Supporting Information Available

The 2-(*N*-Formyl-*N*-methyl) aminoethyl Group as a Potential Phosphate/Thiophosphate Protecting Group in Solid-phase Oligodeoxyribonucleotide Synthesis.

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Preparation of 2-(*N*-formyl-*N*-methyl)aminoethan-1-ol

2-(Methylamino)ethanol (51.0 g, 0.68 mol) is placed in a 250 mL round-bottom flask equipped with a reflux condenser, and cooled to 5 °C by immersion in an ice bath. Ethyl formate (75.0 g, 1.01 mol) is then added, in portions through the condenser to the stirred amino alcohol over a period of 5 min at 5 °C. The solution is removed from the ice bath and brought to reflux for 1 h. The solution is then distilled at atmospheric pressure to remove excess ethyl formate, and then carefully distilled under high-vacuum to afford 2-(*N*-formyl-N-methyl)aminoethan-1-ol as a clear colorless liquid (63.1 g, 0.61 mol, 90%) boiling at 120-122 °C @ 0.15 mm Hg. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [2.75 (s) and 2.94 (s, 30%) (3H)], 3.27 (m, 2H), 3.47 (m, 3H), [7.94 (s) and 7.99 (s, 30%) (1H)]. ^{13}C-NMR (75 MHz, DMSO-d_6): δ 29.2, 34.9, 46.2, 51.2, 57.8, 57.9, 58.1, 58.2, 162.7, 163.0. EI-MS: calcd for $C_4H_9NO_2$ ($M^{\bullet+}$) 103, found 103.

Preparation of *N,N,N',N'*-tetraisopropyl-*O*-[2-[(*N*-formyl-*N*-methyl)amino]ethyl]phosphor-diamidite 20.

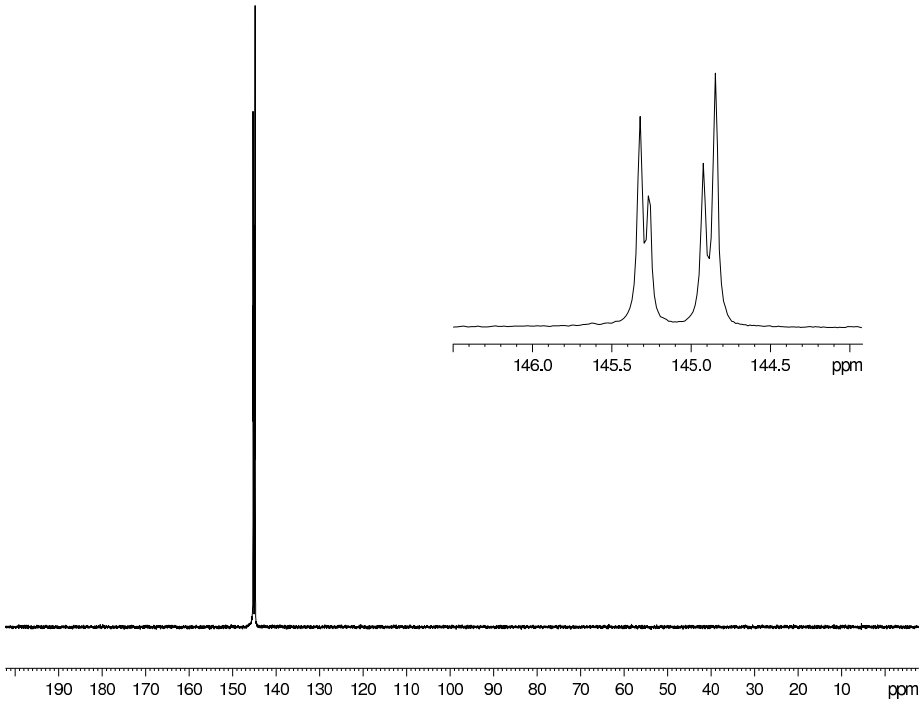
To an oven-dried 100 mL round-bottom flask containing 50 mL of dry benzene under a dry argon atmosphere, 876 μL of freshly distilled phosphorus trichloride (10 mmol) is added by syringe through a rubber septum. The stirred solution is cooled to 5 °C by immersion in an ice bath and then, 7.7 mL of anhydrous *N,N*-diisopropylamine (55 mmol) is added by syringe under argon over a period of 30 min. The reaction mixture is removed from the ice bath and allowed to warm to 25 °C under a positive pressure of argon until the formation of bis(*N,N*-diisopropylamino)chlorophosphine is complete. The rate of the reaction is monitored by ^{31}P NMR spectroscopy; after ~ 48 h, the expected chlorophosphine is observed as the major (> 96%) reaction product (132.0 ppm downfield relative to a phosphoric acid external standard). 2-(*N*-Formyl-*N*-methyl)aminoethan-1-ol (1.03 mL, 10 mmol) is the added to the suspension. The resulting mixture is stirred for 2 h at 25 °C under a positive pressure of argon. ^{31}P NMR analysis of the reaction mixture indicates that the generation of **20** is essentially quantitative (~ 96%) and reveals two singlets at 118.0 and 118.7 ppm in C_6D_6. The suspension is filtered through a sintered glass funnel (coarse porosity, 60 mL) and washed with 20 mL of dry benzene. The filtrates are evaporated under reduced pressure to an oil and dissolved in a minimum amount (~ 3 mL) of benzene and triethylamine (95:5 v/v). The viscous solution is then applied uniformly to the top of a chromatography column (3 × 20 cm) packed with a Silica Gel 60Å (Merck 230-400 mesh, 30 g) slurry in a solution of benzene:triethylamine (95:5 v/v). The column is eluted

isocratically with benzene:triethylamine (95:5 v/v) and fractions of 8 mL each are collected. Fractions containing the phosphordiamidite **20** are evaporated to an oil. Residual triethylamine is removed from the product by co-evaporation with toluene (4 × 10 mL). The phosphordiamidite is then left under high vacuum for at least 3 h. Yield: 2.43 g (7.3 mmol, 73%). ^{1}H-NMR (300 MHz, C_6D_6): δ [1.14 (d, *J* = 6.9 Hz), 1.16 (d, *J* = 6.7 Hz) 1.18 (d, *J* = 6.7 Hz) (24H)], [2.40 (s, 34%) and 2.64 (s, 66%) (3H)], 2.80 (t, *J* = 5.4 Hz, 2H), 3.43 (m, 4H), [3.29 (dt, *J* =5.4 Hz, J_{HP} = 6.6 Hz) and 3.60 (dt, *J* =5.4 Hz, J_{HP} = 6.6 Hz)(2H)], [7.82 (s, 34%) and 7.98 (s, 66%) (1H)]. ^{13}C-NMR (75 MHz, C_6D_6): δ 24.1, 24.2, 24.6, 24.7, 44.7, 44.9, 45.8 (d, $^{2}J_{CP}$ = 8.5 Hz), 50.4(d, $^{2}J_{CP}$ = 8.5 Hz), 61.3, 61.5, 61.9, 62.2, 161.9, 162.3. ^{31}P-NMR (121 MHz, C_6D_6): δ 118.0, 118.7. EI-HRMS: calcd for $C_{16}H_{36}N_3O_2P$ ($M^{\bullet+}$) 333.2545, found 333.2528.

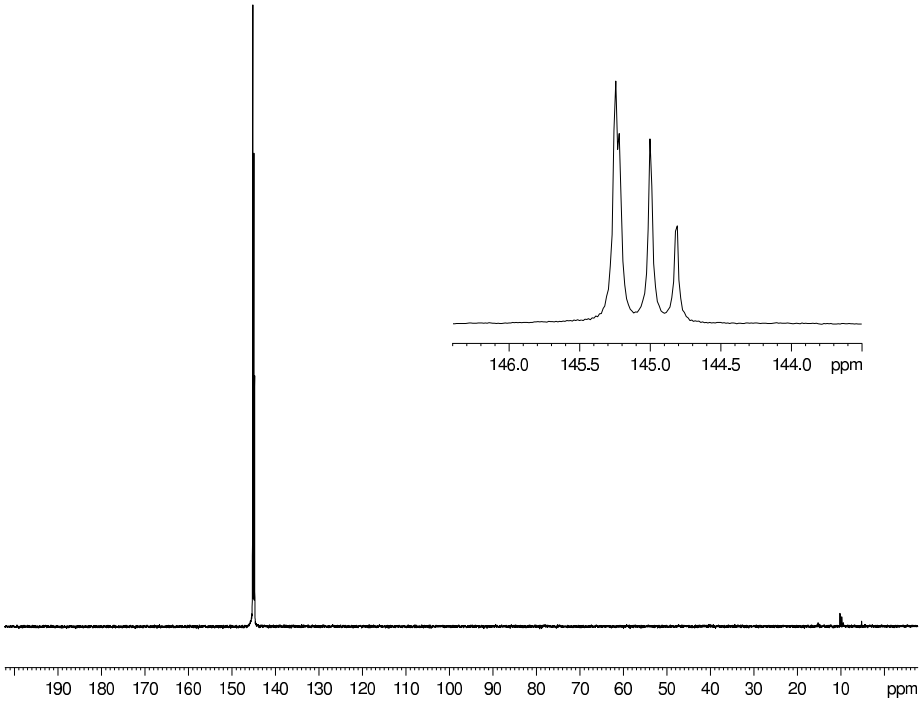
General prepraration of 5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-(*N,N*-diisopropylamino)[2-[(*N*-formyl-*N*-methyl)amino]ethoxy]phosphinyl-2'-deoxyribonucleosides.

A suitably potected deoxyribonucleoside (2 mmol) is dried under high vacuum for 2 h in a 50 mL round-bottom flask. Anhydrous acetonitrile (10 mL) is added to the dried nucleoside followed by *N,N,N',N'*-tetraisopropyl-*O*-[2-[(*N*-formyl-*N*-methyl)amino]ethyl]phosphor-diamidite **20.**(730 mg, 2.2 mmol). To this solution is added by syringe 4.4 mL of 0.45 M 1*H*-tetrazole in acetonitrile (2 mmol), dropwise, over a period of 0.5 h. The rates of the reaction are monitored by TLC using benzene:triethylamine (9:1 v/v) as an eluent. Phosphinylation of suitably protected 2'-deoxynucleosides is usually complete within 1 h at 25°C (for best results, phosphinylation of properly protected 2'-deoxyguanosine is allowed to proceed for 12 h). The reaction mixture is then concentrated under reduced pressure, dissolved in benzene:triethylamine (9:1 v/v), and chromatographed on a silica gel column (4 cm × 10 cm) using the same solvent for equilibration and elution. Appropriate fractions are pooled, concentrated, and each of the deoxyribonucleoside phosphoramidites is isolated as a white amorphous powder in yields exceeding 90%. **5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-(*N,N*-diisopropylamino)[2-[(*N*-formyl-*N*-methyl)amino]ethoxy]phosphinyl-2'-deoxythymidine 1.** ^{31}P-NMR (121 MHz, C_6D_6): δ 145.3, 145.2, 145.0, 144.8. FAB-HRMS: calcd for $C_{41}H_{53}N_4O_9P$ $(M+Cs)^+$ 909.2604, found 909.2544. ***N*^{6}-benzoyl-5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-(*N,N*-diisopropylamino)[2-[(*N*-formyl-*N*-methyl)amino] ethoxy]phosphinyl-2'deoxyadenosine.** ^{31}P-NMR (121 MHz, C_6D_6): δ 145.7, 145.6, 144.9. FAB-HRMS: calcd for $C_{48}H_{56}N_7O_8P$ $(M+Na)^+$ 912.3827, found 912.3843. ***N*^{2}-isobutyryl-5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-(*N,N*-diisopropylamino)[2-[(*N*-formyl-*N*-methyl)amino]ethoxy] phosphinyl-2'deoxyguanosine.** ^{31}P-NMR (121 MHz, C_6D_6): δ 145.7, 140.9. FAB-HRMS: calcd for $C_{45}H_{58}N_7O_9P$ $(M+Na)^+$ is 894.3933, found 894.3978.

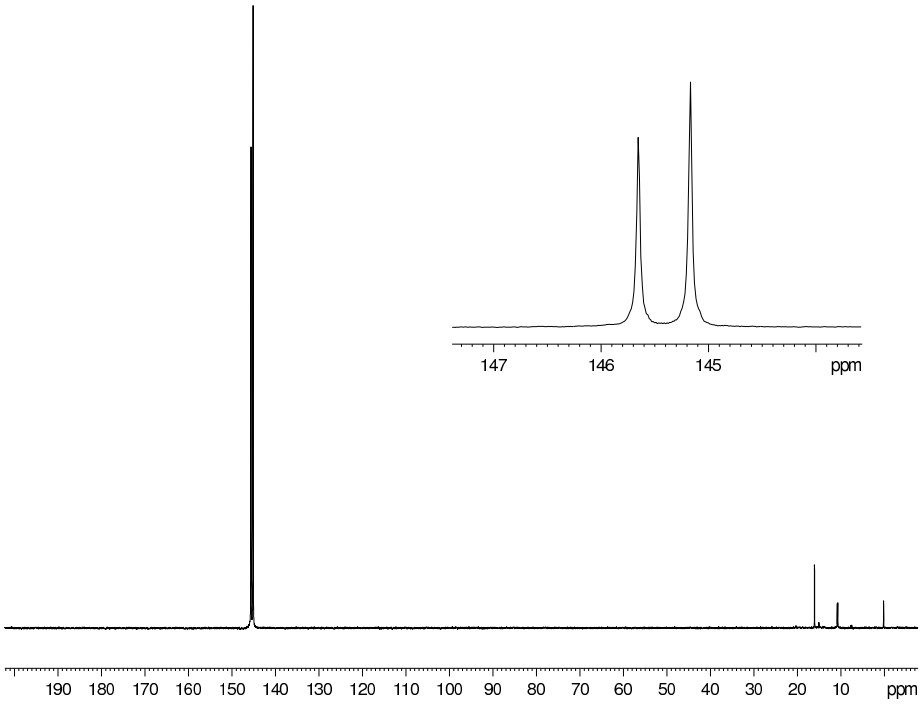
121 MHz ^{31}P-NMR spectrum of **3** in C_6D_6



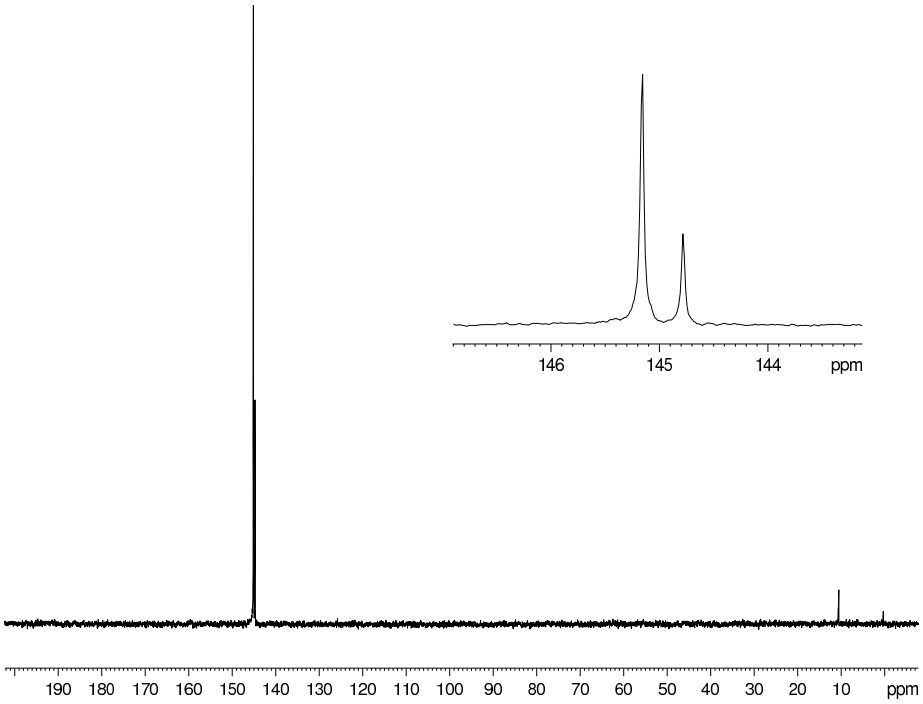
121 MHz ^{31}P-NMR spectrum of **4** in C_6D_6



121 MHz ^{31}P-NMR spectrum of **5** in C_6D_6

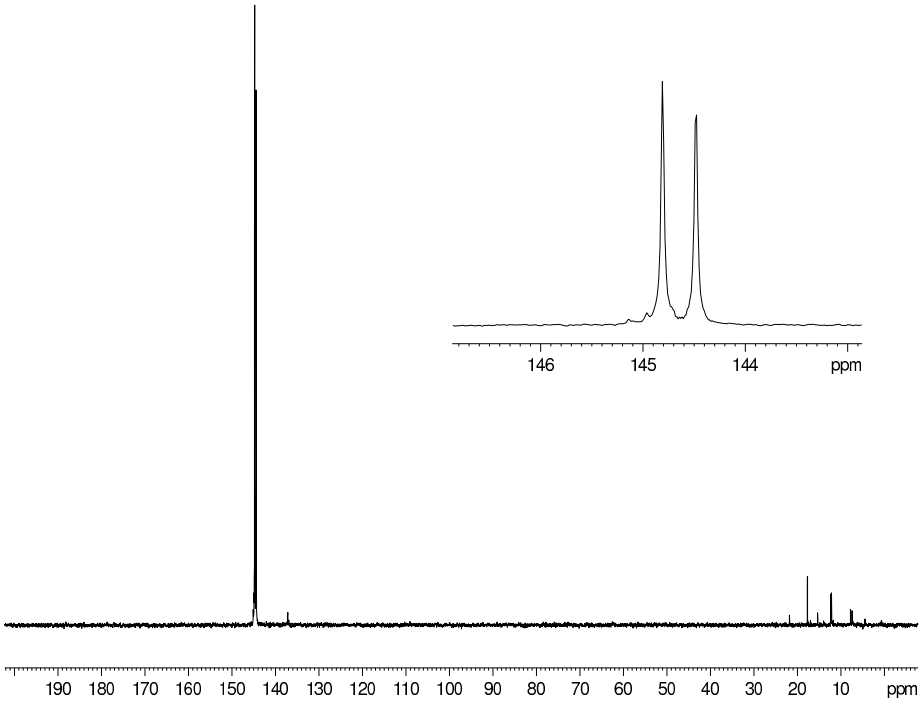


121 MHz ^{31}P-NMR spectrum of **6**[1] in C_6D_6

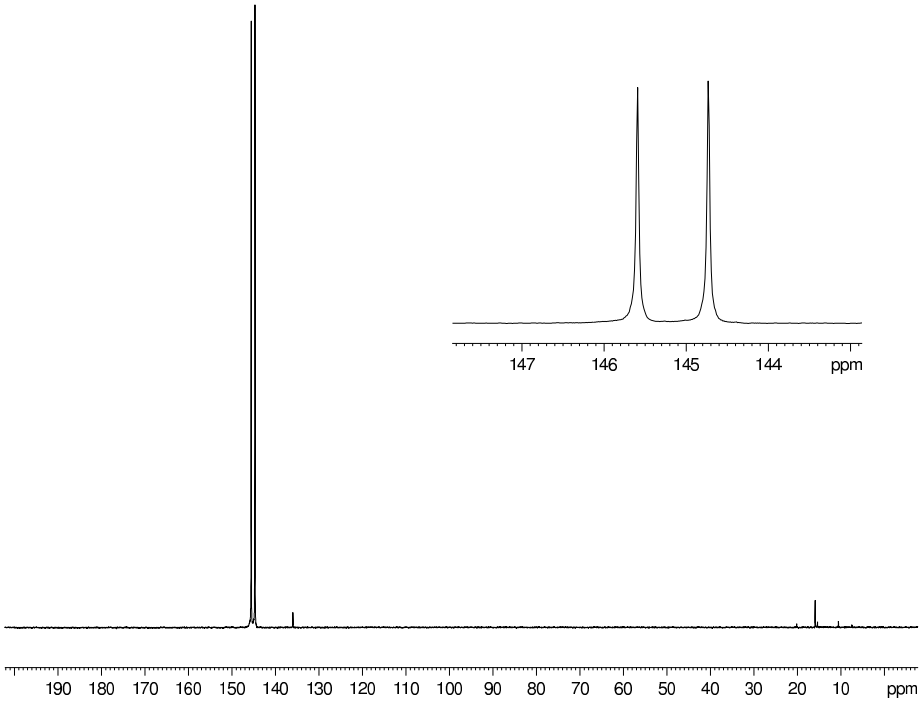


[1]Enrichment in one of the *P*-diastereomers occurred during purification by silica gel chromatography.

121 MHz ^{31}P-NMR spectrum of **7** in C_6D_6



121 MHz ^{31}P-NMR spectrum of **9** in C_6D_6

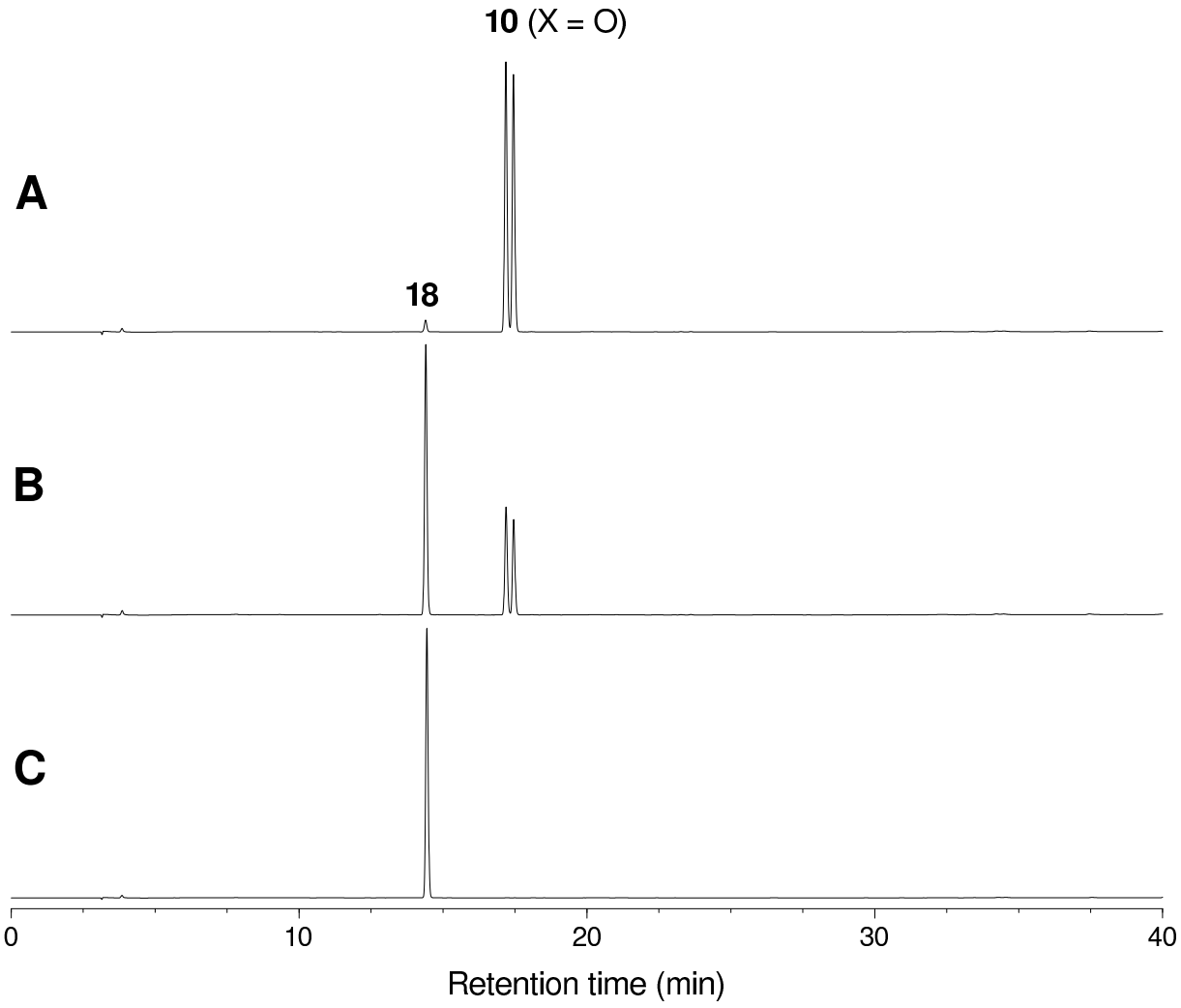


RP-HPLC analysis of the deprotection of **10** (X=O). Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **10** (X=O).

Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 20 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 40 minutes in 0.1 M triethylammonium acetate pH 7.0.

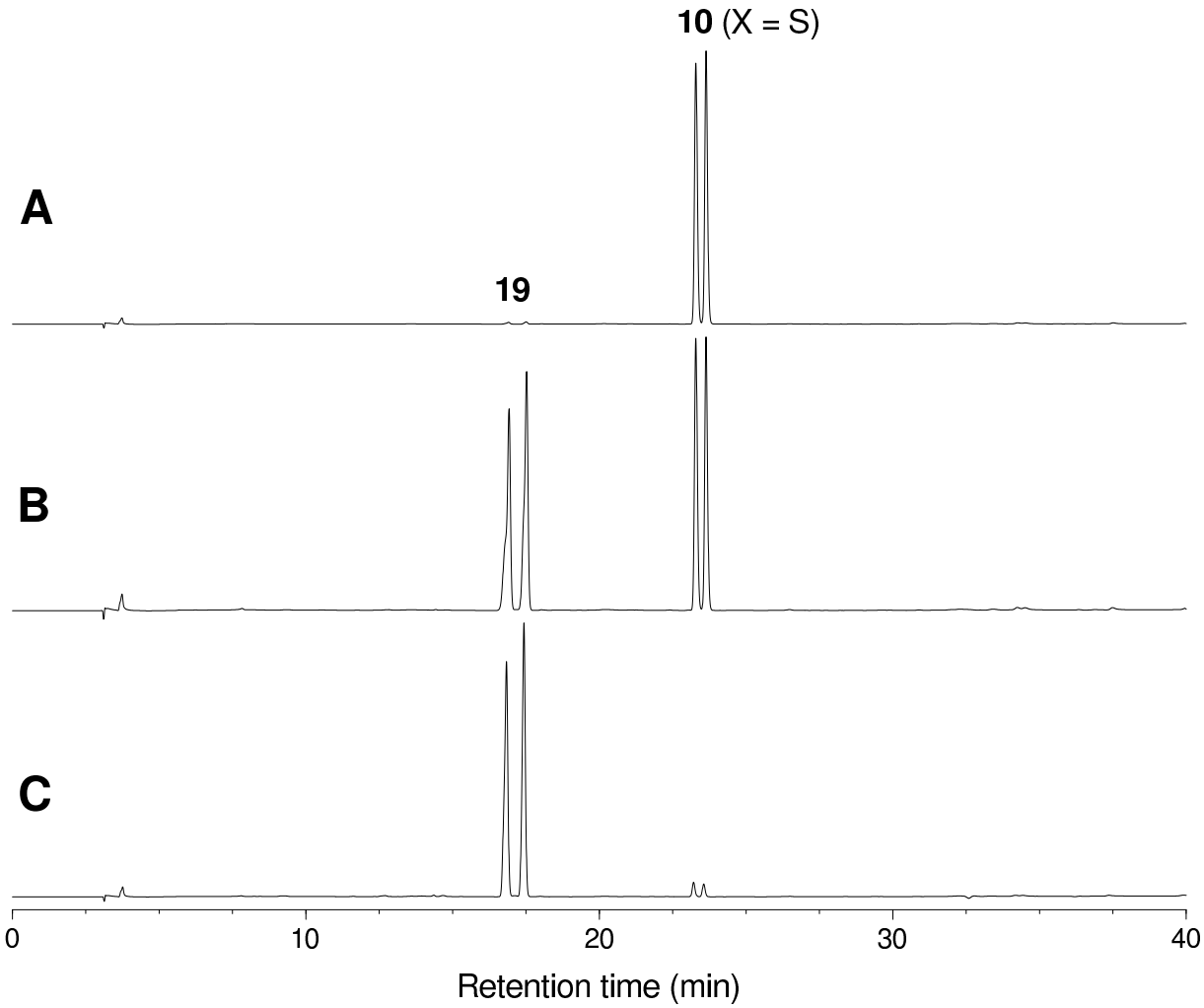


RP-HPLC analysis of the deprotection of **10** (X=S). Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **10** (X=S).

Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 20 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 40 minutes in 0.1 M triethylammonium acetate pH 7.0.

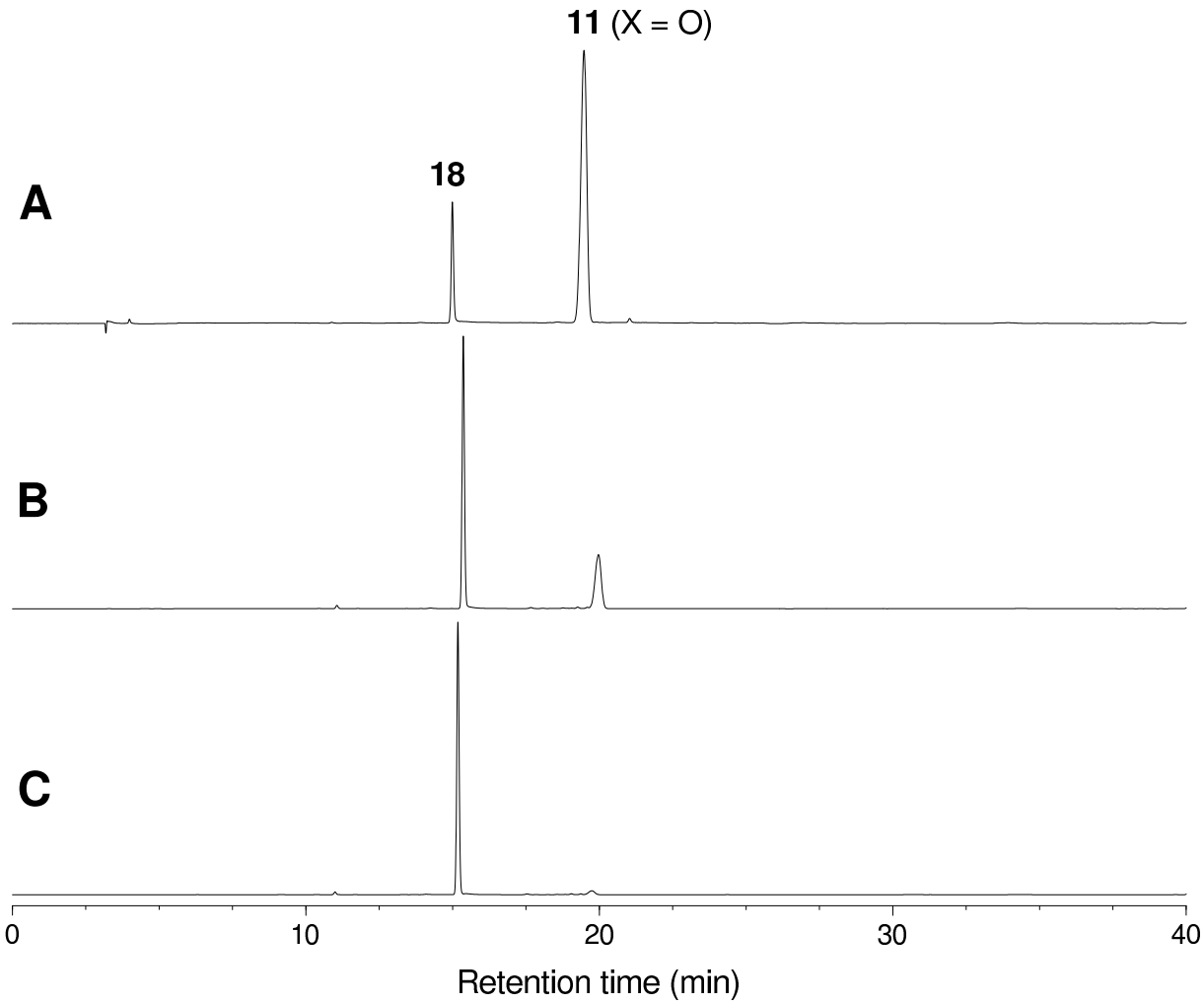


RP-HPLC analysis of the deprotection of **11** (X=O). Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **11** (X=O).

Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 20 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 40 minutes in 0.1 M triethylammonium acetate pH 7.0.

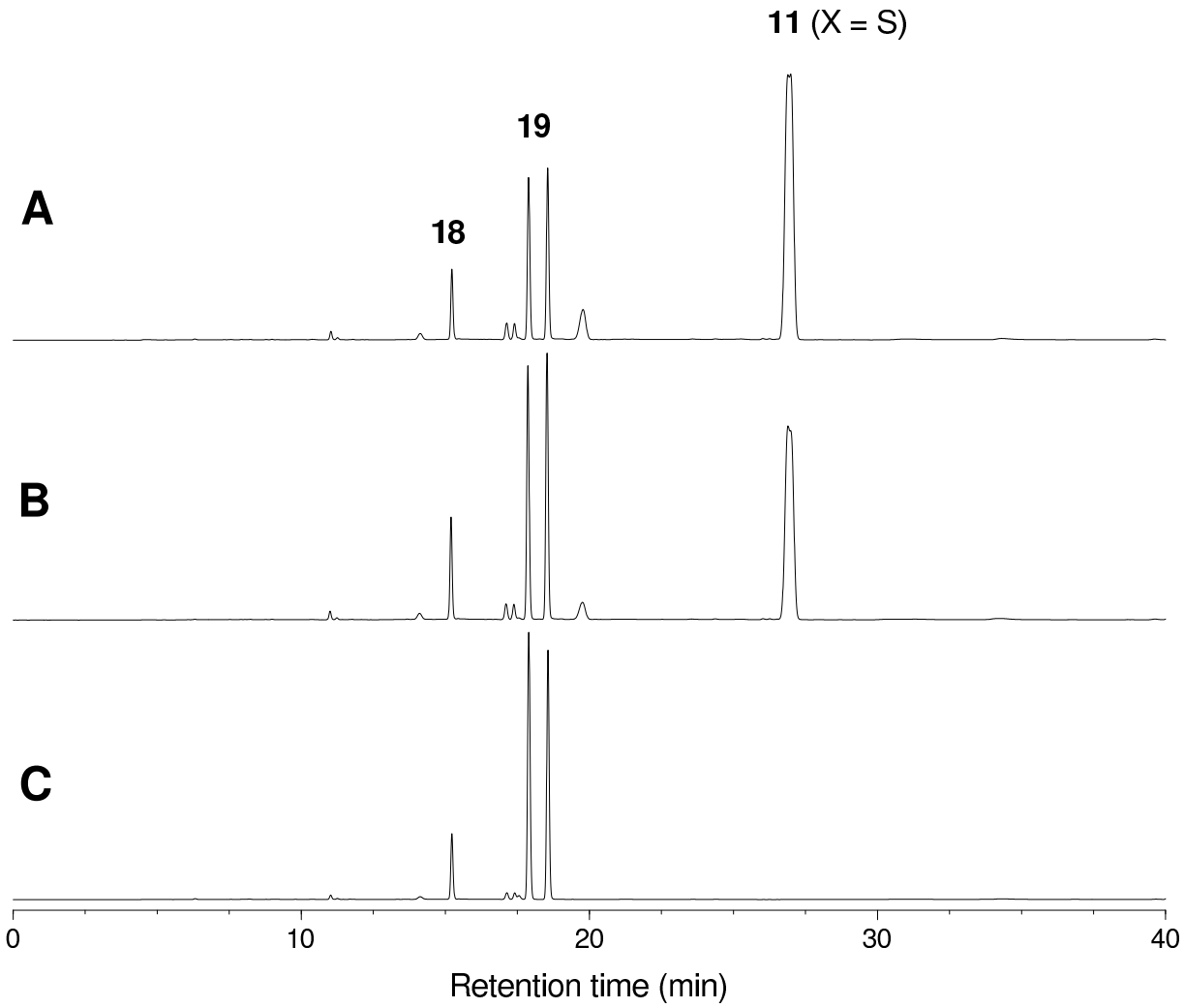


RP-HPLC analysis of the deprotection of **11** (X=S). Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **11** (X=S) stored at room temperature for ~2h in 0.1 M triethylammonium acetate pH 7.0.

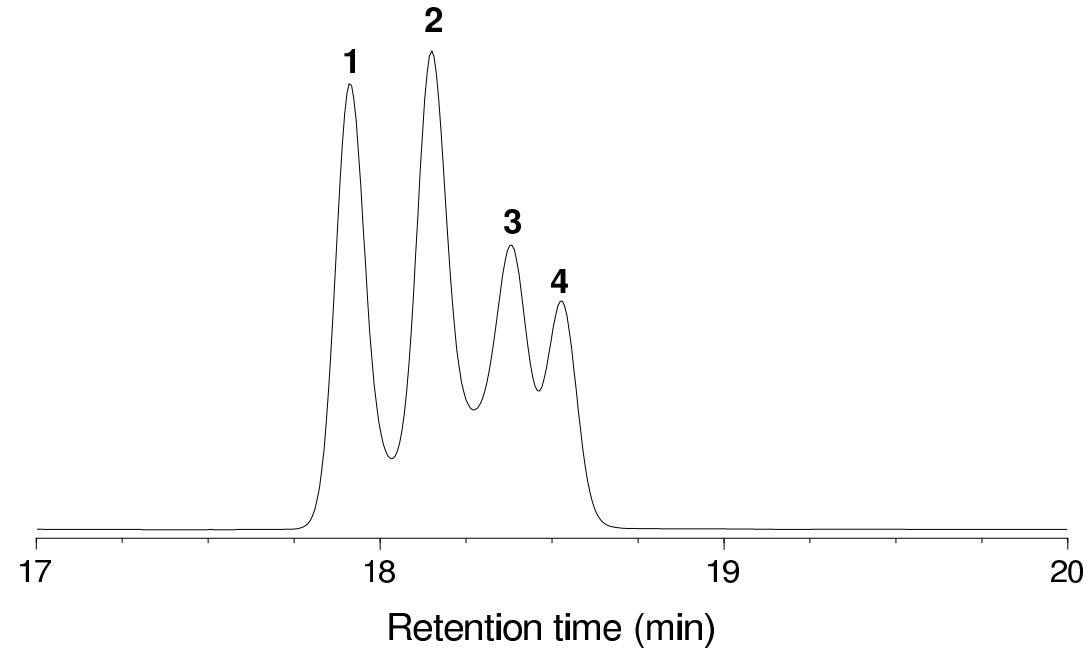
Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 20 minutes in 0.1 M triethylammonium acetate pH 7.0.

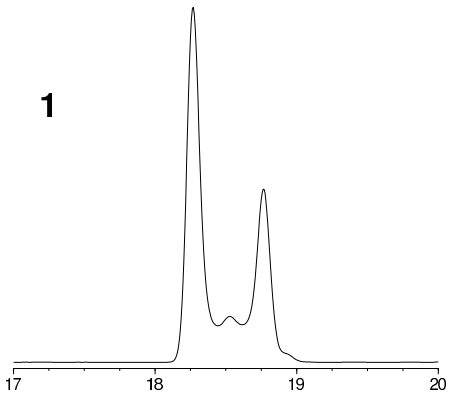
Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 40 minutes in 0.1 M triethylammonium acetate pH 7.0.

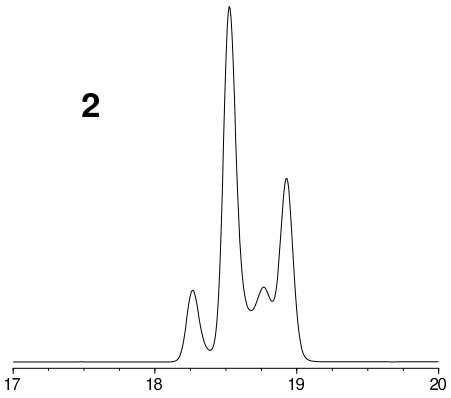


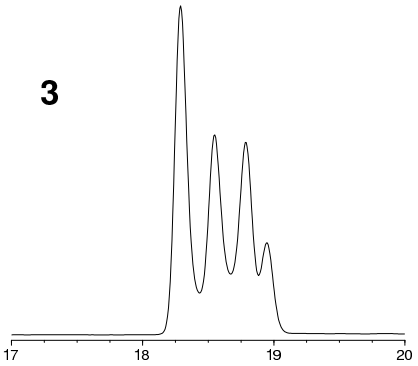
Attempted purification of each rotameric form of diastereomeric **12** (X = O) by RP-HPLC under conditions identical to those used for the analysis of crude **12** (X = O).

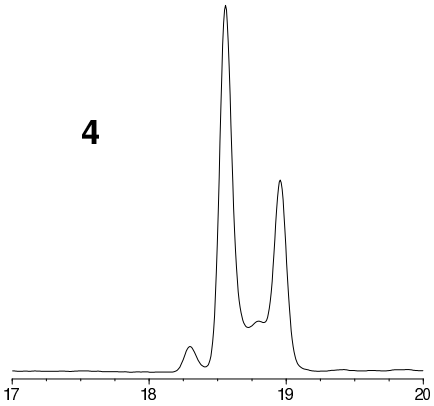
Chromatograms 1 and 3: Peaks 1 and 3 are equilibrating rotamers of one P-diastereomer. *Chromatograms 2 and 4*: Peaks 2 and 4 are equilibrating rotamers of the other P-diastereomer.











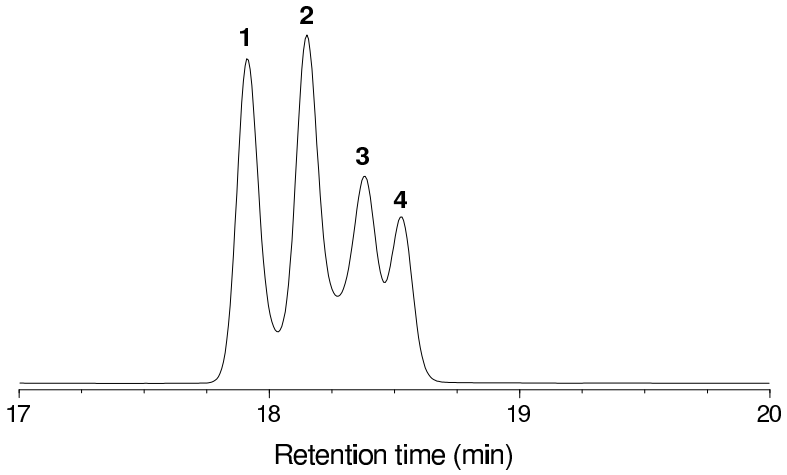
RP-HPLC analysis of the deprotection of **12** (X = O). Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

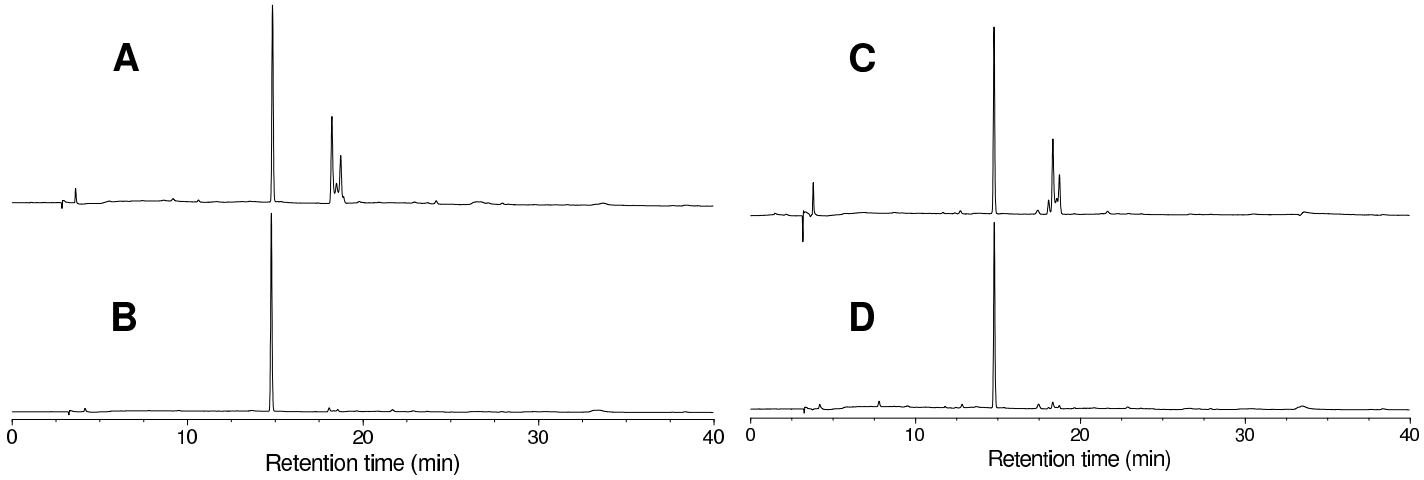
Chromatogram A: Sample composed of mostly peaks 1 and 3 that has been heated at 90°C for 15 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram B: Sample composed of mostly peaks 1 and 3 that has been heated at 90°C for 75 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: Sample composed of mostly peaks 2 and 4 that has been heated at 90°C for 15 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram D: Sample composed of mostly peaks 2 and 4 that has been heated at 90°C for 75 minutes in 0.1 M triethylammonium acetate pH 7.0.



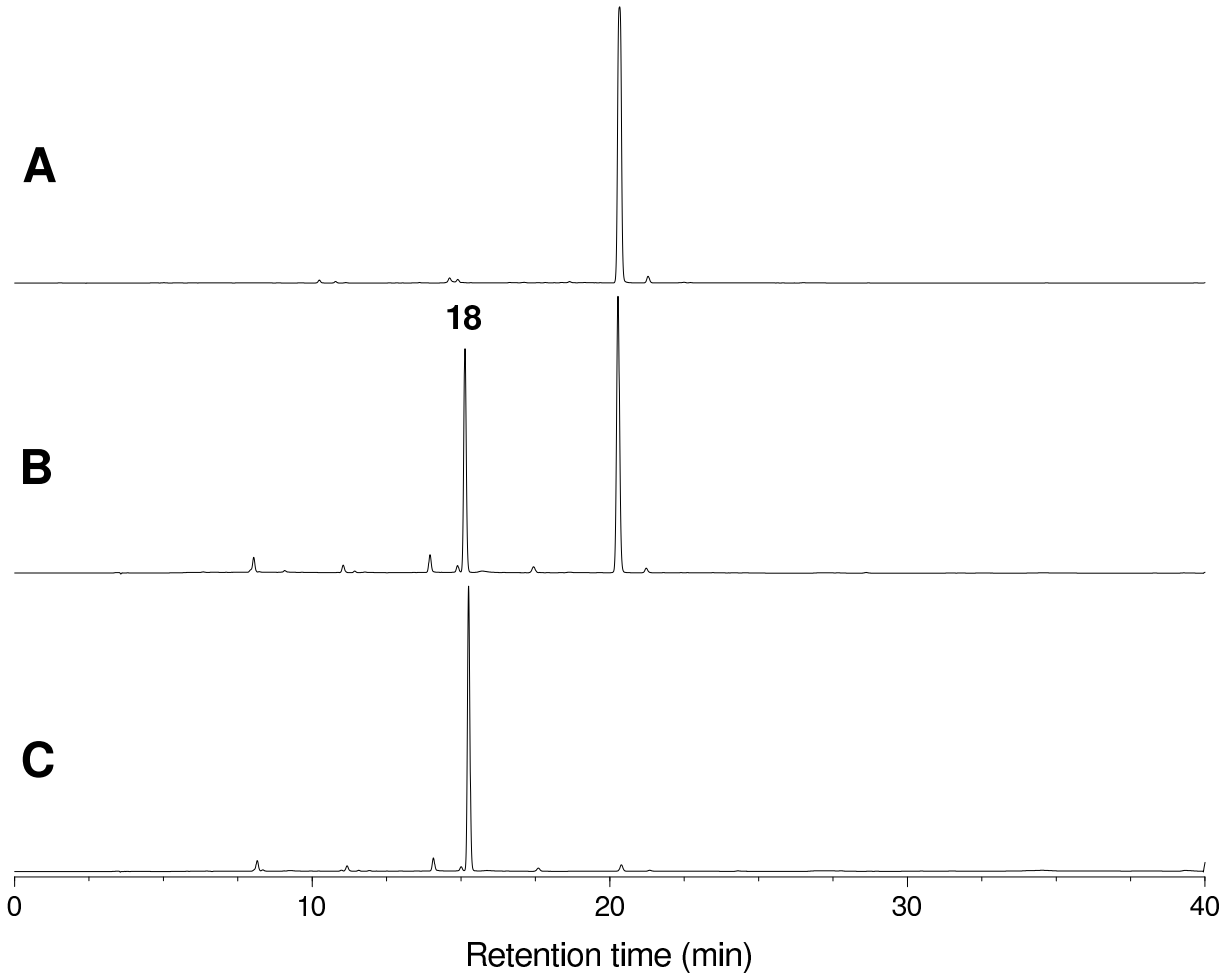


RP-HPLC analysis of the deprotection of **13** (X=O). Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **13** (X=O).

Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 90 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 10 hours in 0.1 M triethylammonium acetate pH 7.0.

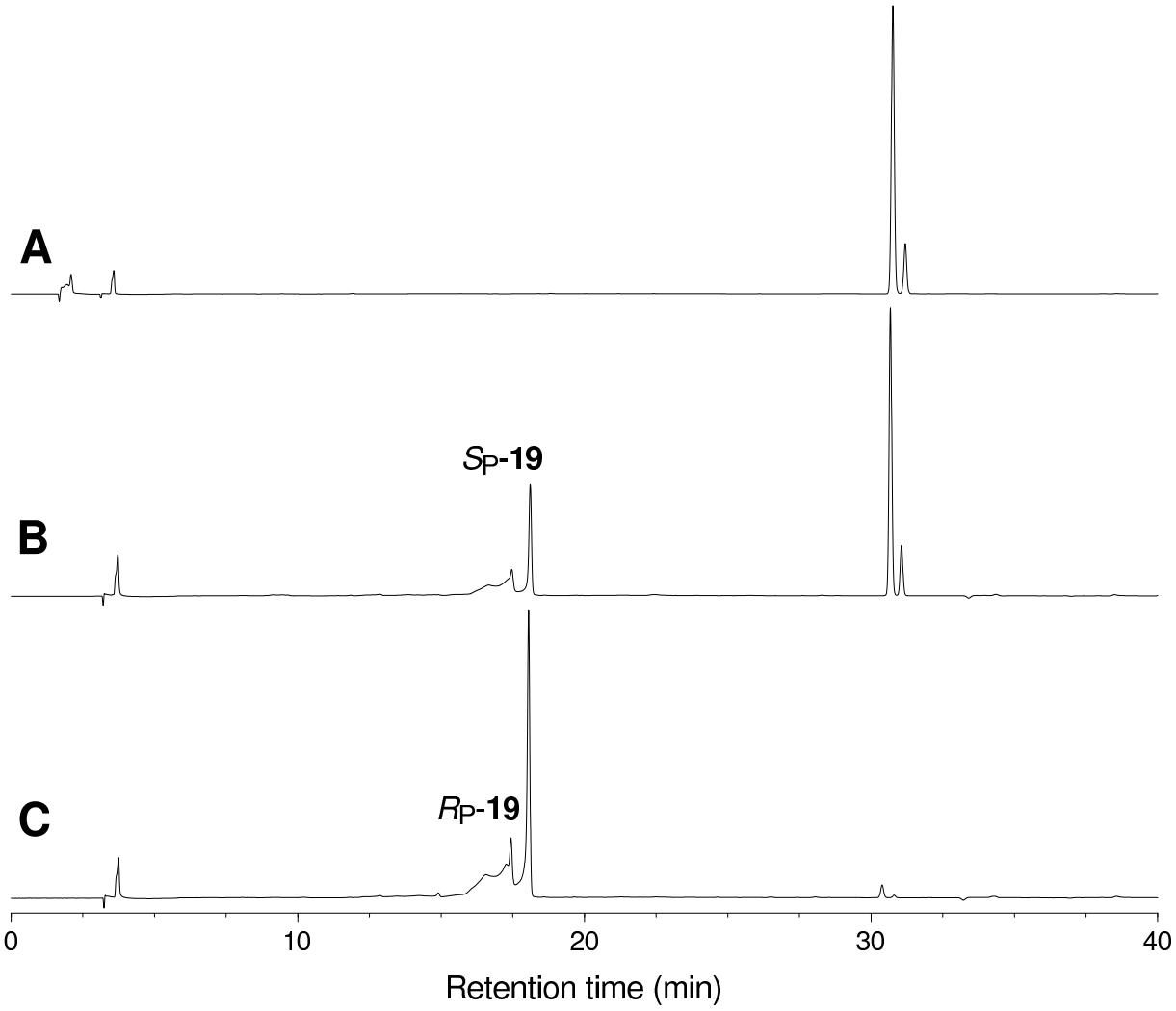


RP-HPLC analysis of the deprotection of **14** (X=S, 85% enriched in "fast"-eluting diastereomer). Deprotection of this diastereomer affords S_P-**19.** Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **14** (X=S, 85% enriched in "fast"-eluting diastereomer).

Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 30 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 3.5 hours in 0.1 M triethylammonium acetate pH 7.0.

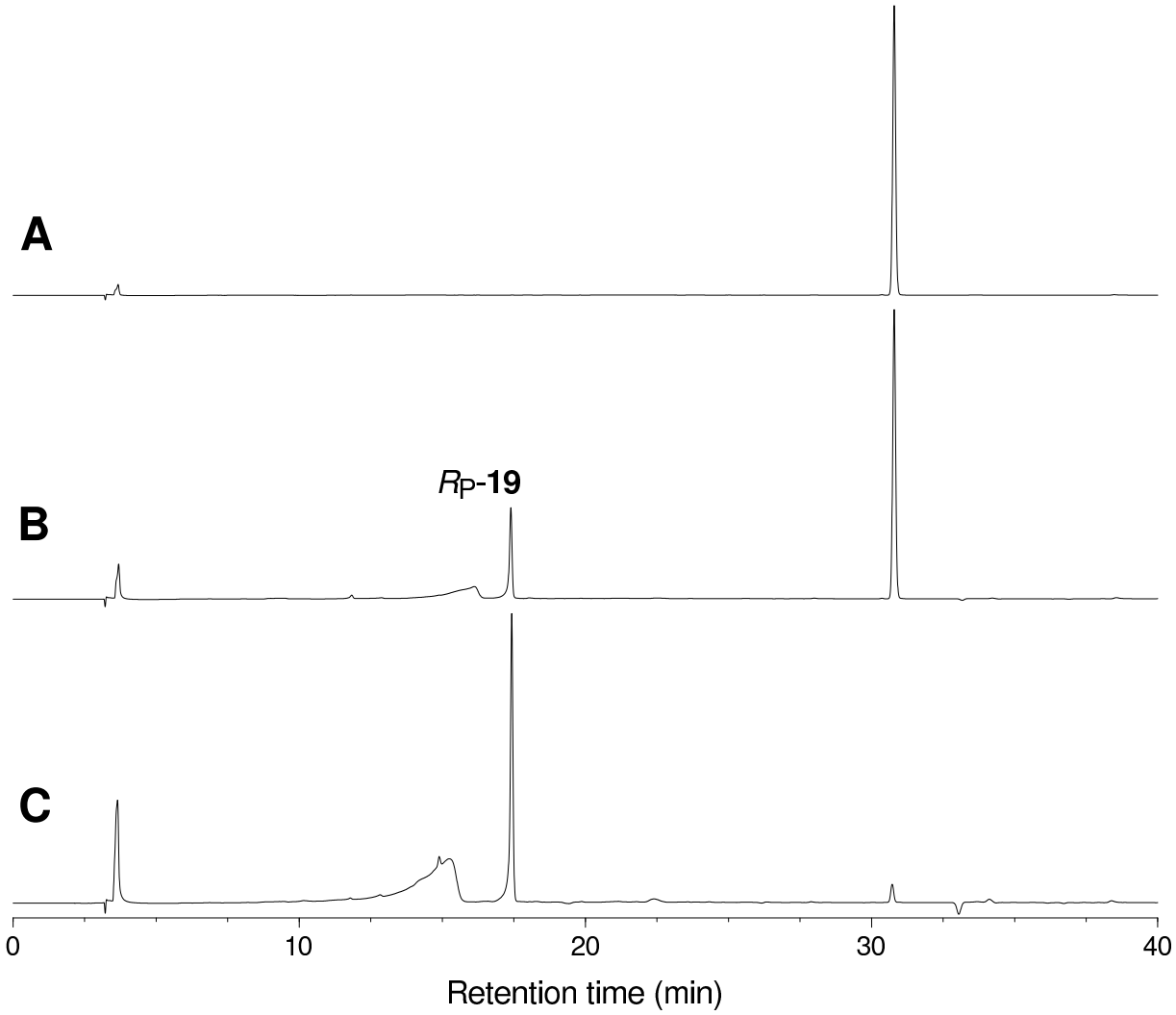


RP-HPLC analysis of the deprotection of **14** (X=S, "slow"-eluting diastereomer). Deprotection of this diastereomer affords R_P-**19.** Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **14** (X=S, "slow"-eluting diastereomer).

Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 30 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 3.5 hours in 0.1 M triethylammonium acetate pH 7.0.

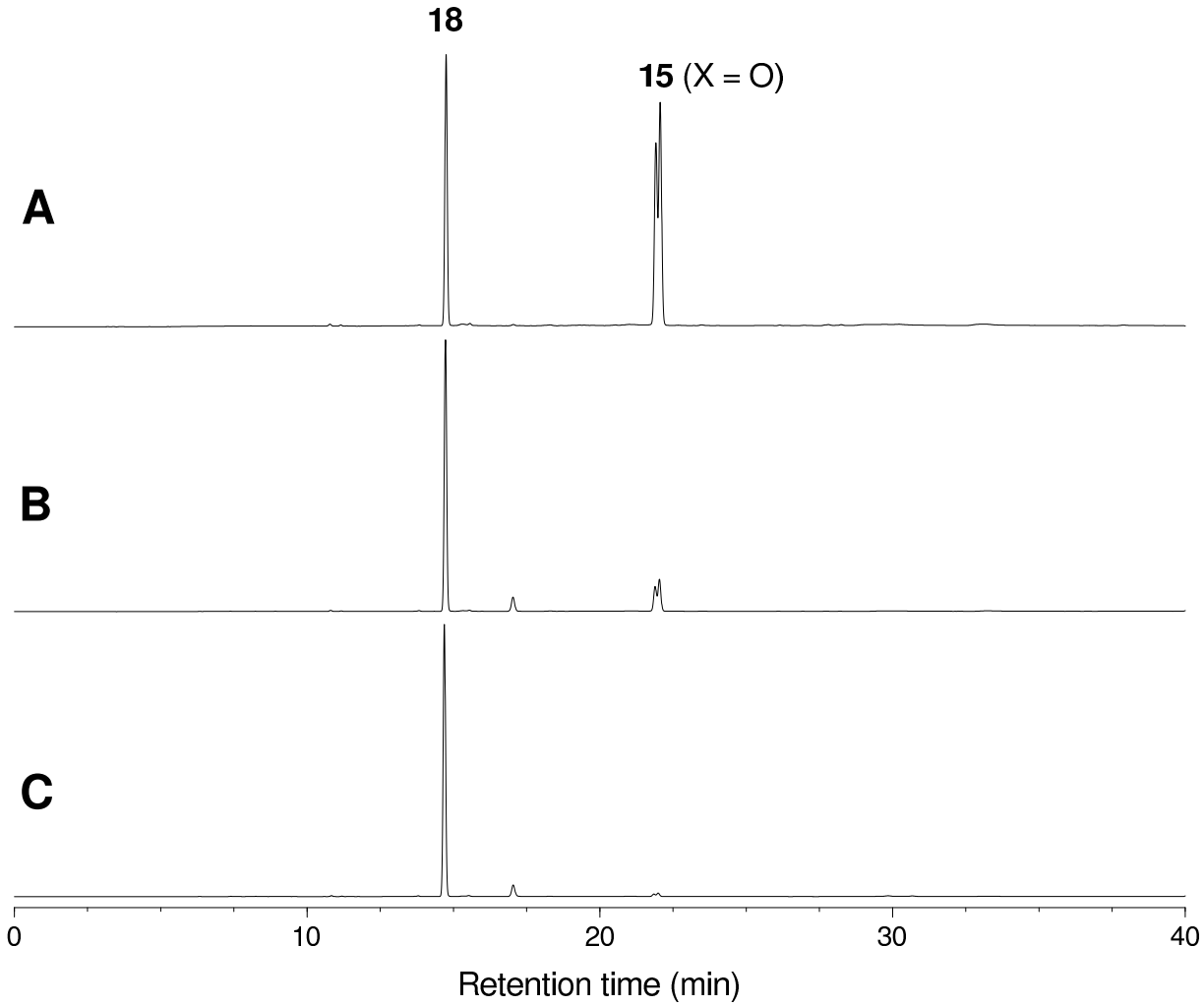


RP-HPLC analysis of the deprotection of **15** (X=O). Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **15** (X=O) stored at room temperature for ~2hours.

Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 20 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 40 minutes in 0.1 M triethylammonium acetate pH 7.0.

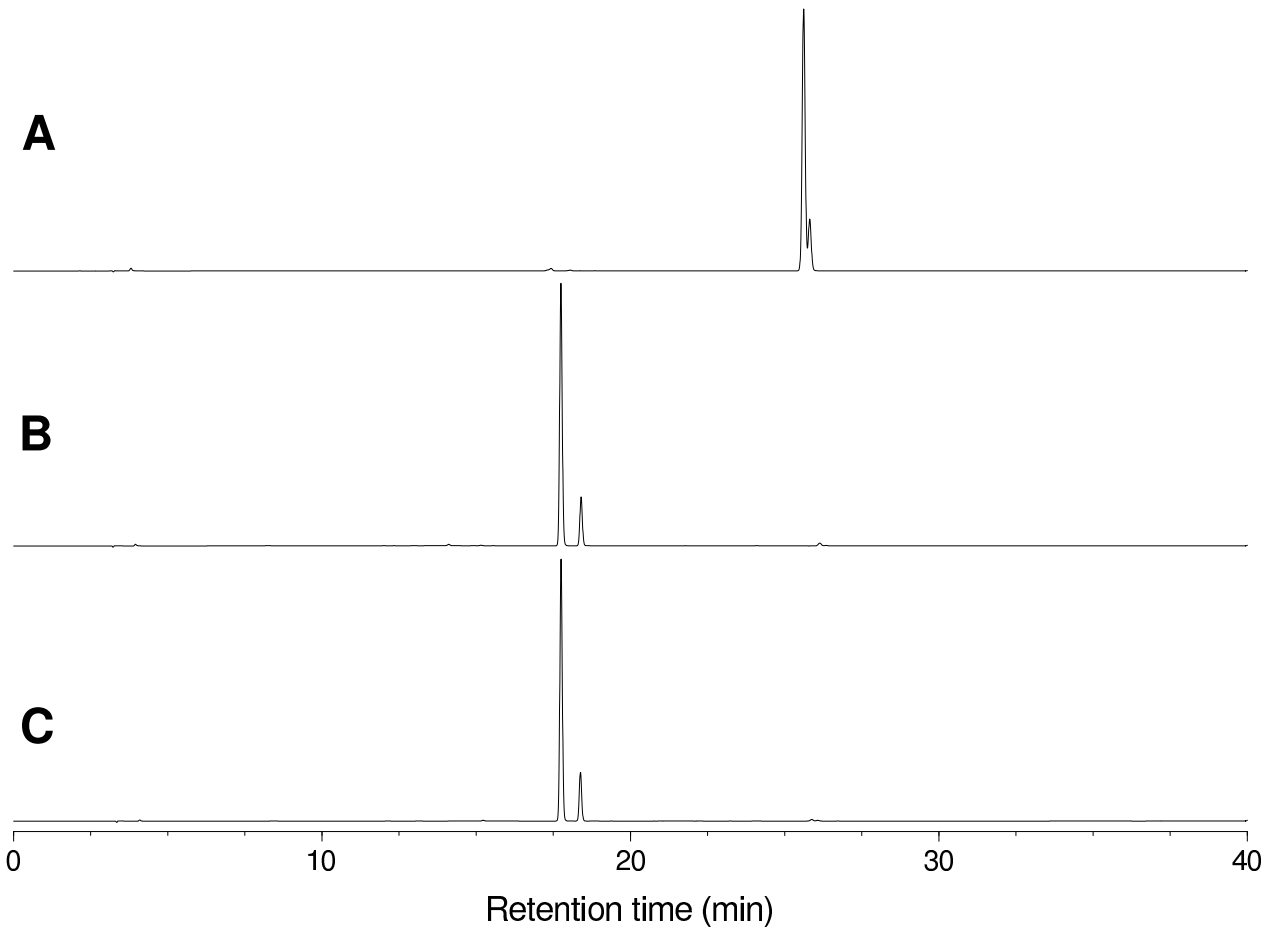


RP-HPLC analysis of the deprotection of **16** (X=S, 90% enriched in "fast"-eluting diastereomer). Deprotection of this diastereomer affords R_P-**19**. Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **16** (X=S, 90% enriched in "fast"-eluting diastereomer).

Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 20 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 30 minutes in 0.1 M triethylammonium acetate pH 7.0.

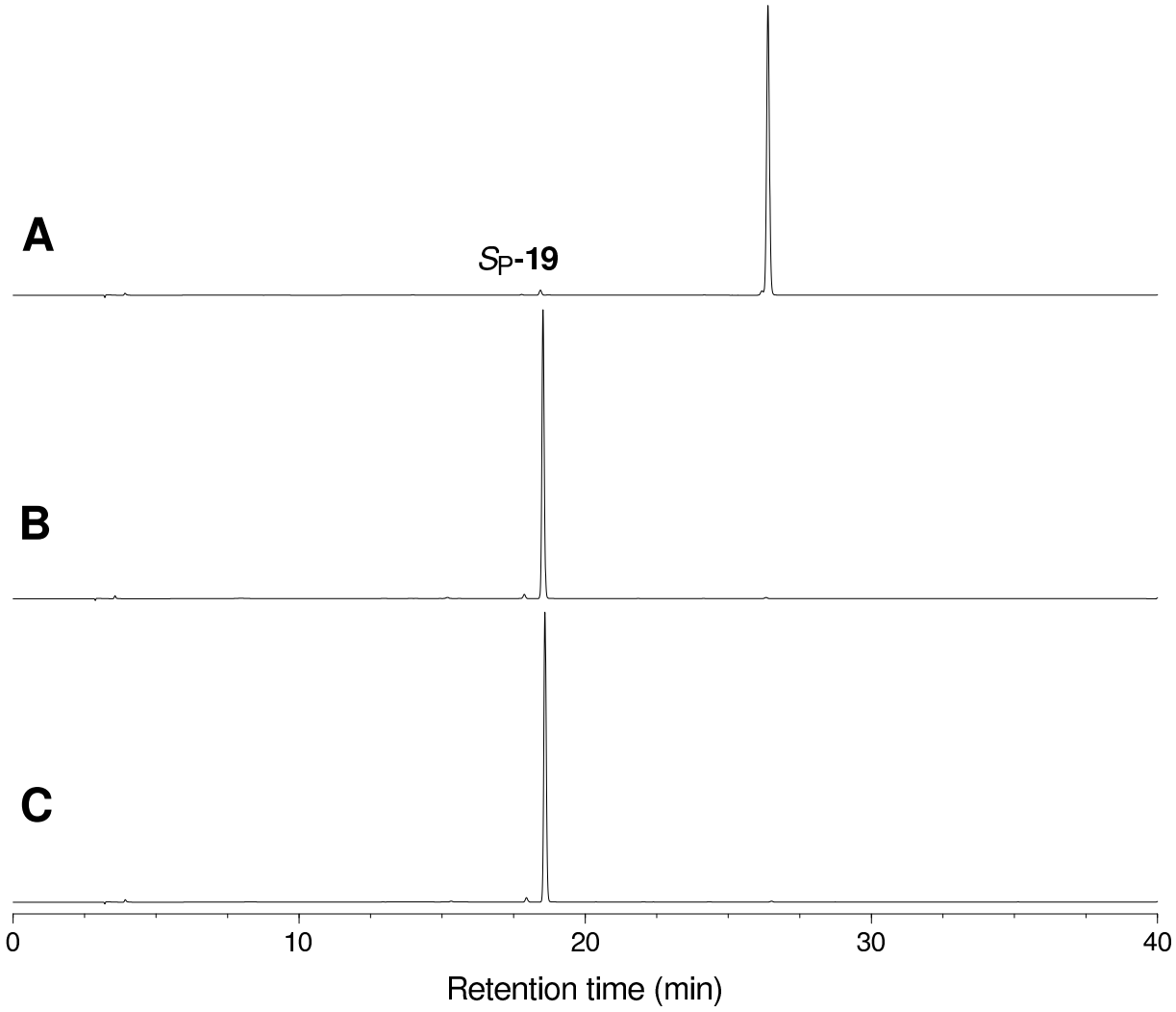


RP-HPLC analysis of the deprotection of **16** (X=S, 98% enriched in "slow"-eluting diastereomer). Deprotection of this diastereomer affords *S*P-**19**. Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **16** (X=S, 98% enriched in "slow"-eluting diastereomer).

Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 20 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 30 minutes in 0.1 M triethylammonium acetate pH 7.0.

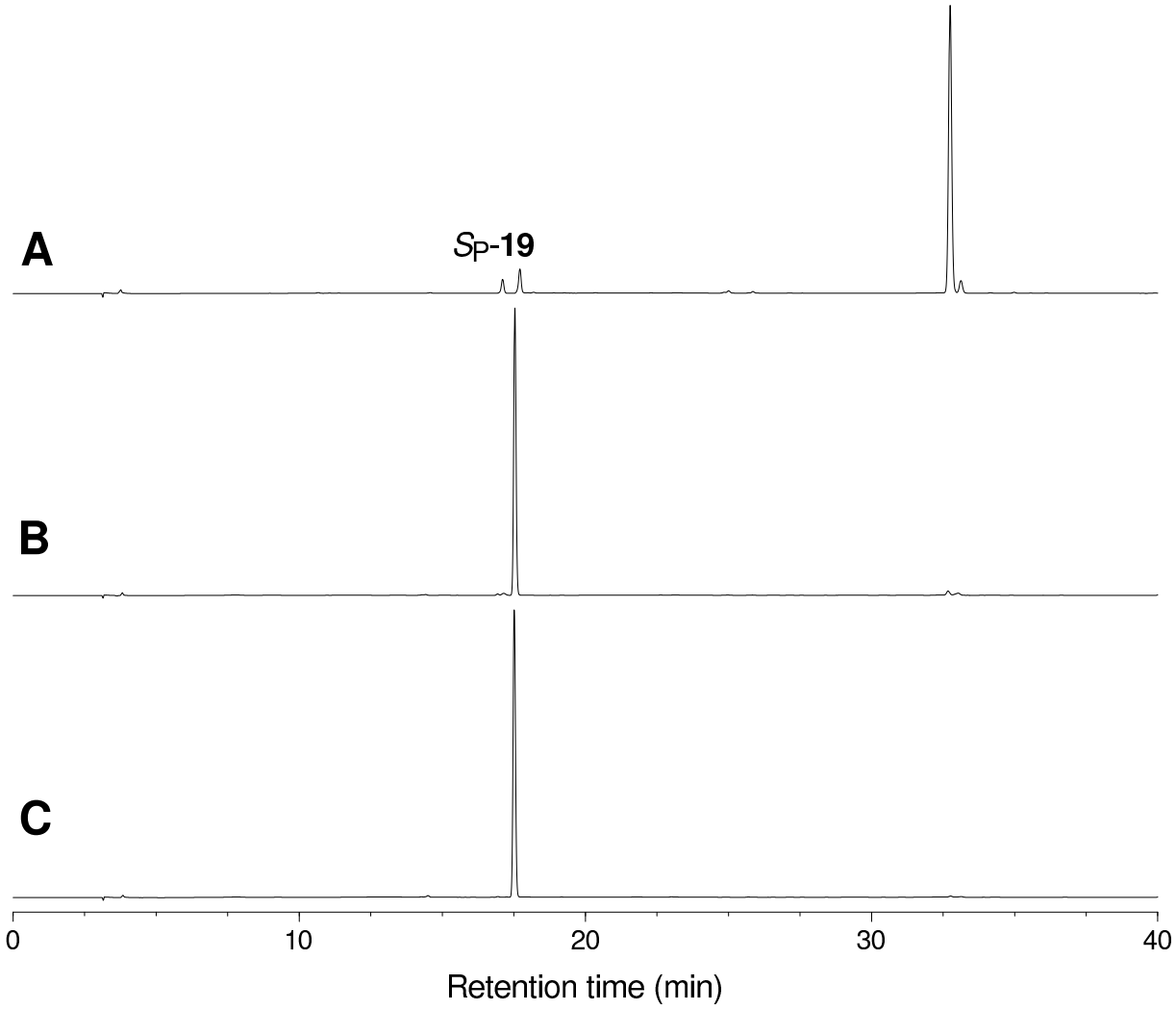


RP-HPLC analysis of the deprotection of **17** (X=S, 99% enriched in "fast"-eluting diastereomer). Deprotection of this diastereomer affords *S*P-**19.** Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **17** (X=S, 99% enriched in "fast"-eluting diastereomer).

Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 10 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 20 minutes in 0.1 M triethylammonium acetate pH 7.0.

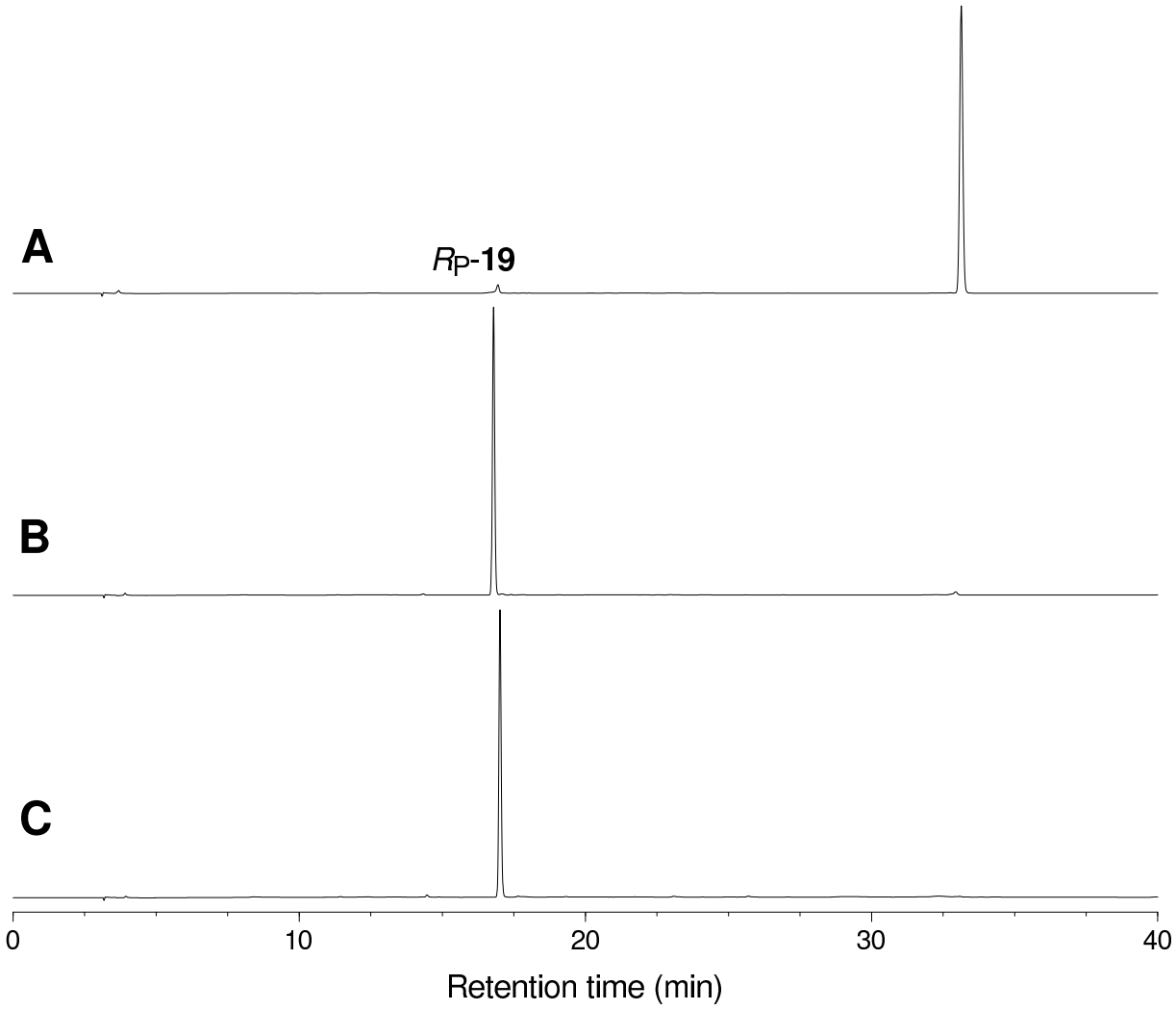


RP-HPLC analysis of the deprotection of **17** (X=S, "slow"-eluting diastereomer). Deprotection of this diastereomer affords R_P-**19**. Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Purified **17** (X=S, "slow"-eluting diastereomer).

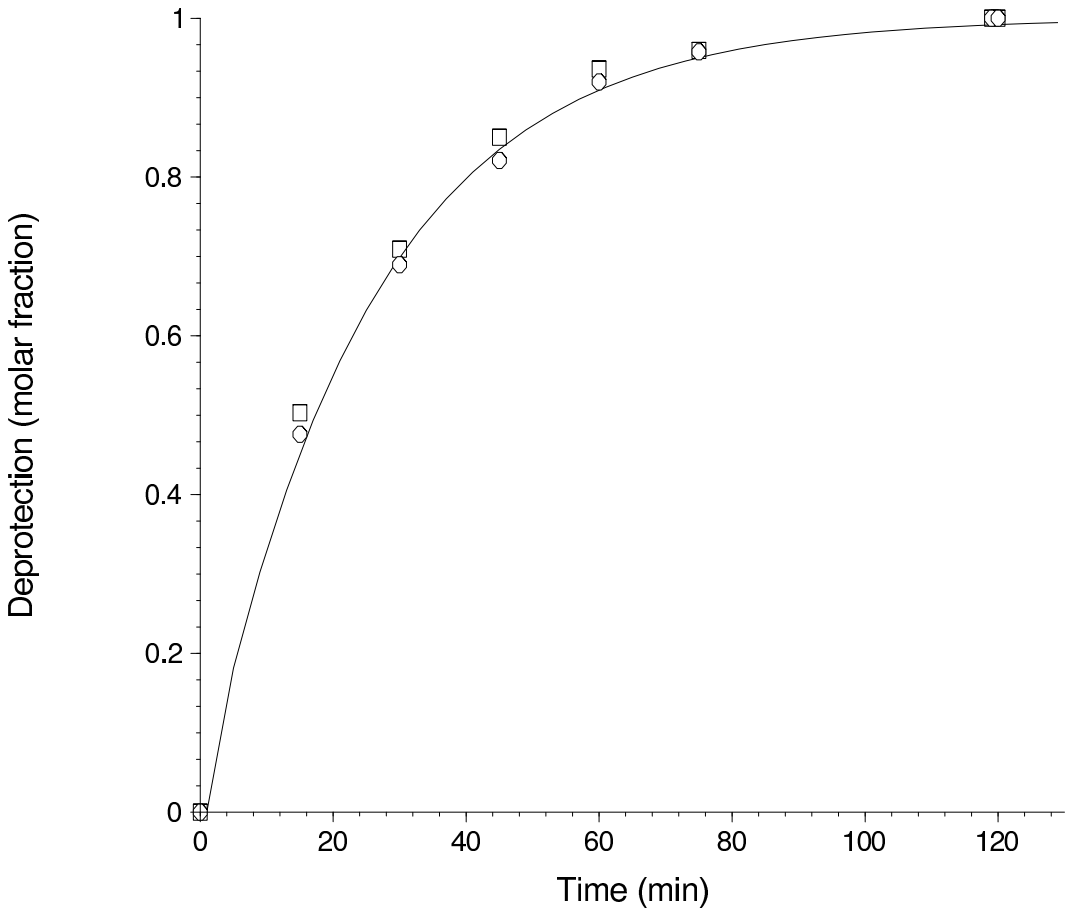
Chromatogram B: *Chromatogram A* sample that has been incubated at 90°C for 10 minutes in 0.1 M triethylammonium acetate pH 7.0.

Chromatogram C: *Chromatogram A* sample that has been incubated at 90°C for 20 minutes in 0.1 M triethylammonium acetate pH 7.0.



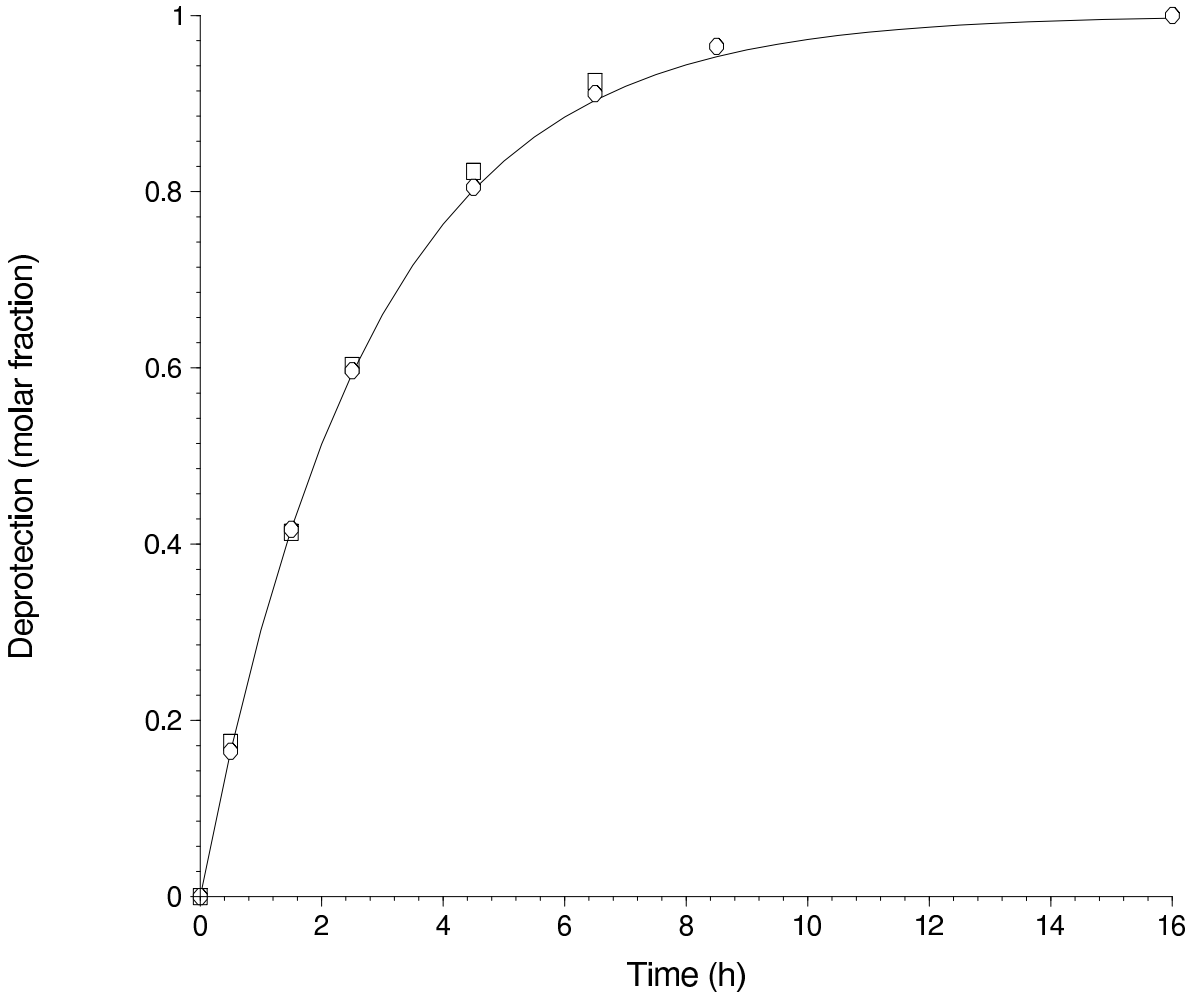
Deprotection of RP-HPLC purified **12** (X=O) in water:acetonitrile (1:1, v/v). Aliquots (100 µl) of **12** were incubated in flame-sealed ampules at 90°C for predetermined time points. The deprotection kinetics of **12** was obtained by integrating the peak area corresponding to **18** on RP-HPLC chromatograms for each time point. RP-HPLC analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

-○- "Fast"-eluting diastereomer of 12 (X=O) as a mixture of two rotamers.
-□- "Slow"-eluting diastereomer of 12 (X=O) as a mixture of two rotamers.

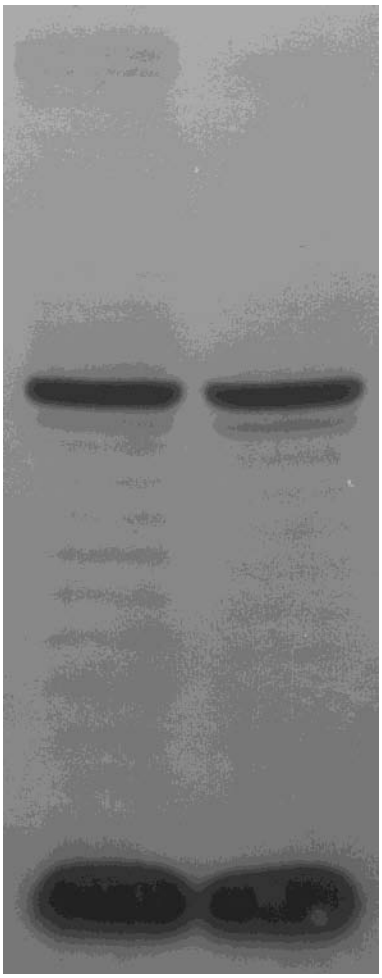


Deprotection of RP-HPLC purified **13** (X=O) in water:acetonitrile (1:1, v/v). Aliquots (100 µl) of **13** were incubated in flame-sealed ampules at 90°C for predetermined time points. The deprotection kinetics of **13** was obtained by integrating the peak area corresponding to **18** on RP-HPLC chromatograms for each time point. RP-HPLC analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

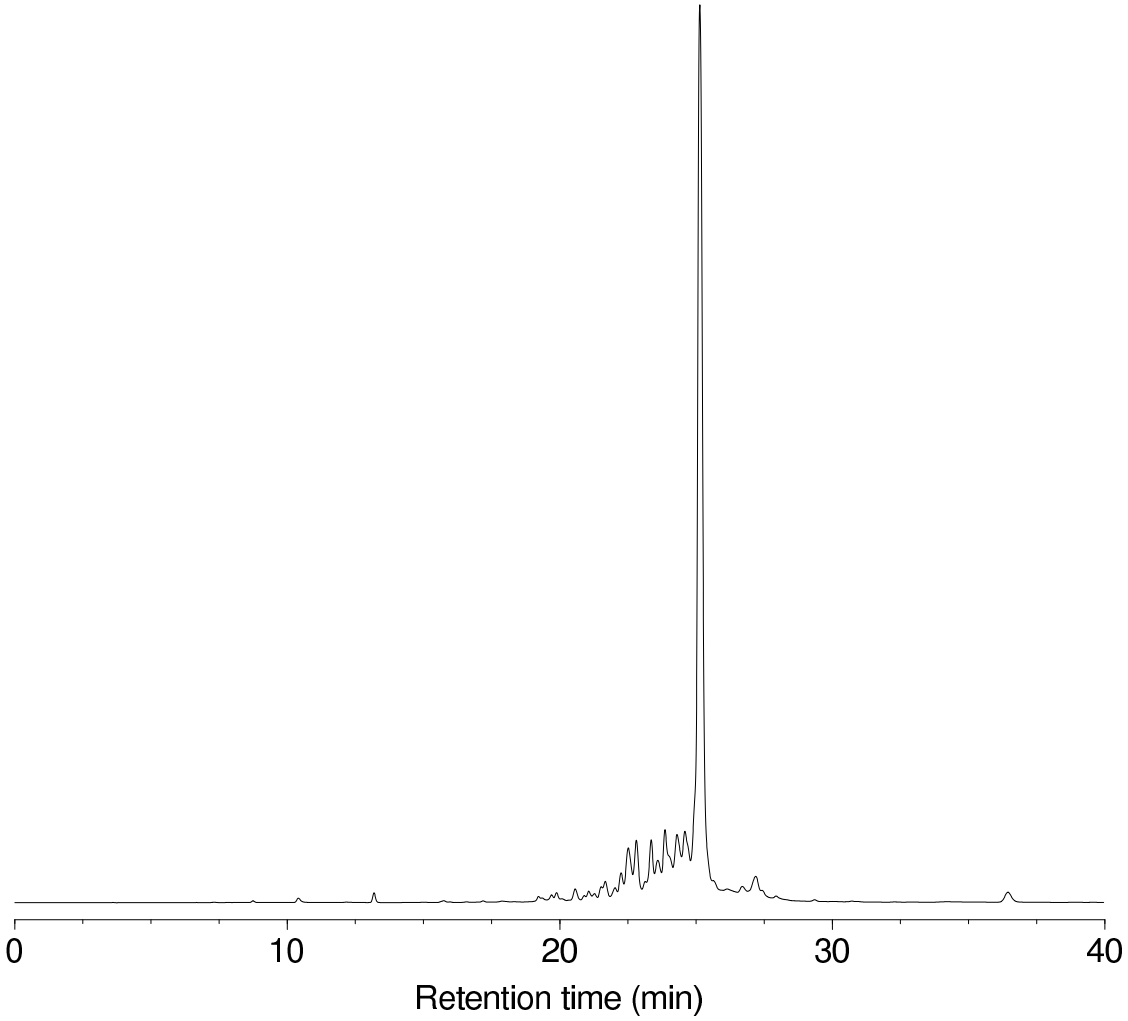
-○- Deprotection performed in H_2O:MeCN (1:1, v/v).
-□- Deprotection performed in D_2O:MeCN (1:1, v/v).



Electrophoretic analysis of dT_{18} using a 20% polyacrylamide - 7M urea gel at pH 8.3 (1X TBE buffer). *Left lane:* crude 18-mer that was prepared from a commercial 2-cyanoethyl phosphoramidite and then treated with concentrated ammonium hydroxide for 1h at 25°C. Right lane: crude 18-mer that was prepared from the 2-(*N*-formyl-*N*-methyl)aminoethyl phosphoramidite **1**, and then treated with pressurized methylamine gas (~2.5 bar) for 3 min at 25°C followed by heating in 0.1 M triethylammonium acetate pH 7.0 for 3 h at 90°C. Bromophenol blue is used as a marker and shows as the large band, in each lane, at the bottom of the gel.



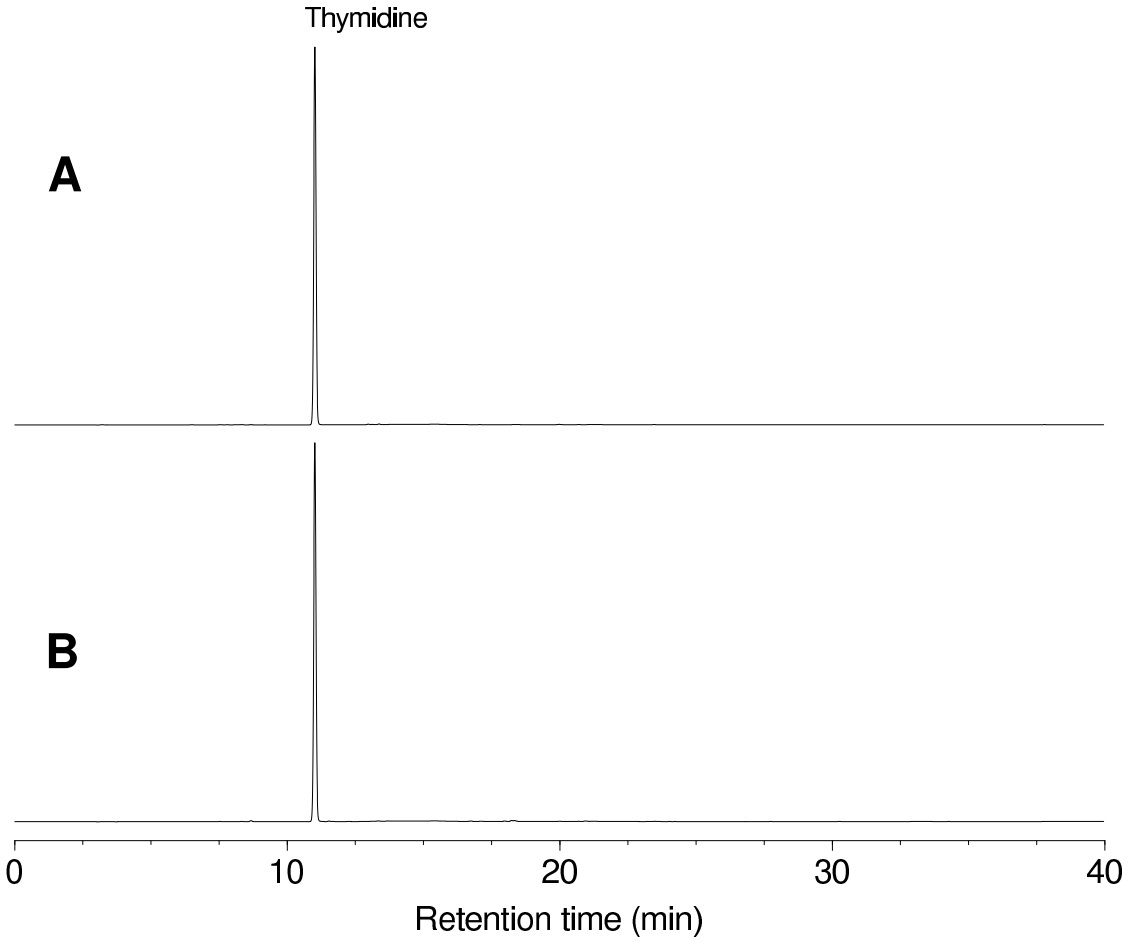
RP-HPLC analysis of fully deprotected $d(AG)_{10}$. The crude 20-mer was prepared from 2-(*N*-formyl-*N*-methyl) aminoethyl phosphoramidites. The analysis was performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.



RP-HPLC analysis of the hydrolysis of crude dT_{18} catalyzed by snake venom phosphodiesterase and bacterial alkaline phosphatase. Analyses were performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.

Chromatogram A: Hydrolysis of dT_{18} that was prepared from the 2-(*N*-formyl-*N*-methyl) aminoethyl phosphoramidite **1.**

Chromatogram B: Hydrolysis of dT_{18} that was prepared from a commercial 2-cyanoethyl phosphoramidite.



RP-HPLC analysis of the hydrolysis of crude $d(AG)_{10}$ catalyzed by snake venom phosphodiesterase and bacterial alkaline phosphatase. The crude 20-mer was prepared from 2-(*N*-formyl-*N*-methyl) aminoethyl phosphoramidites. The analysis was performed with a 5 µm Supelcosil LC-18S column (25 cm x 4.6 mm) and a linear gradient of 1% acetonitrile/min starting from 0.1 M triethylammonium acetate pH 7.0 pumped at a flow rate of 1 mL/min.