**S12** (75 MHz, $CDCl_3$)



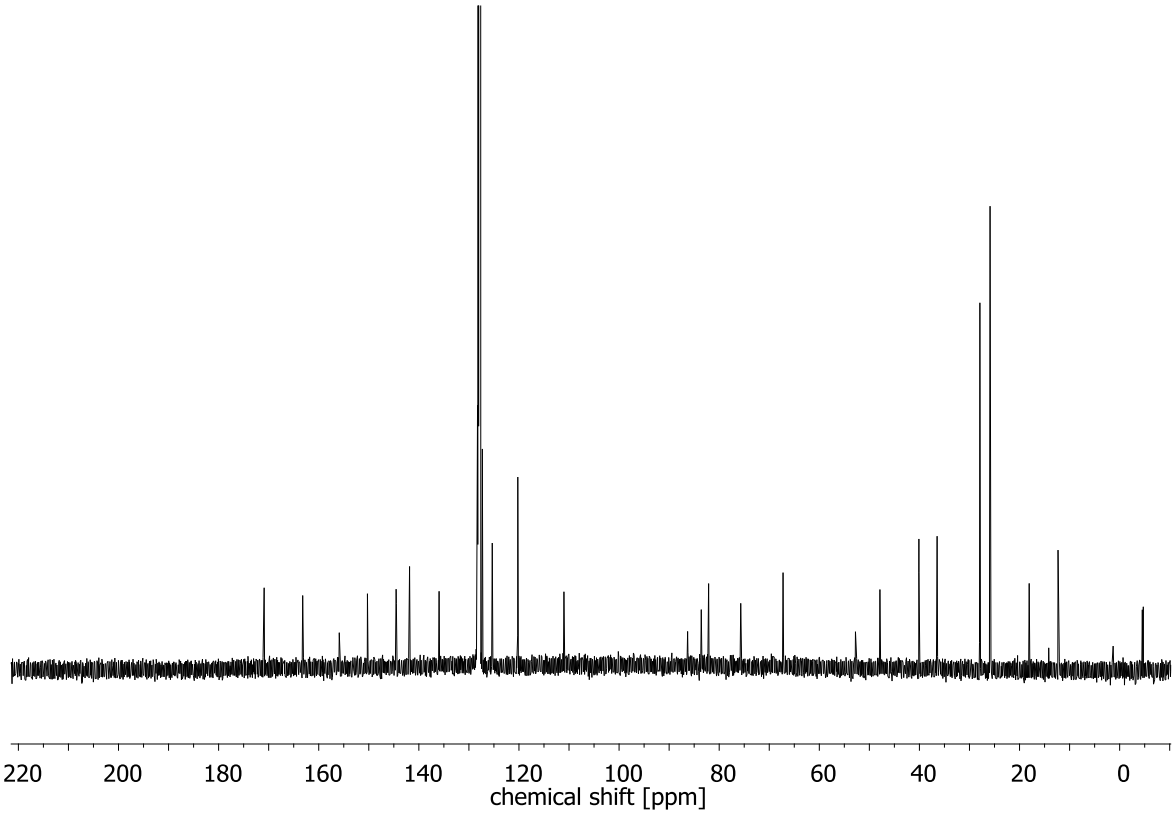
^{1}H NMR spectrum of (*R*)-**S12** (300 MHz, $CDCl_3$)



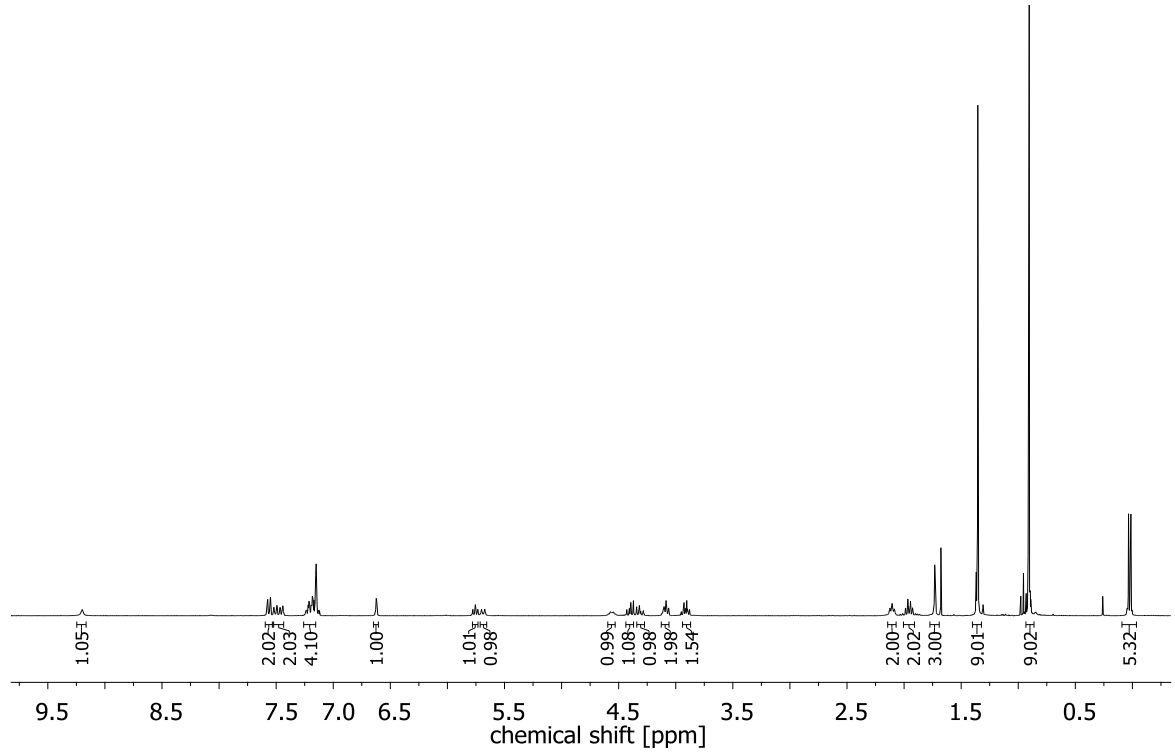
^{13}C NMR spectrum of (*R*)-**S12** (75 MHz, $CDCl_3$)



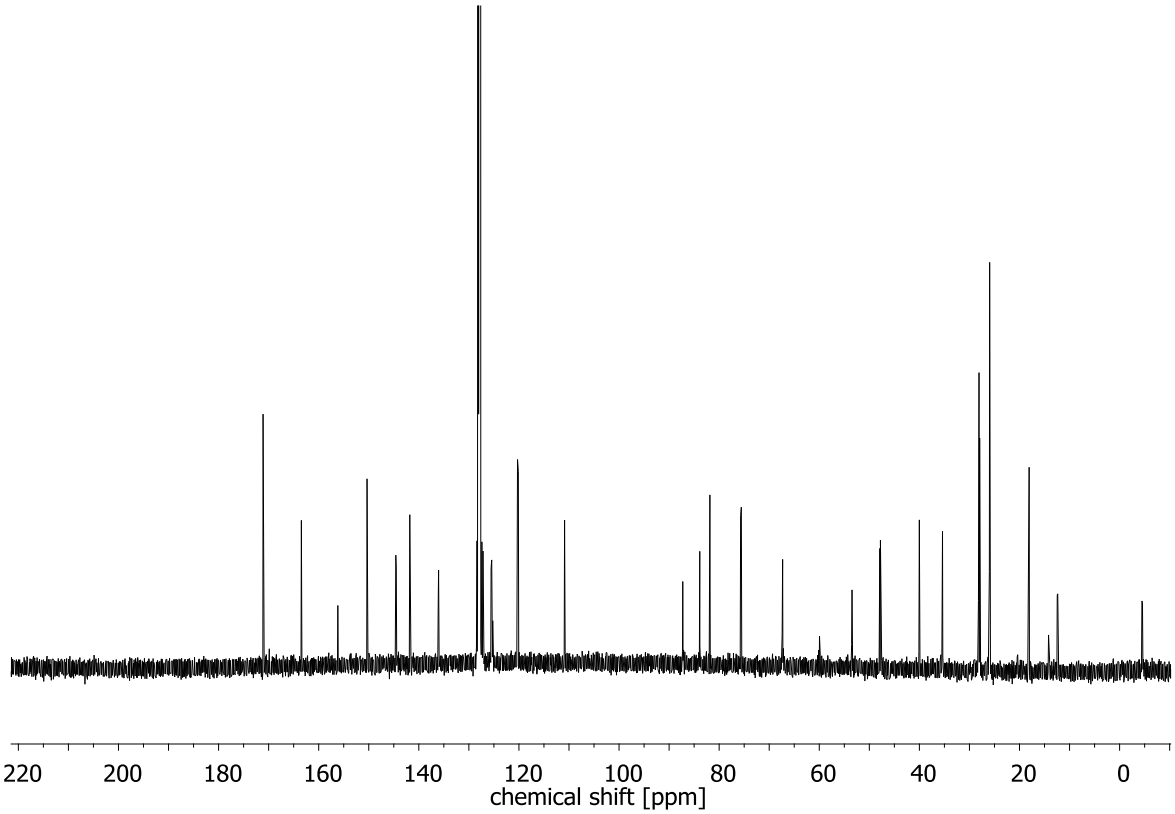
^{1}H NMR spectrum of (*S*)-**S13** (300 MHz, C_6D_6, 70 °C)



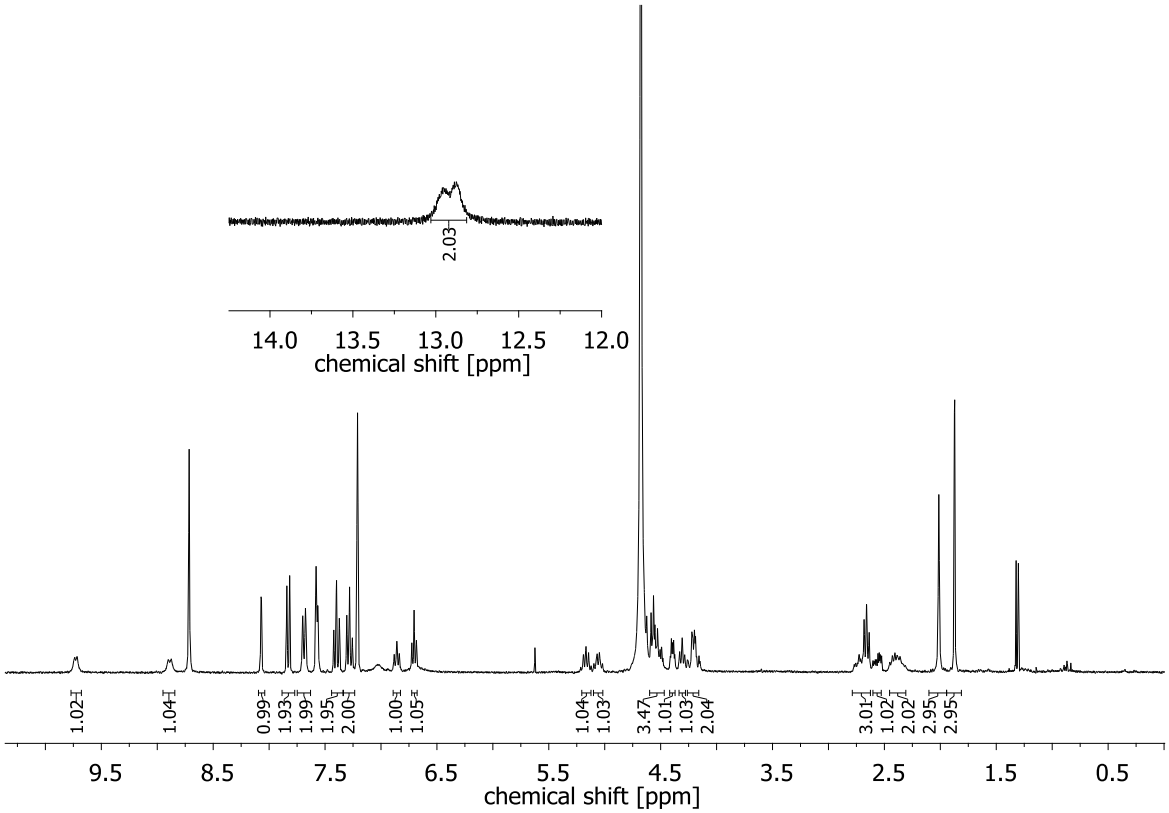
^{13}C NMR spectrum of (*S*)-**S13** (75 MHz, C_6D_6, 70 °C)



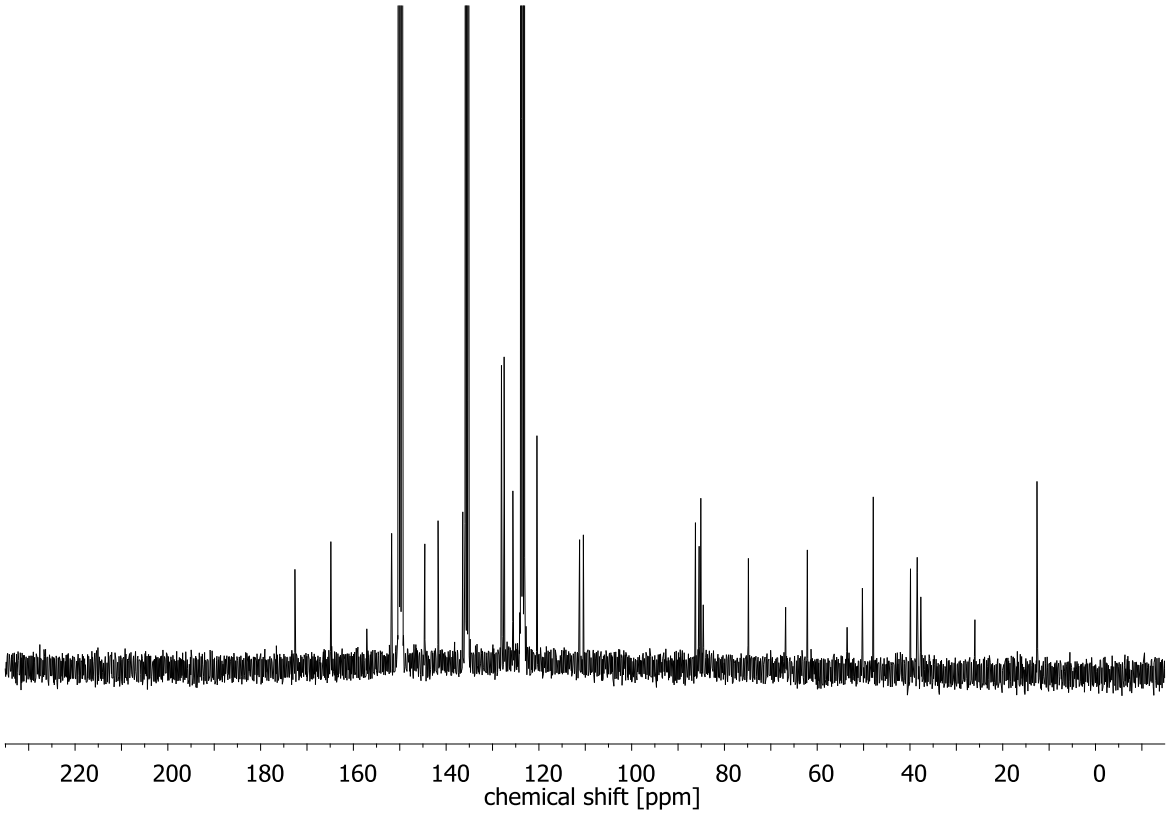
^{1}H NMR spectrum of (*R*)-**S13** (300 MHz, C_6D_6, 70 °C)



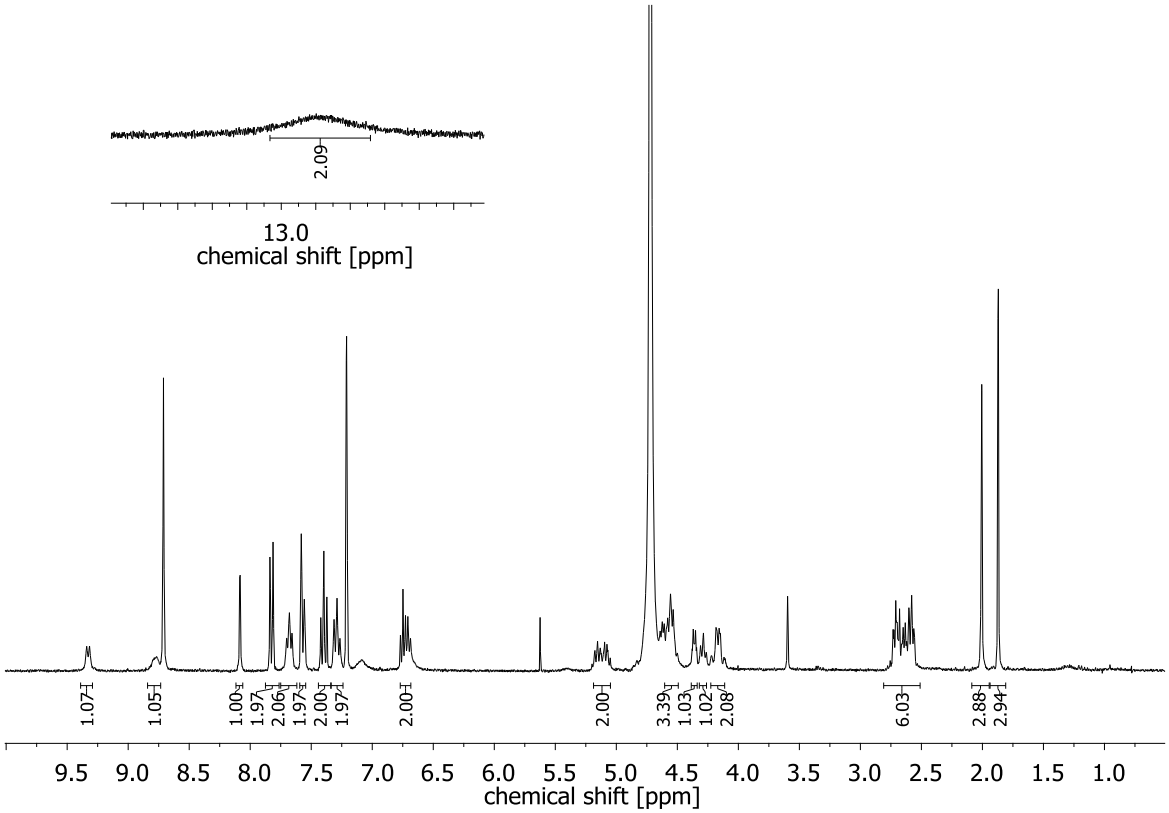
^{13}C NMR spectrum of (*R*)-**S13** (75 MHz, C_6D_6, 70 °C)



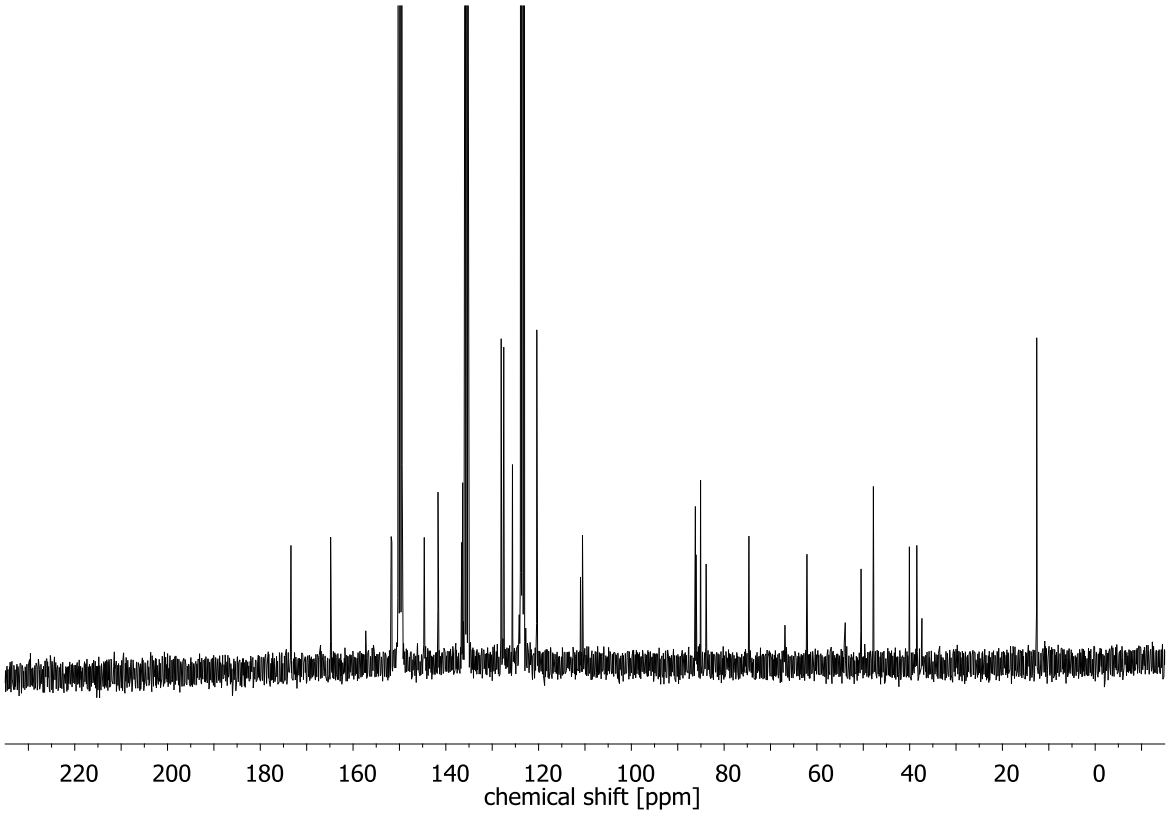
^{1}H NMR spectrum of (*S*)-**S14** (300 MHz, pyridine-d_5, 50 °C)



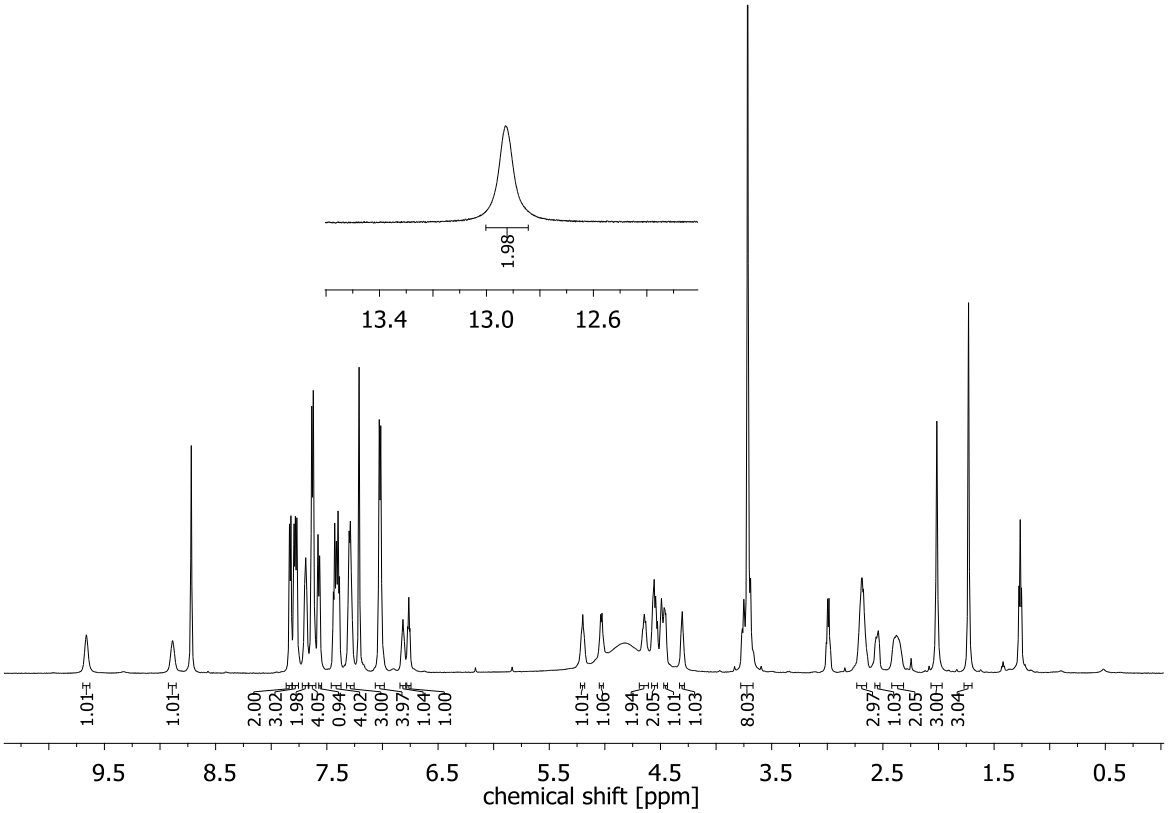
^{13}C NMR spectrum of (*S*)-**S14** (75 MHz, pyridine-d_5, 50 °C)



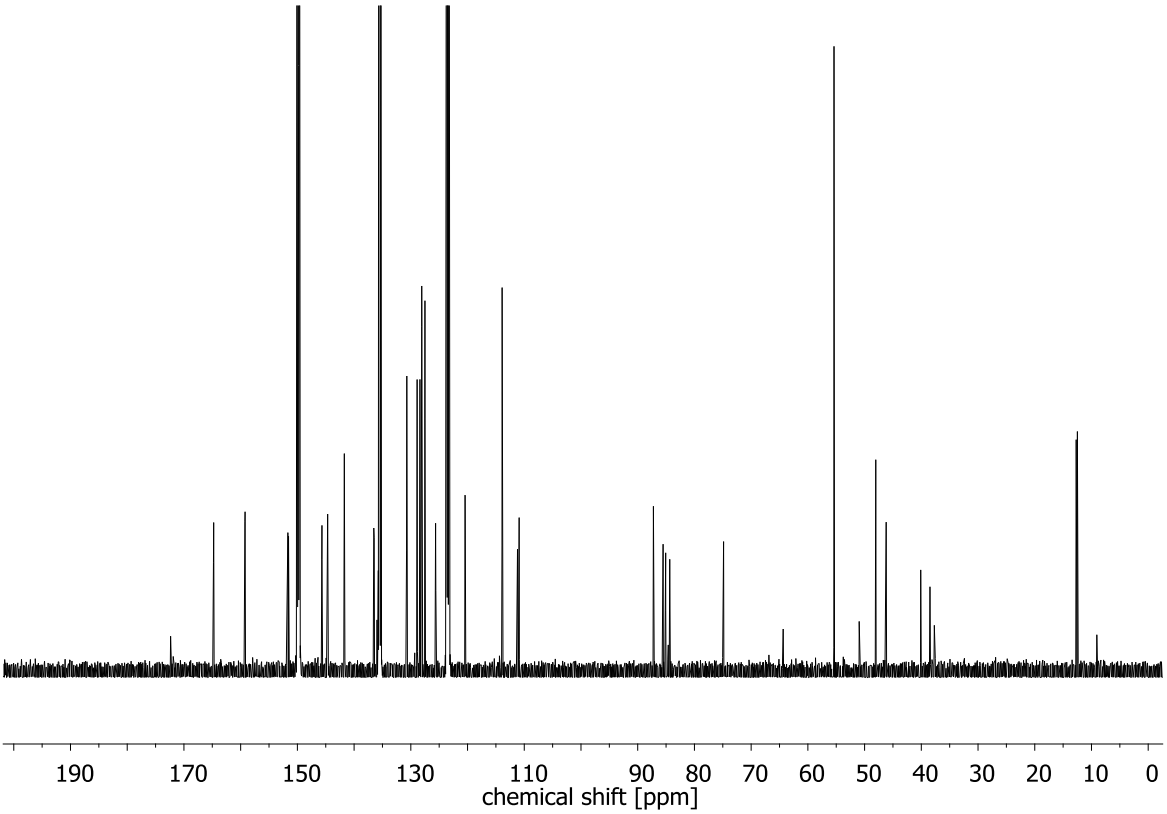
^{1}H NMR spectrum of (*R*)-**S14** (300 MHz, pyridine-d_5, 50 °C)



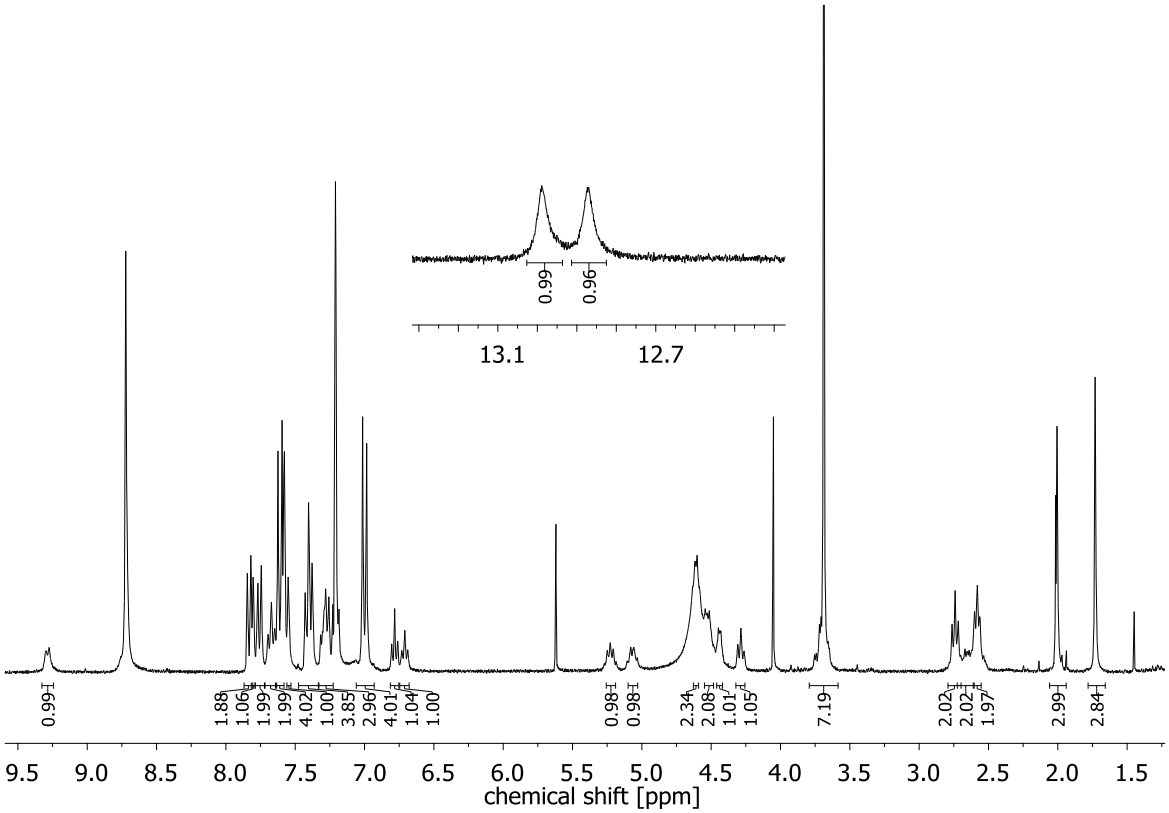
^{13}C NMR spectrum of (*R*)-**S14** (75 MHz, pyridine-d_5, 50 °C)



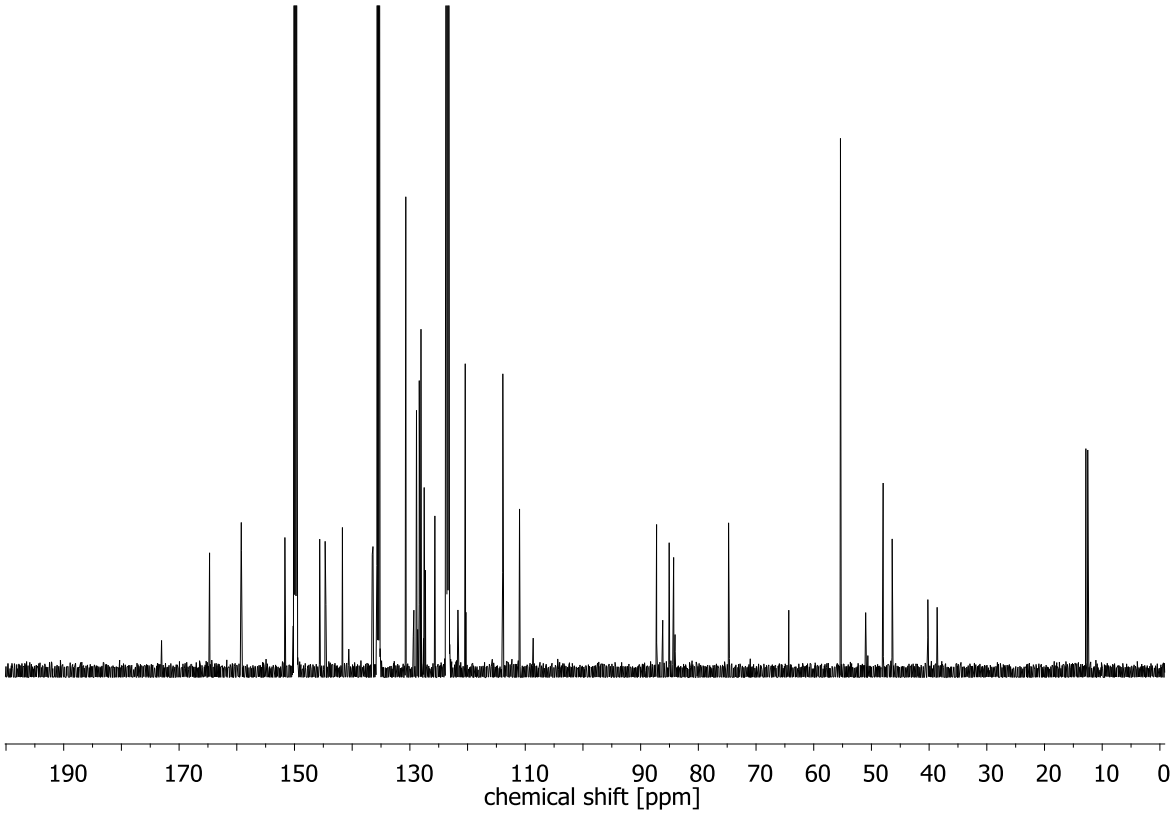
^{1}H NMR spectrum of (*S*)-**S15** (600 MHz, pyridine-d_5, 50 °C)



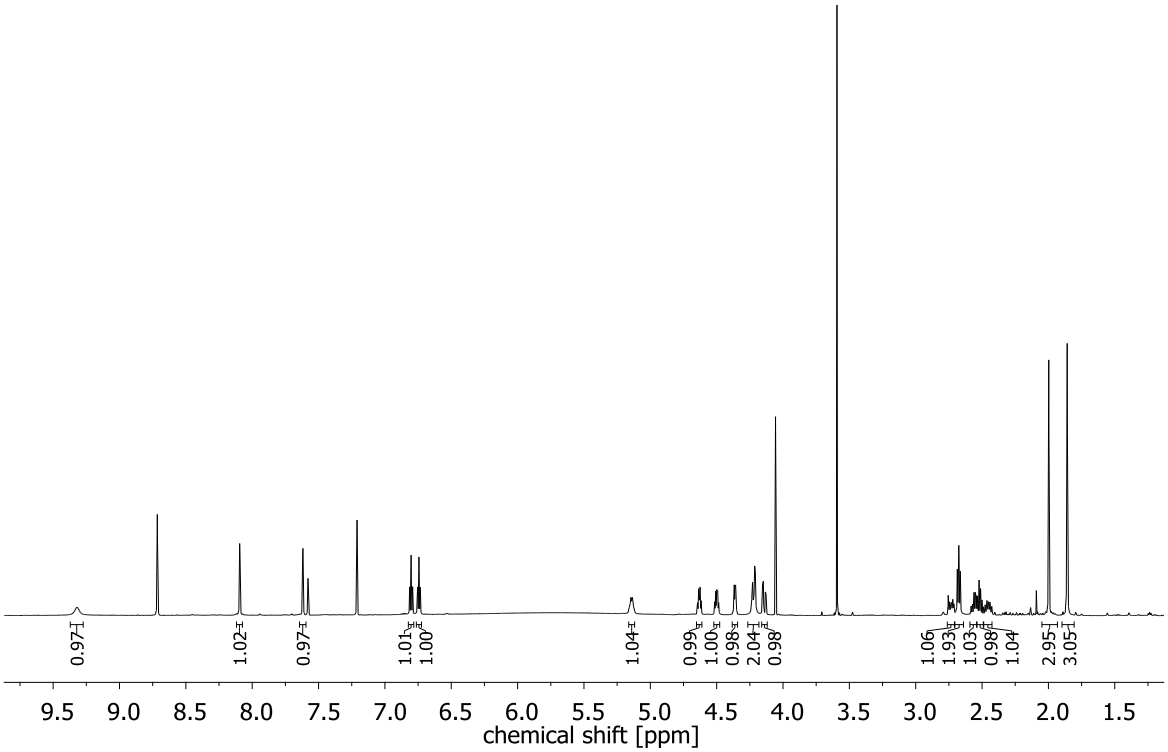
^{13}C NMR spectrum of (*S*)-**S15** (75 MHz, pyridine-d_5, 50 °C)



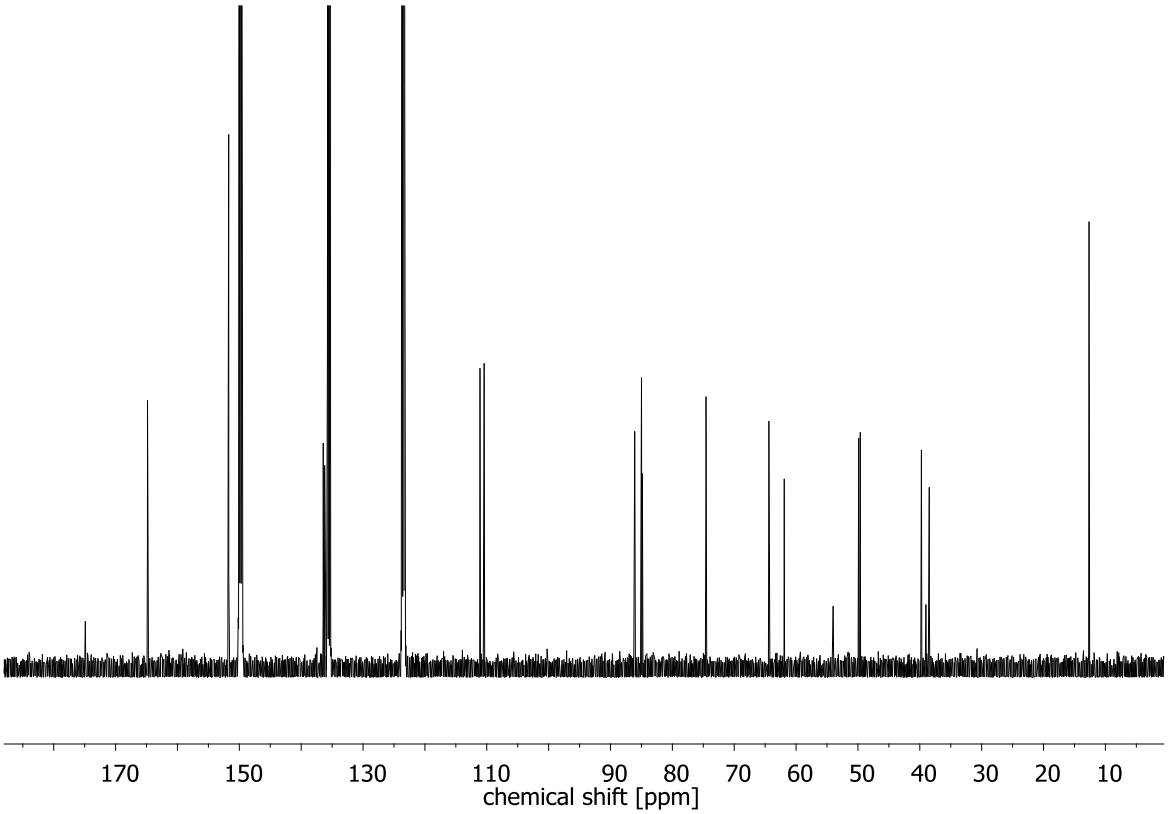
^{1}H NMR spectrum of (*R*)-**S15** (600 MHz, pyridine-d_5, 50 °C)



^{13}C NMR spectrum of (*R*)-**S15** (75 MHz, pyridine-d_5, 50 °C)



^{1}H NMR spectrum of (*S*)-**S16** (600 MHz, pyridine-d_5, 35 °C)



^{13}C NMR spectrum of (*S*)-**S16** (75 MHz, pyridine-d_5, 35 °C)

Oligonucleotide synthesis and analytical data of oligonucleotides

Automated synthesis of oligonucleotides

The syntheses of oligonucleotides were performed on a Pharmacia LKB (Gene Assembler Plus) For each oligonucleotide synthesis, 18 mg of nucleoside-charged polystyrene resin (39 μmol 5'-*O*-DMTr-nucleoside/g matrix) purchased from GE Healthcare were used. Anhydrous MeCN was used as the solvent. For the cleavage of DMTr protecting groups, the resin was purged with 3% dichloroacetic acid in anhydrous dichloroethane. The removal of the acid was carried out by purging with anhydrous dichloroethane. The activation of the phsosphoramidite functionality was effected by a 0.25 M benzylthiotetrazole solution in anhydrous MeCN. The coupling time for standard phosphoramidites was 2 min and for NAA-modified building blocks (*S*)-**S1** and (*R*)-**S1** 4 min. Oxidation of P(III)-species was attained by alkaline iodine solution (10 mM I_2 in MeCN/2,4,6-collidine/H_2O 10:1:5). For the capping of residual 5'-OH-groups, a mixture of solution A (0.5 M DMAP in anhydrous MeCN) and solution B (Ac_2O/2,4,6-collidine/anhydrous MeCN 2:3:5) was used. After completion of the synthesis, the oligonucleotides were cleaved from the solid support with concomitant removal of the Fmoc and β-cyanoethyl protecting groups by reacting the oligonucleotide-charged solid support with 25% aq. NH_3/EtOH (3:1) at 55 °C for 20 h. The thus obtained suspension was filtered and the filtrate was concentrated *in vacuo*. The resultant residue was dissolved in 750 μL water. For purification of this crude oligonucleotide solution, a volume containing ~ 40 nmol crude oligonucleotide was applied to gel electrophoresis (0.7 mm, 20% polyacrylamide). The oligonucleotide-containing segments of the gel were visualised by UV-light (260 nm) and separated from the rest of the gel. Oligonucleotides were extracted from the gel by incubating each gel segment in 300 μL TEN-buffer (1.0 M TRIS, 0.5 M EDTA, 3.0 M NaCl) at 0 °C for 16 h. The thus obtained TEN-solutions were diluted with 900 μL

EtOH and stored for 20 min at -80 °C for precipitation. Centrifugation at 4 °C and careful removal of the supernatant gave the precipitated pure oligonucleotides.

Control experiment regarding the NAA-stereochemistry: in order to demonstrate that no epimerisation of the NAA-moiety occurred under the basic cleavage conditions after oligonucleotide synthesis, a control experiment was performed. A preparative amount of bis-*O*-deprotected (6'*S*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*S*)-**S14** was treated under the basic conditions of oligonucleotide deprotection and cleavage (25% aq. NEt_3/EtOH (3:1) at 55 °C) for 6 h, thus furnishing fully deprotected (6'*S*)-thymidinyl amino acid (3'-deoxy-3'-aminothymidin-3'-yl)-amide (*S*)-**S16** in quantitative yield (*vide supra*). Product (*S*)-**S16** was fully characterised, and NMR spectroscopy unambiguously proved that only one diastereomer had been obtained. Hence, it could be concluded that no partial epimerisation had occurred under the basic cleavage conditions.

Analytical data of oligonucleotides

UV spectra of oligonucleotide solutions were measured on a Varian (Cary 100 Bio) within a range $\Delta\lambda$ of 320-190 nm. The concentration of the oligonucleotide solutions was ~ 2.0 µM. ESI mass spectra of oligonucleotides were measured in the negative mode on a Thermo Fisher LTQ XL. For the measurements, aqueous 25 µM oligonucleotide solutions with 30% MeCN and 5% NEt_3 were used. Analytical HPLC: the purity analysis of modified and unmodified oligonucleotides by HPLC was performed on a GE Äktapurifier composed of a Dionex P580 HPLC pump, a Dionex ASI-100 fraction sampler, a heating device for the column and a Dionex UV170U UV-detector with four UV-Vis channels. For the seperation of oligonucleotides, a Dionex DNAPac PA100 anion exchange column (4 x 250 mm) was used with a flow rate of 1 mL/min and a temperature of 80 °C or 60 °C. The oligonucleotides were eluted using a gradient of 0-60% (during 45 min) of eluent B (25 mM TRIS-HCl, 0.5 M

$NaClO_4$, 6 M urea, pH = 8) in eluent A (25 mM TRIS-HCl, 6 M urea, pH = 8). The purity analysis of palindromic oligonucleotides was perfomed on a VWR EliteLaChrom system composed of a VWR L-2130 pump, a VWR L-2300 heating device for the column, an autosampler and a UV-Detector. For oligonucleotide separation, a Merck LiChroCART® RP-column was used. The oligonucleotides were eluted using a 5%-8%-12% gradient (during 30 min) of MeCN in aq. 10 mM NEt_3/AcOH. In both cases, oligonucleotides were detected by the absorption at λ = 260 nm, and retention times t_R [min] are not corrected.

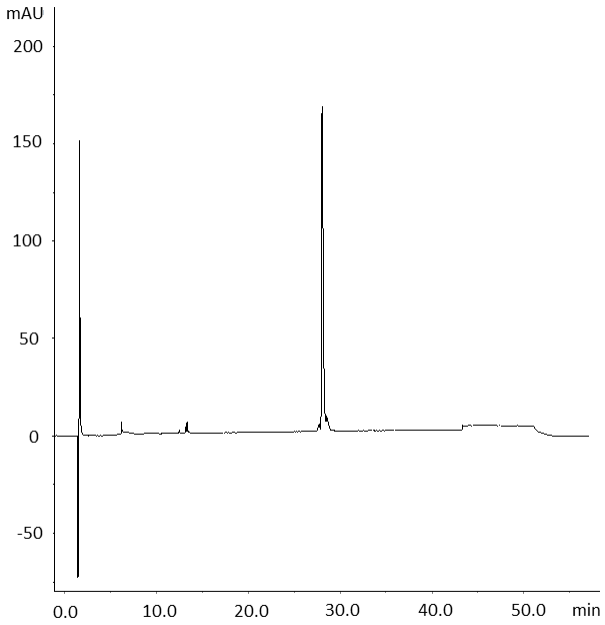
Table S1. Retention times of synthesised oligonucleotides (HPLC).

no.	sequence	NAA-6'-config.	retention time [min]
1	5'-GGCACGG **TxT TT TT TxT** GGCACGG-3'	*S*	29.5
2		*R*	28.5
3	5'-GGCACGG **TxT TT TxT TT** GGCACGG-3'	*S*	29.5
4		*R*	29.2
5	5'-GGCACGG **TxT TxT TT TT** GGCACGG-3'	*S*	29.6
6		*R*	27.0 *
7	5'-GGCACGG **TxT TT TT TT** GGCACGG-3'	*S*	28.3 *
8		*R*	28.1 *
9	5'-GGCACGG **TxT TxT TxT TxT** GGCACGG-3'	*S*	25.2 *
10		*R*	24.8 *
11	5'-G **TxT** GACG **TT** GACG **TT** GACG **TT** G-3'	*S*	30.6
12		*R*	29.9
13	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*S*	30.0
14		*R*	29.9
15	5'-G **TT** GACG **TT** GACG **TxT** GACG **TT** G-3'	*S*	30.0
16		*R*	29.8
17	5'-G **TT** GACG **TT** GACG **TT** GACG **TxT** G-3'	*S*	29.8
18		*R*	29.7
19	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*S*	24.7 *
20		*R*	24.5 *
21	5'-GCGC **TxT** GC **TT** AAGCAAGCGC-3'	*S*	13.3**
22		*R*	14.6**
23	5'-GCGC **TT** GC **TxT** AAGCAAGCGC-3'	*S*	14.0**
24		*R*	14.5**

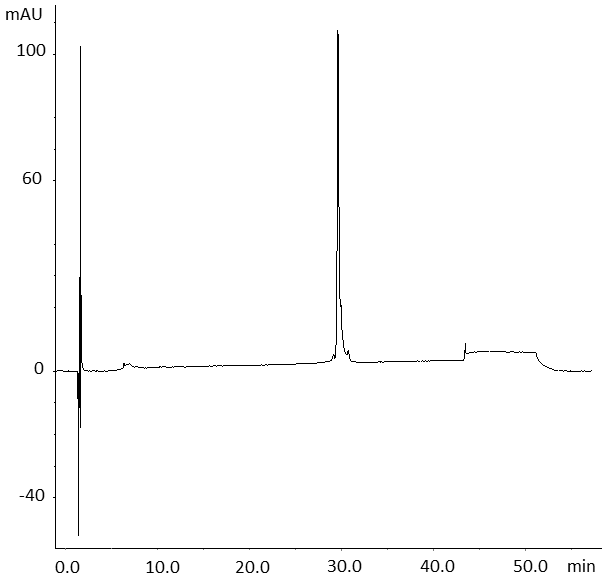
* These separations were performed at 60 °C; all other HPLC separations were performed at 80 °C.

** In these cases of palindromic sequences (nos. 21-24), the RP column was employed for HPLC separations.

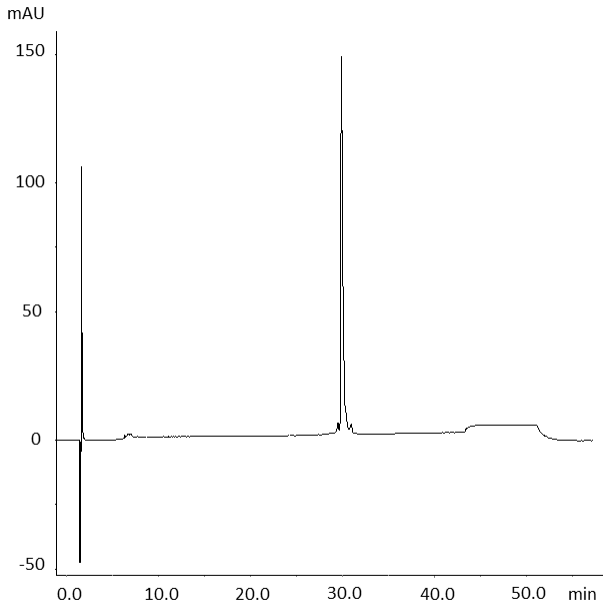
Representative HPLC chromatograms of purified oligonucleotides



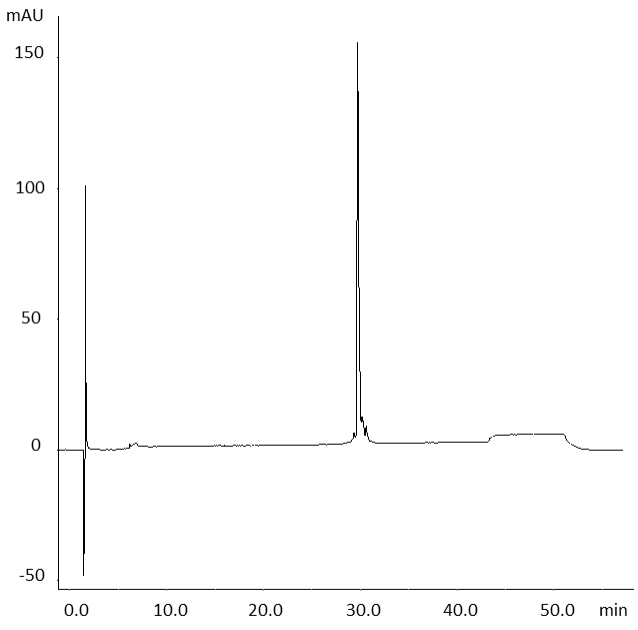
HPLC chromatogram of **6a** (5'-GGCACGGT**x**TTTTTTTGGCACGG-3', **x** = (6'*R*)-NAA)



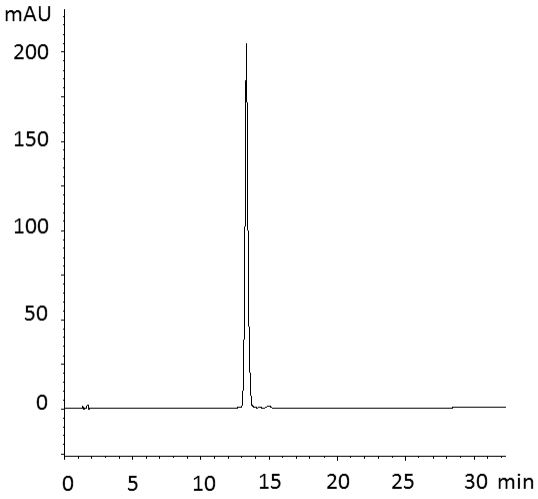
HPLC chromatogram of **6b** (5'-GGCACGGT**x**TT**x**TTTTTGGCACGG-3', **x** = (6'*S*)-NAA)



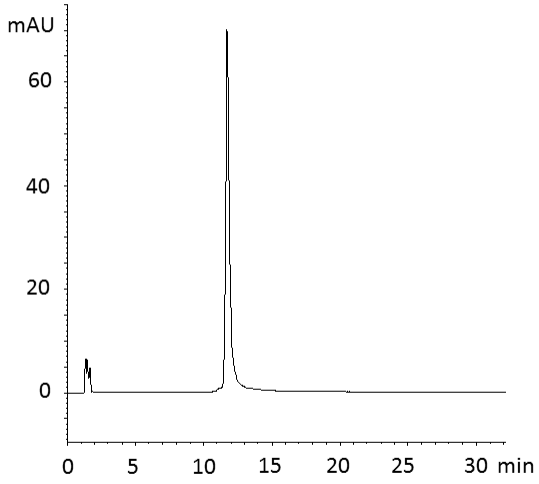
HPLC chromatogram of **7a** (5'-GT**x**TGACGTTGACGTTGACGTTG-3', **x** = (6'*R*)-NAA)



HPLC chromatogram of **7d** (5'-GTTGACGTTGACGTTGACGT**x**TG-3', **x** = (6'*R*)-NAA)



HPLC chromatogram of **8f** (5'-GCGCT**x**TGCTTAAGCAAGCGC-3', **x** = (6'*S*)-NAA)



HPLC chromatogram of **8g** (5'-GCGCTTGCT**x**TAAGCAAGCGC-3', **x** = (6'*R*)-NAA)

Table S2. Mass spectral data of synthesised oligonucleotides.

no.	sequence	NAA-6'-config.	calculated	found
1	5'-GGCACGG **TxT TT TT TxT** GGCACGG-3'	*S*	6740.6	6741.6
2		*R*		6742.0
3	5'-GGCACGG **TxT TT TxT TT** GGCACGG-3'	*S*		6741.8
4		*R*		6742.3
5	5'-GGCACGG **TxT TxT TT TT** GGCACGG-3'	*S*		6741.5
6		*R*		6741.1
7	5'-GGCACGG **TxT TT TT TT** GGCACGG-3'	*S*	6764.6	6764.7
8		*R*		6765.2
9	5'-GGCACGG **TxT TxT TxT TxT** GGCACGG-3'	*S*	6714.9	6715.1*
10		*R*	6692.8	6692.7
11	5'-G **TxT** GACG **TT** GACG **TT** GACG **TT** G-3'	*S*	6789.1	6789.6
12		*R*		6789.6
13	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*S*		6789.4
14		*R*		6789.3
15	5'-G **TT** GACG **TT** GACG **TxT** GACG **TT** G-3'	*S*		6789.6
16		*R*		6789.5
17	5'-G **TT** GACG **TT** GACG **TT** GACG **TxT** G-3'	*S*		6789.7
18		*R*		6789.8
19	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*S*	6717.3	6717.0
20		*R*		6717.2
21	5'-GCGC **TxT** GC **TT** AAGCAAGCGC-3'	*S*	6094.1	6094.3
22		*R*		6094.3
23	5'-GCGC **TT** GC **TxT** AAGCAAGCGC-3'	*S*	6094.1	6094.5
24		*R*		6094.6

* In this case, only the $[M+NH_4^+]$-peak was observed. The calculated mass therefore also refers to the $[M+NH_4^+]$-peak.

Melting temperature data and CD spectra of oligonucleotide duplexes

Melting temperatures of duplexes

Melting curves of duplexes were measured on a Varian (Cary 100 Bio). For these measurements, buffered aqueous solutions of oligonucleotides were prepared (pH = 7, 10 mM NaH_2PO_4/Na_2HPO_4 1:1, 150 mM NaCl) with oligonucleotide concentrations of 2.0 µM. The volume of each sample was 450 µL. The samples were heated/cooled at a rate of 0.7 °C/min. For each sample, five melting curves (three heating curves, two cooling curves) were recorded at four different wavelengths (λ = 250, 260, 270, 280 nm). The cooling curves were used for melting temperature calculations. Melting temperatures were obtained by calculating the inflexion points of the melting curves.

In Tables S3 to S7, the red **x** indicates the NAA-modification with its configuration listed in the adjacent column (all other linkages are phosphates). The color code of Table S8 is explained in its footnote. All T_m and ΔT_m values (see Fig. 2 to 4) are reported in °C.

Table S3. Melting points and ΔT_m values for sequences of type **6 (**DNA/DNA duplexes).

no.	sequence	NAA-6'-config.	T_m	ΔT_m	ΔT_m/mod.
a	5'-GGCACGG **TxT TT TT TT** GGCACGG-3'	*S*	69.5	-1.2	
	5'-GGCACGG **TxT TT TT TT** GGCACGG-3'	*R*	69.2	-1.5	
b	5'-GGCACGG **TxT TxT TT TT** GGCACGG-3'	*S*	66.6	-4.1	-2.05
	5'-GGCACGG **TxT TxT TT TT** GGCACGG-3'	*R*	69.8	-0.9	-0.45
c	5'-GGCACGG **TxT TT TxT TT** GGCACGG-3'	*S*	66.3	-4.4	-2.2
	5'-GGCACGG **TxT TT TxT TT** GGCACGG-3'	*R*	69.1	-1.6	-0.8
d	5'-GGCACGG **TxT TT TT TxT** GGCACGG-3'	*S*	66.4	-4.3	-2.15
	5'-GGCACGG **TxT TT TT TxT** GGCACGG-3'	*R*	68.0	-2.7	-1.35
e	5'-GGCACGG **TxT TxT TxT TxT** GGCACGG-3'	*S*	63.9	-6.8	-1.7
	5'-GGCACGG **TxT TxT TxT TxT** GGCACGG-3'	*R*	68.8	-1.9	-0.48
	5'-GGCACGG **TT TT TT TT** GGCACGG-3' (reference)		70.7		

Table S4. Melting points and ΔT_m values for sequences of type **7 (**DNA/DNA duplexes).

no.	sequence	NAA-6'-config.	T_m	ΔT_m	ΔT_m/mod.
a	5'-G **TxT** GACG **TT** GACG **TT** GACG **TT** G-3'	*S*	67.1	-0.9	
	5'-G **TxT** GACG **TT** GACG **TT** GACG **TT** G-3'	*R*	68.0	±0.0	
b	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*S*	67.3	-0.7	
	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*R*	67.7	-0.3	
c	5'-G **TT** GACG **TT** GACG **TxT** GACG **TT** G-3'	*S*	66.9	-1.1	
	5'-G **TT** GACG **TT** GACG **TxT** GACG **TT** G-3'	*R*	67.1	-0.9	
d	5'-G **TT** GACG **TT** GACG **TT** GACG **TxT** G-3'	*S*	68.3	+0.3	
	5'-G **TT** GACG **TT** GACG **TT** GACG **TxT** G-3'	*R*	68.4	+0.4	
e	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*S*	63.2	-4.8	-1.2
	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*R*	66.3	-1.7	-0.43
	5'-G **TT** GACG **TT** GACG **TT** GACG **TT** G-3' (reference)		68.0		

Table S5. Melting points and ΔT_m values for palindromic sequences of type **8** (DNA/DNA duplexes).

no.	sequence	NAA-6'-config.	T_m	ΔT_m	ΔT_m/mod.
f	5'-GCGC **TxT** GC **TT** AAGCAAGCGC-3'	*R*	83.8	-1.8	
g	5'-GCGC **TT** GC **TxT** AAGCAAGCGC-3'	*S*	85.4	-0.2	
	5'-GCGC **TT** GC **TxT** AAGCAAGCGC-3'	*R*	85.0	-0.6	
	5'-GCGC **TT** GC **TT** AAGCAAGCGC-3' (reference)		85.6		

Table S6. Melting points and ΔT_m values for sequences of type **6** (DNA/RNA duplexes).

no.	sequence	NAA-6'-config.	T_m	ΔT_m	ΔT_m/mod.
a	5'-GGCACGG **TxT TT TT TT** GGCACGG-3'	*S*	64.7	-3.8	
	5'-GGCACGG **TxT TT TT TT** GGCACGG-3'	*R*	64.4	-4.1	
b	5'-GGCACGG **TxT TxT TT TT** GGCACGG-3'	*S*	61.7	-6.8	-3.4
	5'-GGCACGG **TxT TxT TT TT** GGCACGG-3'	*R*	63.3	-5.2	-2.3
c	5'-GGCACGG **TxT TT TxT TT** GGCACGG-3'	*S*	61.4	-7.1	-3.55
	5'-GGCACGG **TxT TT TxT TT** GGCACGG-3'	*R*	63.4	-5.1	-2.55
d	5'-GGCACGG **TxT TT TT TxT** GGCACGG-3'	*S*	61.6	-6.9	-3.45
	5'-GGCACGG **TxT TT TT TxT** GGCACGG-3'	*R*	62.2	-6.3	-3.15
e	5'-GGCACGG **TxT TxT TxT TxT** GGCACGG-3'	*S*	55.3	-13.2	-3.3
	5'-GGCACGG **TxT TxT TxT TxT** GGCACGG-3'	*R*	59.3	-9.2	-2.3
	5'-GGCACGG **TT TT TT TT** GGCACGG-3' (reference)		68.5		

Table S7. Melting points and ΔT_m values for sequences of type **7 (**DNA/RNA duplexes).

no.	sequence	NAA-6'-config.	T_m	ΔT_m	ΔT_m/mod.
a	5'-G **TxT** GACG **TT** GACG **TT** GACG **TT** G-3'	*S*	65.2	-0.3	
	5'-G **TxT** GACG **TT** GACG **TT** GACG **TT** G-3'	*R*	64.9	-0.6	
b	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*S*	61.8	-3.7	
	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*R*	62.9	-2.4	
c	5'-G **TT** GACG **TT** GACG **TxT** GACG **TT** G-3'	*S*	62.0	-3.5	
	5'-G **TT** GACG **TT** GACG **TxT** GACG **TT** G-3'	*R*	63.1	-2.4	
d	5'-G **TT** GACG **TT** GACG **TT** GACG **TxT** G-3'	*S*	64.0	-1.5	
	5'-G **TT** GACG **TT** GACG **TT** GACG **TxT** G-3'	*R*	64.0	-1.5	
e	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*S*	53.7	-11.8	-2.95
	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*R*	57.7	-7.8	-1.95
	5'-G **TT** GACG **TT** GACG **TT** GACG **TT** G-3' (reference)		65.5		

Table S8. Melting points and ΔT_m values for mismatched duplexes (DNA/DNA).

no.	sequence	NAA-6'-config.	mismatch	T_m	ΔT_m
a	5'-G **TT** GACG **TT** GACG **TT** GACG **TT** G-3' (reference)		G14	65.9	-2.1
			C14	62.3	-5.7
			T14	64.2	-3.8
b	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*S*	G14	62.7	-4.6
			C14	61.4	-5.9
			T14	62.7	-4.6
c	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*R*	G14	63.8	-3.9
			C14	61.6	-6.1
			T14	62.6	-5.5
d	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*S*	G14	58.4	-4.8
			C14	56.8	-6.4
			T14	58.8	-4.4
e	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*R*	G14	61.5	-4.8
			C14	59.8	-6.5
			T14	61.0	-5.3
f	5'-GGCACGG **TTTTTTTT** GGCACGG-3' (reference)		G12	65.8	-4.9
			C12	64.1	-6.6
			T12	65.5	-5.2
g	5'-GGCACGG **TxTTxTTxTTxT** GGCACGG-3'	*S*	G12	59.8	-4.1
			C12	59.3	-4.6
			T12	60.7	-3.2
h	5'-GGCACGG **TxTTxTTxTTxT** GGCACGG-3'	*R*	G12	64.8	-4.0
			C12	62.4	-6.4
			T12	63.5	-5.3

x: (6'*S*)-configured NAA-modification; **x**: (6'*R*)-configured NAA-modification; **T**: position with base-pairing mismatch in the counterstrand; all linkages not noted as **x** are phosphates.

Melting temperatures at elevated NaCl concentrations

Melting temperatures have also been measured at NaCl concentrations of 0.5 M and 1.0 M, respectively. In Tables S9 to S12, the red **x** indicates the NAA-modification with its configuration listed in the adjacent column (all other linkages are phosphates). All T_m and ΔT_m values are reported in °C. The following ΔT_m values are listed in the Tables:

ΔT_m (1): Difference between the T_m value of the NAA-modified duplex and the corresponding unmodified reference duplex at the same NaCl concentration.

ΔT_m (2): Difference between the T_m value of the respective duplex at an elevated NaCl concentration and the T_m value of the same modified duplex at [NaCl] = 150 mM (*i.e.* under standard conditions).

1) [NaCl] = 0.5 M

Table S9. Melting points and ΔT_m values for various sequences at [NaCl] = 0.5 M (DNA/DNA duplexes).

no.	sequence	6'-config.	T_m	ΔT_m (1)	ΔT_m /mod	ΔT_m (2)
1	5'-GGCACGG **TxT TxT TxT TxT** GGCACGG-3'	*R*	72.6	-3.9	-1.0	+3.8
2	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*R*	71.0	-3.3	-0.8	+4.7
	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*S*	68.7	-5.6	-1.4	+5.5
3	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*R*	73.1	-1.2	-1.2	+5.4
	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*S*	72.8	-1.5	-1.5	+5.5
4	5'-G **TT** GACG **TT** GACG **TT** GACG **TT** G-3' (reference)		74.3			+6.3
5	5'-GGCACGG **TT TT TT TT** GGCACGG-3' (reference)		76.5			+5.8

Table S10. Melting points and ΔT_m values for various sequences at [NaCl] = 0.5 M (DNA/RNA duplexes).

no.	sequence	6'-config.	T_m	ΔT_m (1)	ΔT_m/mod	ΔT_m (2)
1	5'-GGCACGG **TxT TxT TxT TxT** GGCACGG-3'	*R*	64.5	-9.2	-2.3	+5.2
2	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*R*	62.5	-9.3	-2.3	+4.8
	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*S*	59.4	-12.4	-3.1	+5.7
3	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*R*	68.7	-3.1	-3.1	+5.8
	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*S*	66.9	-4.9	-4.9	+5.8
4	5'-G **TT** GACG **TT** GACG **TT** GACG **TT** G-3' (reference)		71.8			+6.3
5	5'-GGCACGG **TT TT TT TT** GGCACGG-3' (reference)		73.7			+5.2

2) [NaCl] = 1.0 M

Table S11. Melting points and ΔT_m values for various sequences at [NaCl] = 1.0 M (DNA/DNA duplexes).

no.	sequence	6'-config.	T_m	ΔT_m (1)	ΔT_m/mod	ΔT_m (2)
1	5'-GGCACGG **TxT TxT TxT TxT** GGCACGG-3'	*R*	74.0	-4.5	-1.1	+5.2
2	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*R*	72.4	-4.0	-1.0	+6.1
		S	69.4	-7.0	-1.8	+6.2
3	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*R*	74.6	-1.8	-1.8	+6.9
		S	74.3	-2.1	-2.1	+7.0
4	5'-G **TT** GACG **TT** GACG **TT** GACG **TT** G-3' (reference)		76.4			+8.4
5	5'-GGCACGG **TT TT TT TT** GGCACGG-3' (reference)		78.5			+7.8

Table S12. Melting points and ΔT_m values for various sequences at [NaCl] = 1.0 M (DNA/RNA duplexes).

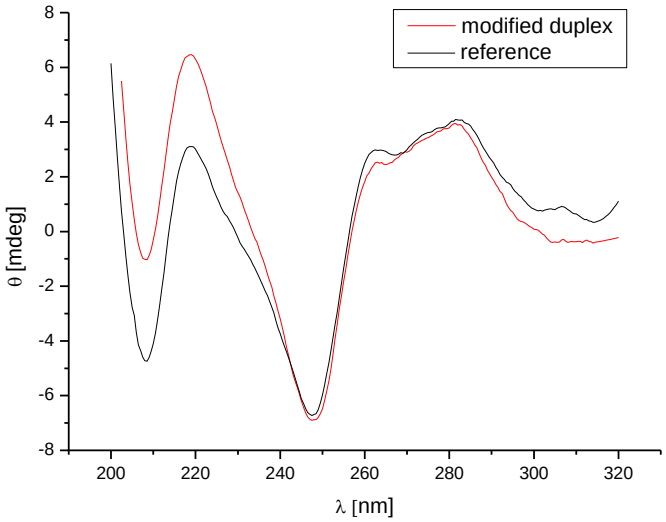
no.	sequence	6'-config.	T_m	ΔT_m (1)	ΔT_m/mod	ΔT_m (2)
1	5'-GGCACGG **TxT TxT TxT TxT** GGCACGG-3'	*R*	65.9	-9.7	-1.4	+6.6
2	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*R*	63.8	-9.7	-2.4	+6.1
	5'-G **TxT** GACG **TxT** GACG **TxT** GACG **TxT** G-3'	*S*	61.0	-12.5	-3.1	+7.3
3	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*R*	69.6	-3.9	-3.9	+6.7
	5'-G **TT** GACG **TxT** GACG **TT** GACG **TT** G-3'	*S*	68.7	-4.8	-4.8	+6.9
4	5'-G **TT** GACG **TT** GACG **TT** GACG **TT** G-3' (reference)		73.5			+8.0
5	5'-GGCACGG **TT TT TT TT** GGCACGG-3' (reference)		75.6			+7.1

Representative CD spectra of duplexes

Circular dichroism (CD) spectra were measured on an Applied Photophysics (Chirascan) spectrometer in the wavelength range of 200-320 nm. For these measurements, the same solutions were used as in the melting point experiments. All measurements were performed at 25 °C. The signals were recorded in a wavelength distance of 0.5 nm. The delay in time between the data points was 2 s. For each sample, 3 CD spectra were measured and a median curve was calculated. For each curve, a background correction was performed.

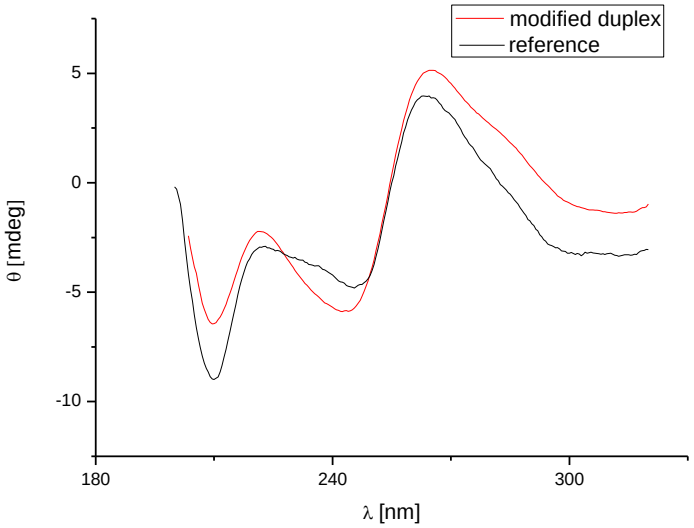
Sequence: 5'-GGCACGG **T**x**T TT TT TT** GGCACGG-3', NAA-6'-configuration: (*R*)

Complementary sequence: DNA



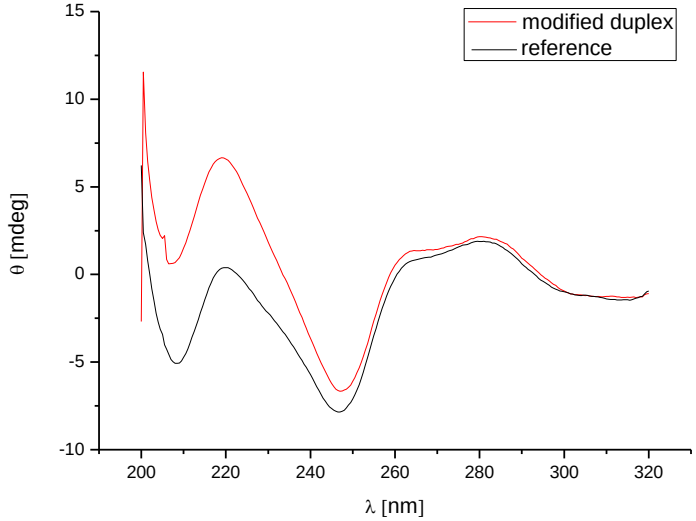
Sequence: 5'-GGCACGG **TxT TT TT TT** GGCACGG-3', NAA-6'-configuration: (*R*)

Complementary sequence: RNA



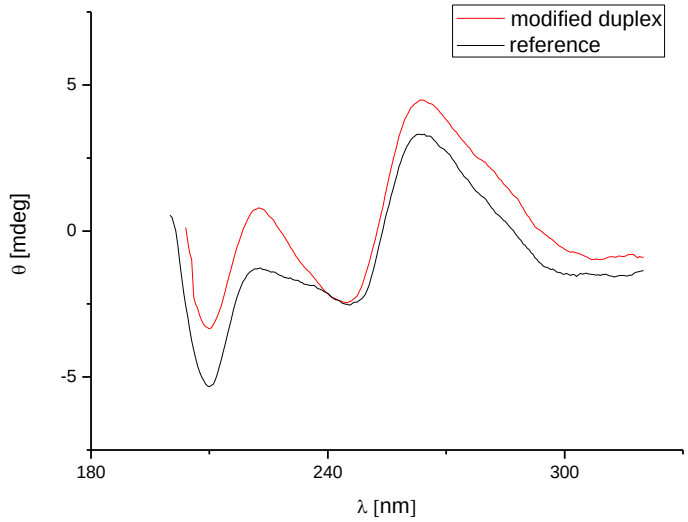
Sequence: 5'-GGCACGG **TxT TT TT TT** GGCACGG-3', NAA-6'-configuration: (*S*)

Complementary sequence: DNA



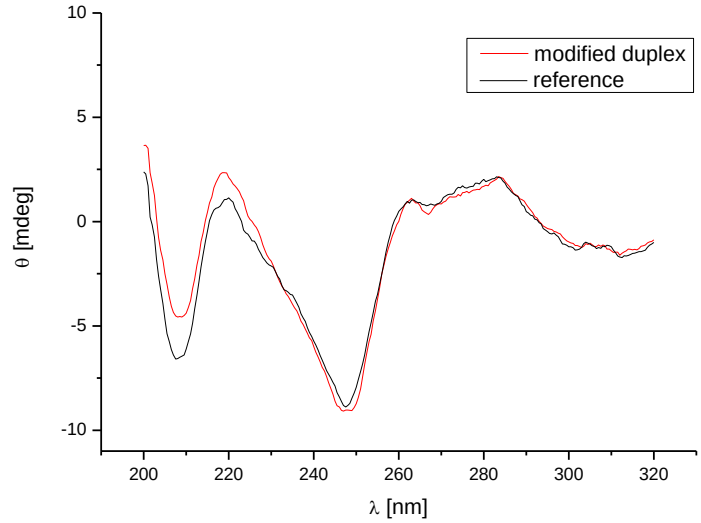
Sequence: 5'-GGCACGG **T**x**T TT TT TT** GGCACGG-3', NAA-6'-configuration: (*S*)

Complementary sequence: RNA



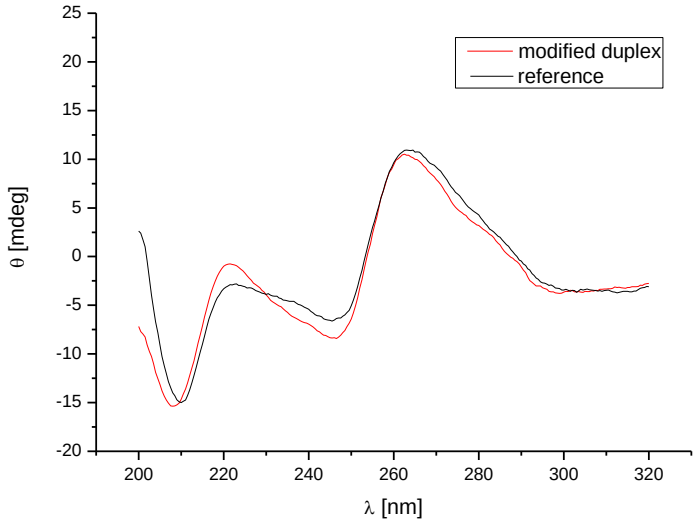
Sequence: 5'-GGCACGG **T**x**T TT T**x**T TT** GGCACGG-3', NAA-6'-configuration: (*R*)

Complementary sequence: DNA



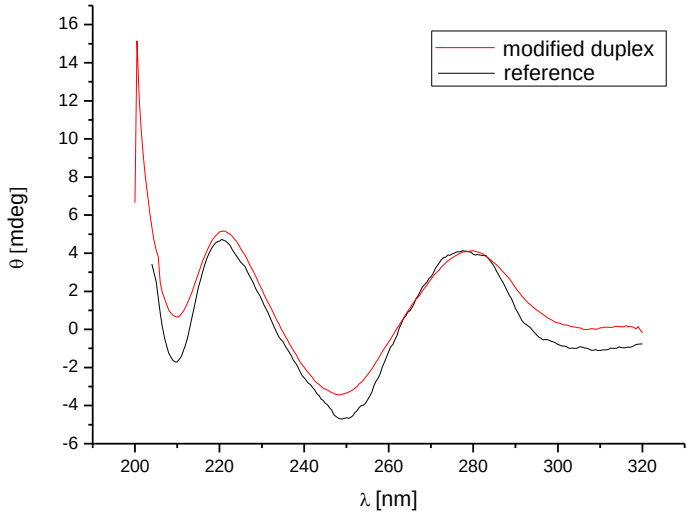
Sequence: 5'-GGCACGG **T**<u>**x**</u>**T TT T**<u>**x**</u>**T TT** GGCACGG-3', NAA-6'-configuration: (*R*)

Complementary sequence: RNA



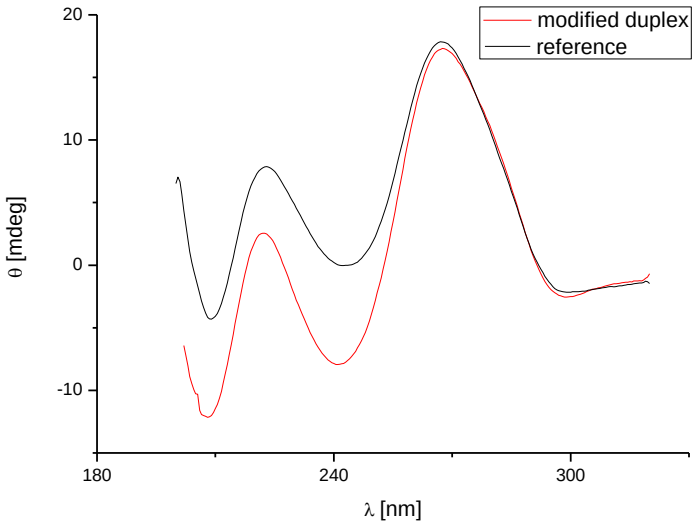
Sequence: 5'-G **T**<u>**x**</u>**T** GACG **T**<u>**x**</u>**T** GACG **T**<u>**x**</u>**T** GACG **T**<u>**x**</u>**T** G-3', NAA-6'-configuration: (*R*)

Complementary sequence: DNA



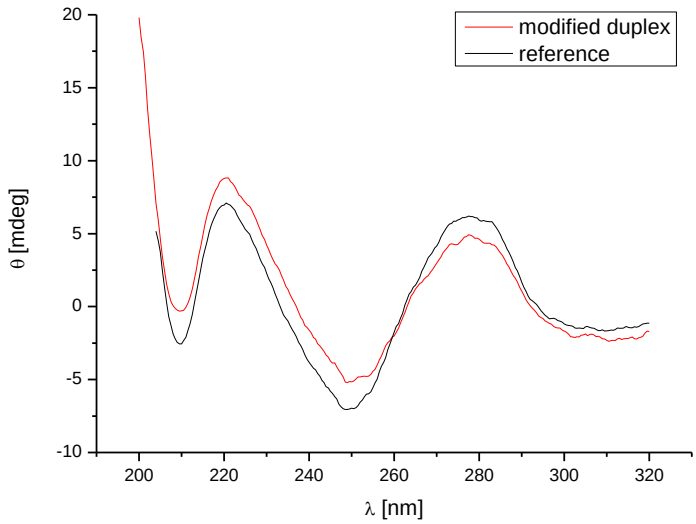
Sequence: 5'-G **T**x**T** GACG **T**x**T** GACG **T**x**T** GACG **T**x**T** G-3', NAA-6'-configuration: (*R*)

Complementary sequence: RNA



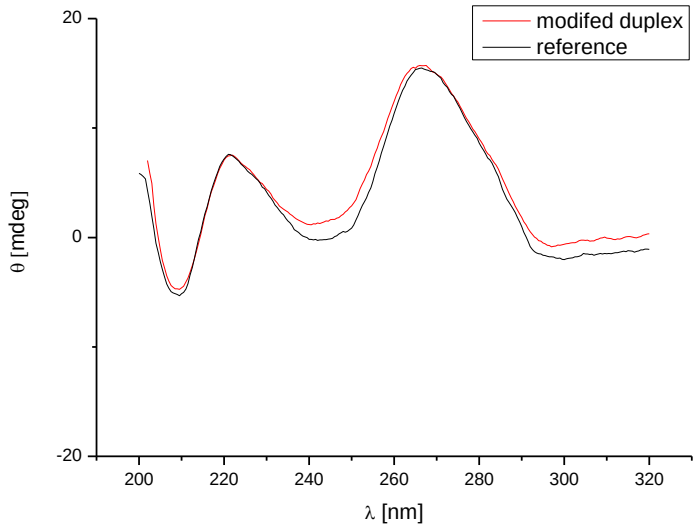
Sequence: 5'-G **TT** GACG **T**x**T** GACG **TT** GACG **TT** G-3', NAA-6'-configuration: (*S*)

Complementary sequence: DNA



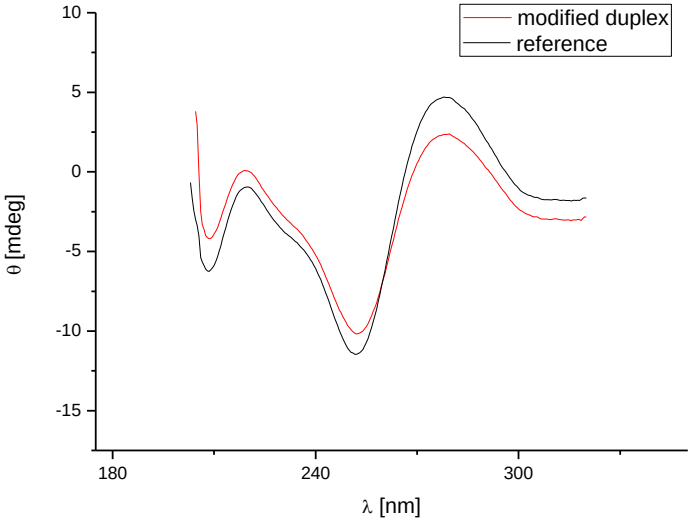
Sequence: 5'-G **TT** GACG **T**x**T** GACG **TT** GACG **TT** G-3', NAA-6'-configuration: (*S*)

Complementary sequence: RNA



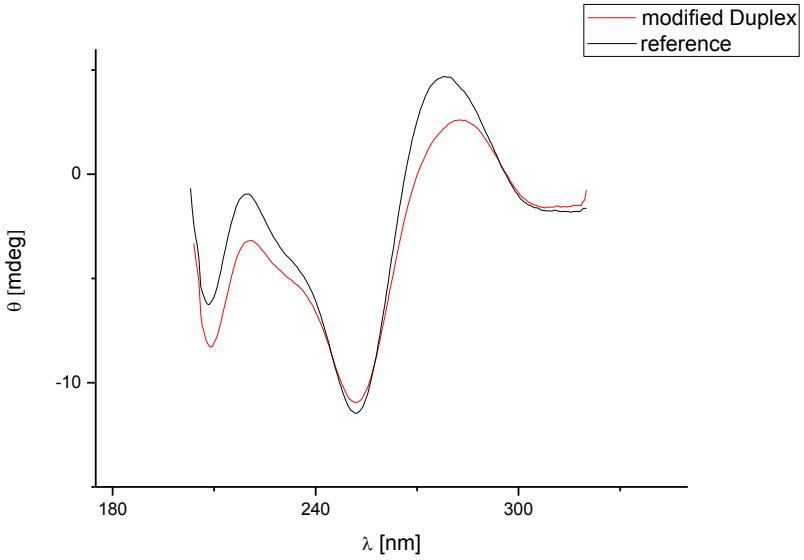
Sequenz: 5'-GCGC **T**x**T** GC **TT** AAGCAAGCGC-3', NAA-6'-configuration: (*S*)

Complementary sequence: DNA



Sequence: 5'-GCGC **T**x**T** GC **TT** AAGCAAGCGC-3', NAA-6'-configuration: (*S*)

Complementary sequence (palindromic): 5'-GCGC **T**x**T** GC **TT** AAGCAAGCGC-3', NAA-6'-configuration: (*S*)



References

S1 S. Czernecki and J.-M. Valéry, *Synthesis*, 1991, 239-240.

S2 R. Eisenhuth and C. Richert, *J. Org. Chem.*, 2008, **74**, 26-37.

S3 U. Schmidt, A. Lieberknecht, U. Schanbacher, T. Beuttler and J. Wild, *Angew. Chem. Int. Ed.*, 1982, **21**, 776-777.

S4 U. Schmidt, A. Lieberknecht and J. Wild, *Synthesis*, 1984, 53-60.

S5 U. Schmidt and J. Wild, *Liebigs Ann. Chem.*, 1985, 1882-1894.

S6 R. Hamzavi, F. Dolle, B. Tavitian, O. Dahl and P. E. Nielsen, *Bioconjugate Chem.*, 2003, **14**, 941-954.

S7 A. P. Spork and C. Ducho, *Org. Biomol. Chem.*, 2010, **8**, 2323-2326.

S8 A. P. Spork, D. Wiegmann, M. Granitzka, D. Stalke and C. Ducho, *J. Org. Chem.*, 2011, **76**, 10083-10098.

S9 A. T. Khan and E. A. Mondal, *Synlett*, 2003, 694-698.

S10 J. D. More and N. S. Finney, *Org. Lett.*, 2002, **4**, 3001-3003.

S11 U. Schmidt, H. Griesser, V. Leitenberger, A. Lieberknecht, R. Mangold, R. Meyer and B. Riedl, *Synthesis*, 1992, 487-490.

S12 R. Mazurkiewicz, A. Kuźnik, M. Grymel and N. H. Kuźnik, *Magn. Reson. Chem.*, 2005, **43**, 36-40.

S13 B. D. Vineyard, W. S. Knowles, M. J. Sabacky, G. L. Bachman and D. J. Wienkauff, *J. Am. Chem. Soc.*, 1977, **99**, 5946-5952.

S14 M. J. Burk, *J. Am. Chem. Soc.*, 1991, **113**, 8518-8519.

S15 T. Masquelin, E. Broger, K. Müller, R. Schmid and D. Obrecht, *Helv. Chim. Acta*, 1994, **77**, 1395-1411.

S16 C. Ducho, R. B. Hamed, E. T. Batchelar, J. L. Sorensen, B. Odell and C. J. Schofield, *Org. Biomol. Chem.*, 2009, **7**, 2770-2779.