Supporting Information

(*R*)-2,4-Dihydroxybutyramide *Seco*-Pseudonucleosides: New Versatile Homochiral Synthons for Synthesis of Modified Oligonucleotides

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Experimental

Materials were obtained from commercial suppliers and used without further purification unless otherwise noted. *N*,*N*-Diisopropylethylamine, ethylenediamine, (*R*)-(+)- -hydroxy- -butyrolactone, (*R*)-(-)-pantolactone, 1-pyrenemethylamine hydrochloride, triethylamine and 4,7,10-trioxa-1,13-tridecanediamine were from Aldrich. 9-Fluorenylmethyl succinimidyl carbonate was purchased from Novabiochem; 4,4'-dimethoxytritylchloride (DMTrCl) was from Avocado; piperidine from Romil; *bis*(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine, di-*tert*-butyl dicarbonate, methyl trifluoroacetate and trifluoroacetic acid were from Fluka. Diisopropylammonium tetrazolide was prepared from diisopropylamine and 1*H*-tetrazole solution in MeCN. Dichloromethane (Fisher) was used freshly distilled over CaH_2. Anhydrous pyridine was from Aldrich; THF (BDH) was stored over 4Å molecular sieves under argon. Other solvents: dioxane, toluene, diethyl ether (BDH); chloroform, ethyl acetate, acetone, acetonitrile, hexane, absolute ethanol, methanol (Fisher) were used as received. *O*,*O*'-Diisobutyryl-5(6)-carboxyfluorescein pentafluorophenyl ester,[1] D-(+)-biotin pentafluorophenyl ester[2] and 2,2'-sulfonyldiethanol 3'-phosphate CPG-500[3,4] were prepared as described.

Oligonucleotides were purified either by PAGE in 20% denaturing (7 M urea) gel in Tris-borate buffer, pH 8.3, or by reverse-phased HPLC on Gilson HPLC system using Phenomenex Bondclone 10 C_{18} column (3.9x300 mm) and dual wavelength detection (215 and 254 nm); buffer A: 5% of MeCN (v/v) in 0.1M triethylammonium acetate, buffer B: MeCN; flow rate 1 mL/min, under gradient of B in A: 0-5%, 5 min, 5-15%, 10 min, 15-40, 30 min, 40-80%, 10 min, 80-0%, 10 min. Appropriate fractions were pooled, evaporated, and precipitated from 0.4 mL of 1M $LiClO_4$ by adding 1.6 mL of acetone. Duplex stability studies were done in a buffer containing 100 mM NaCl, 10 mM Na-phosphate, 0.1 mM EDTA, pH 7.0. MALDI-TOF spectra were recorded on a Voyager DE system (Perseptive Biosystems) in positive ion mode using either 1:1 (v/v) mixture of 2,6-dihydroxyacetophenone (40 mg/mL in MeOH) and aq. diammonium hydrogen citrate (80 mg/mL) for all oligonucleotides or 2,4,6-trihydroxyacetophenone (10 mg/mL in 50% aq. MeCN v/v) for low molecular weight compounds as a matrix. ^{1}H NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer using DMSO-d_6 as a solvent, chemical shifts are given in Ω, ppm, and referenced to DMSO-d_6 (2.5 ppm). ^{1}H NMR coupling constants are reported in Hz and refer to apparent multiplicities. TLC plates (Merck Kieselgel 60 F_{254}) were developed in either system A ($CHCl_3$-EtOAc 1:1 + 1% Et_3N v/v/v) or B ($CHCl_3$-MeOH 9:1 v/v), and visualised under short wave UV and stained by trifluoroacetic acid vapours for DMTr-containing compounds.

Typical procedure for 2-hydroxylactone aminolysis. Preparation of 5e. A solution of (*R*)-(+)- -hydroxy- -butyrolactone (2.00 g, 20 mmol) and 4,7,10-trioxa-1,13-tridecanediamine (21.3 mL, 100 mmol) in 10 mL EtOH was kept at 55°C for 48 h, then 29 mL (300 mmol) CF_3CO_2Me were added, and the mixture was kept at room temperature overnight, evaporated, co-evaporated with toluene (100 mL) and dry pyridine (4 x 100 mL). The residue was dissolved in dry pyridine (100 mL), cooled in an ice bath, and DMTrCl (8.56 g, 25 mmol) was added in seven portions within 1 h. After disappearance of the starting material, the excess of DMTrCl was quenched with MeOH (1 mL), and after 10 min the mixture was diluted with 500 mL $CHCl_3$, washed with 2.5% $NaHCO_3$ (3x300 mL), water (2x300 mL), 20% NaCl (300 mL), then dried (Na_2SO_4), evaporated, co-evaporated with toluene (3x30 mL) and the residue was chromatographed on silica gel column using stepwise gradient of $CHCl_3$ in toluene 1:2 to 1:1 to 2:1 to 100% $CHCl_3$ + 0.25% Et_3N (v/v/v), then gradient of EtOAc in $CHCl_3$ 5% to 10% to 15% EtOAc in $CHCl_3$+ 0.25% Et_3N (v/v/v). Fractions containing product were combined, evaporated, and the residue was dried *in vacuo* to afford 12.4 g (86%) colourless oil, R_f 0.27 (A). MALDI-TOF: $[M+Na]^+$ calc. 743.75, found 744.76; $[M+K]^+$ calc. 759.86, found 760.98. ^{1}H-NMR: 9.34 (br.s., 1H, CF_3CONH), 7.66 (br.s., 1H, CHCONH, exchangeable with D_2O), 7.36 (d, 2H, J = 6.1 Hz, ArH), 7.30 (dd, 2H, J_1 = 7.15 Hz, J_2 = 6.1 Hz, ArH), 7.22 (m, 5H, ArH), 6.87 (d, 4H, J = 7.1 Hz, ArH), 5.35 (d, 1H, J = 5.05 Hz, OH, exchangeable with D_2O), 3.96 (m, 1H, CHO), 3.73 (s, 6H, OCH_3), 3.51–3.22 (m, 16H, CH_2O, CH_2NH), 1.96 (m, 1H), 3.09 (m, 4H, CH_2NH), 2.57 (m, 2H,), 1.96 (m, 1H, CH_2CH_2CH), 1.70 ($CH_2CH_2CH_2$), 1.58 (m, 1H, CH_2CH_2CH).

Typical procedure for side-chain label attachment. Preparation of 5f. To the solution of 2.35 g (3.3 mmol) of **4e** in 30 mL MeOH 7.5 mL 10% Na_2CO_3 were added, and the mixture was stirred at rt for overnight, evaporated, diluted with 100 mL EtOAc, washed with 1.5% $NaHCO_3$ (3 x 150 mL), then dried (Na_2SO_4), evaporated, coevaporated with toluene (3x30 mL) and DCM (2 x 30 mL) to yield 1.56 g (76%) colourless oil, R_f 0.12 (B). MALDI-TOF: $[M+K]^+$ calc. 663.85, found 664.90. ^{1}H-NMR: 7.68 (br.s., 1H, CONH, exchangeable with D_2O), 7.36 (d, 2H, J = 6.1 Hz, ArH), 7.30 (dd, 2H, J_1 = 7.1 Hz, J_2 = 6.1 Hz, ArH), 7.22 (m, 5H, ArH), 6.87 (d, 4H, J = 7.15 Hz, ArH), 5.75 (br.s., 1H, OH, exchangeable with D_2O), 3.96 (dd, 1H, J_1 = 3.05 Hz, J_2 = 7.15 Hz, CHO), 3.73 (s, 6H, OCH_3), 3.51–3.22 (m, 16H, CH_2O, NH_2, exchangeable with D_2O), 1.96 (m, 1H), 3.09 (m, 2H, CH_2NH), 2.57 (m, 2H, CH_2NH_2), 1.96, 1.71 (2m, 2H, CH_2CH_2CH), 1.56 (m, 4H, $CH_2CH_2CH_2$). This intermediate (1.24 g, 2 mmol) was co-evaporated with dry pyridine (2x30 mL), dissolved in 30 mL of dry pyridine, and D-(+)-biotin pentafluorophenyl ester (0.814 g, 2 mmol) was added in one portion. After the reaction was completed (about 1 h), solvent was evaporated and the residue was chromatographed on silica gel column using gradient of 0% to 6% MeOH in $CHCl_3$ + 0.25% Et_3N (v/v/v). Fractions containing product were combined, evaporated, and the residue was dried *in vacuo* to afford 1.56 g (92%) of glassy oil, R_f 0.21 (B). MALDI-TOF: $[M+Na]^+$ calc. 874.04, found 874.67; $[M+K]^+$ calc. 890.15, found 890.89. ^{1}H-NMR: 7.70 (m, 2H, CH_2NH), 7.35 (d, 2H, J = 7.3 Hz, ArH), 7.29 (dd, 2H, J_1 = 7.3 Hz, J_2 = 8.05 Hz, ArH), 7.21 (m, 5H, ArH), 6.87 (d, 4H, J = 8.95 Hz, ArH), 6.39 (s, 1H), 6.33 (s, 1H, NHCONH, exchangeable with D_2O), 5.37 (br.s., 1H, OH, exchangeable with D_2O), 4.29, 4.11 (2m, 2H, CHNHCONHCH), 3.96 (dd, 1H, J_1 = 3.65, J_2 = 8.25, CHO), 3.73 (s, 6H, OCH_3), 3.45–3.34 (m, CH_2OC, CH_2N, CHS, CH_2S), 3.08–3.00 (m, 12H, CH_2OCH_2), 2.04 (t, 2H, J = 7.3 Hz, CH_2CO), 1.96, 1.72 (2m, 2H, CH_2CH_2CH), 1.62–1.28 (m, 10H, $CH_2CH_2CH_2$, $SCHCH_2$).

Typical procedure for solid phase attachment. Reagents 7e,**f preparation**. Solid supports **7e**,**f** were prepared by a modification of the published method[3]. A solution of succinic anhydride (300 mg, 3 mmol) and DMAP (80 mg, 0.66 mmol) in 10 mL of dry pyridine was added to 300 mg of LCAA-CPG-500 Å, and the mixture was left at room temperature for 24 h with occasional swirling. After filtration, successive washes with 10 mL portions of pyridine, CH_2Cl_2, and Et_2O, and drying, the succinylated LCAA-CPG was suspended in 4 mL of DMF pyridine (1:1 v/v); compound **5e**,**f** (0.25 mmol), 1,3-diisopropylcarbodiimide (0.28 mL, 1.8 mmol) and DMAP (20 mg) were added; and the suspension was left for 48 h at room temperature. To block the remaining carboxylic groups, a solution of pentachlorophenol (100 mg) in pyridine (1 mL) was added, and the mixture was kept at

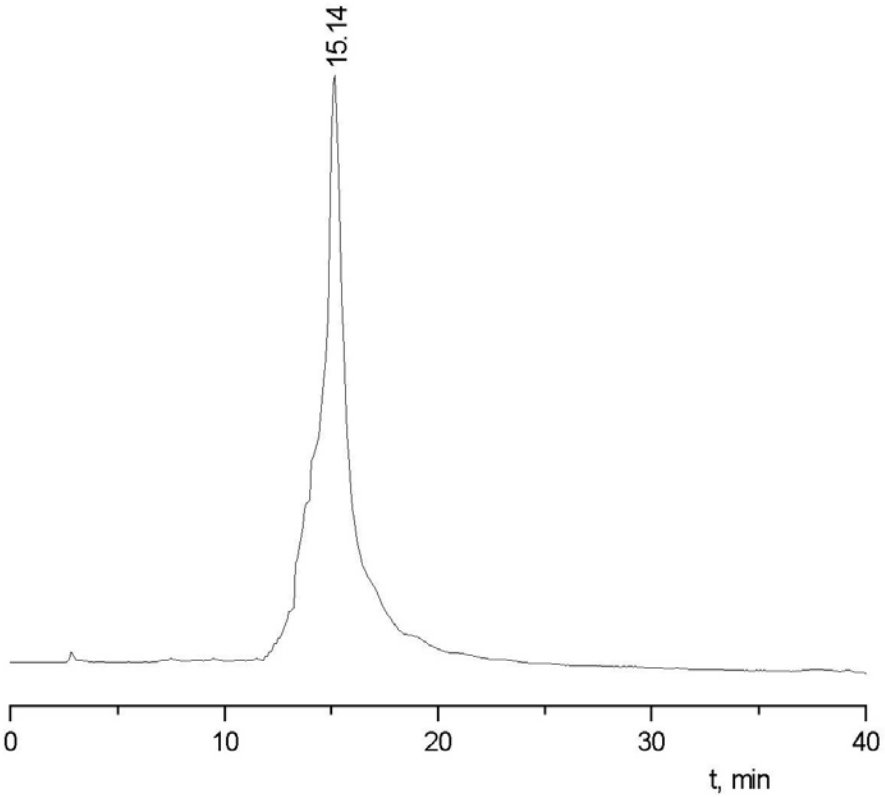
room temperature for a further 12 h. The support was filtered, resuspended in 5% v/v solution of piperidine in pyridine (3 mL), reacted for 10 min, and filtered again and washed successively with 10 ml portions of $CHCl_3$, MeOH, MeCN, and Et_2O, and dried *in vacuo*. Loading was determined by treating a portion (5 mg) of **7e**,**f** with 1 mL of 3% w/v CCl_3CO_2H in 1,2-dichloroethane and measuring the absorbance of DMTr cation at 504 nm (ε = 75 mL cm^{-1} $umol^{-1}$)[3]. These were found to be 37 umol/g for **7e** and 27 umol/g for **7f**.

References

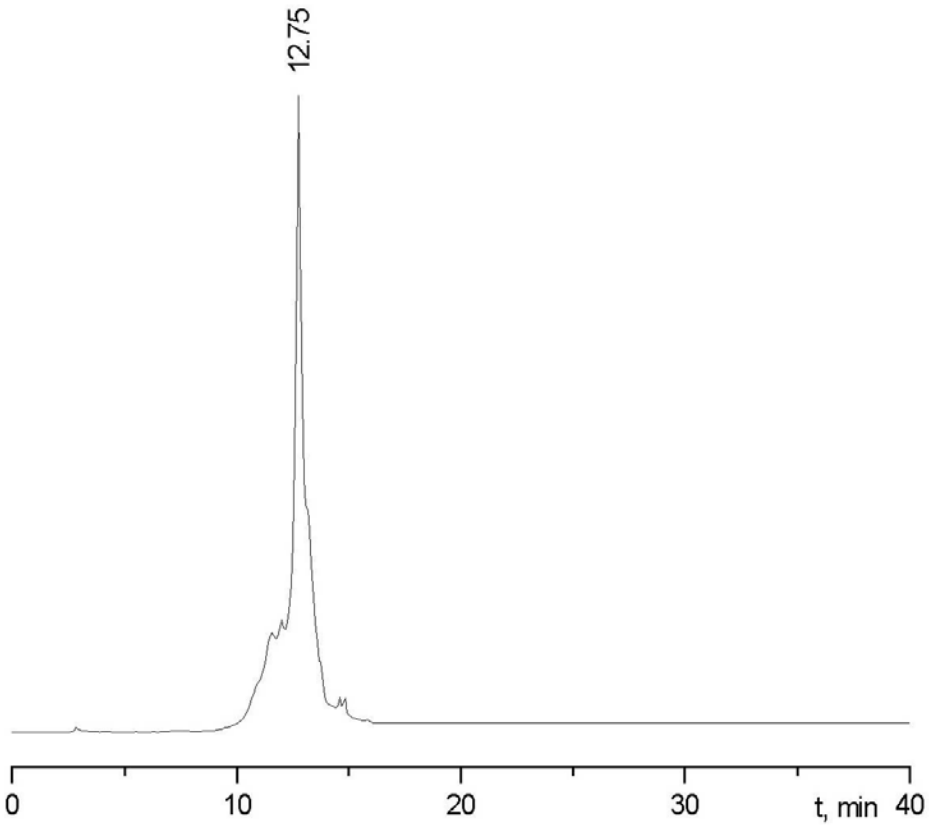
1. Haralambidis, J.; Angus, K.; Pownall, S.; Duncan, L.; Chai, M.; Tregear, G. W. *Nucl. Acids Res.* **1990**, 18, 501.
2. Korshun, V. A.; Pestov, N. B.; Nozhevnikova, E. V.; Prokhorenko, I. A.; Gontarev, S. V.; Berlin, Y. A. *Synth. Commun.* **1996**, 26, 2531.
3. Damha, M. J.; Giannaris,P. A.; Zabarylo, S. V. *Nucleic Acids Res*. **1990**, 18, 3813.
4. Koizumi, M.; Koga, R.; Hotoda, H.; Momota, K.; Ohmine, T.; Furukawa, H.; Agatsuma, T.; Nishigaki, T.; Abe, K.; Kosaka, T.; Tsutsumi, S.; Sone, J.; Kaneko, M.; Kimura, S.; Shimada, K. *Bioorg.Med.Chem.* **1997**, 5; 2235.

RP-HPLC traces of crude oligonucleotides containing (*R*)-2,4-dihydroxybutyramide modifications (Table 2).

CTCCCAGGCTCAAAT(**6a**)p (**III**)



CTCCCAGGCTCAAAT(**7a**) (**IV**)



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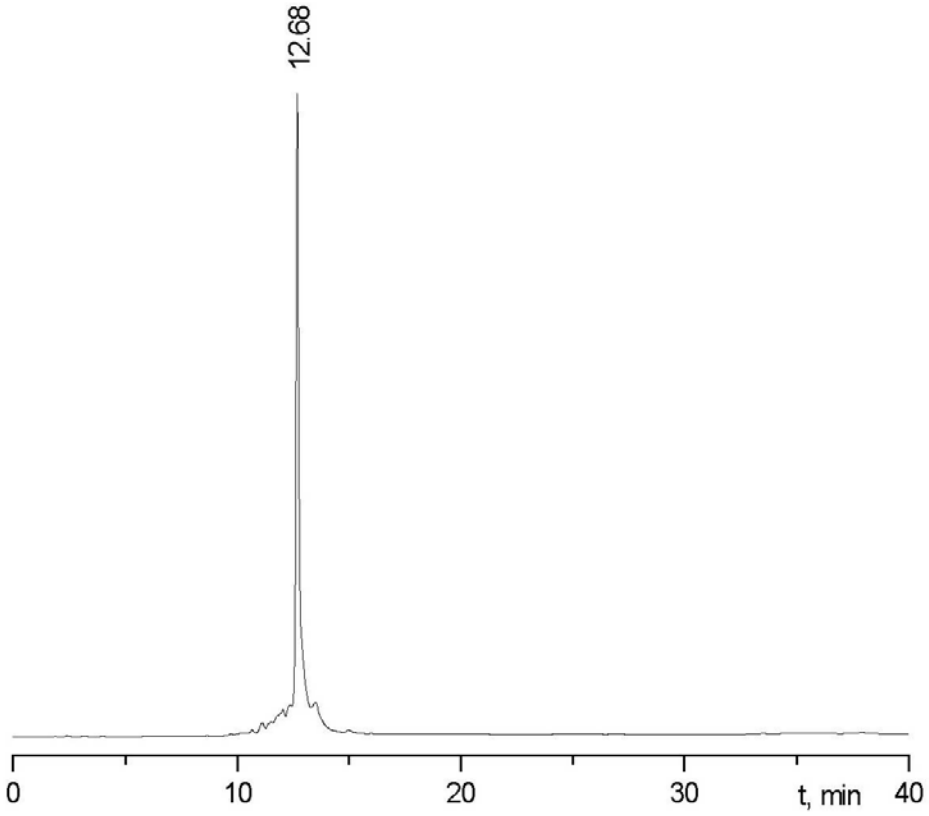
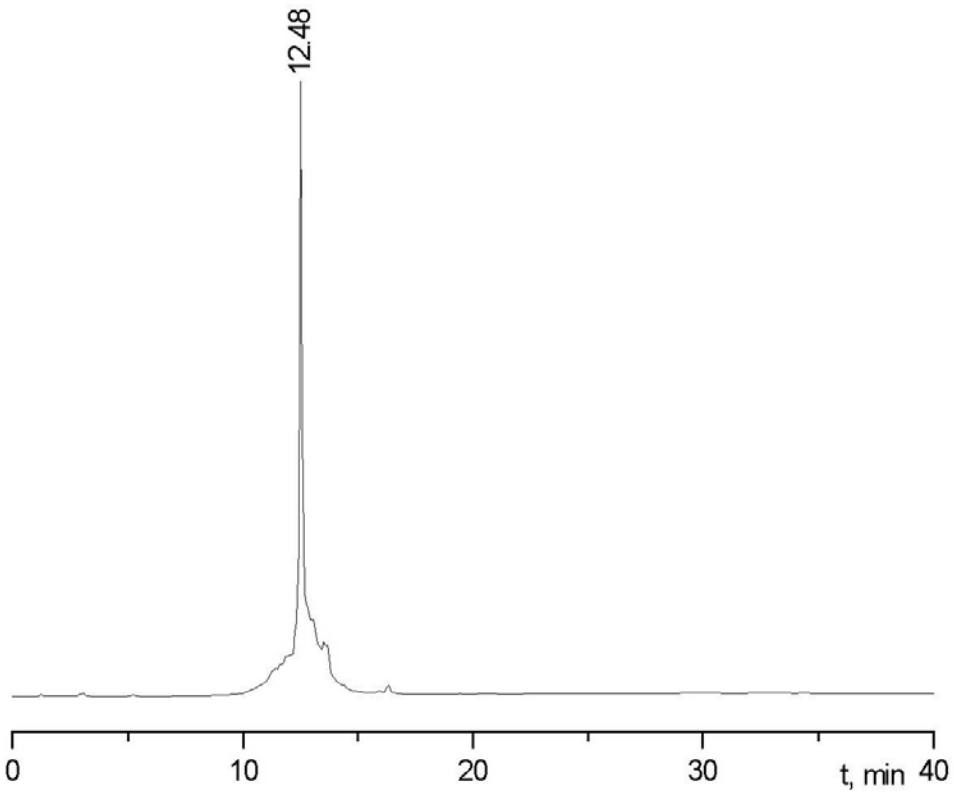


(**6b**)CTCCCAGGCTCAAAT (**VI**)



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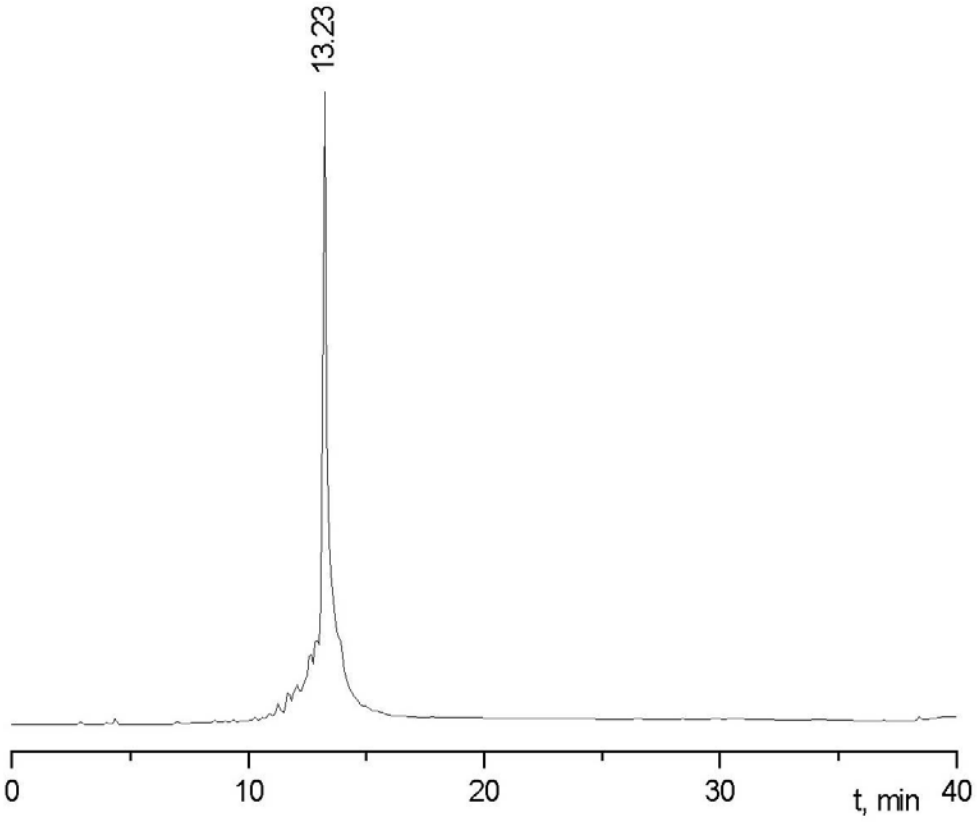

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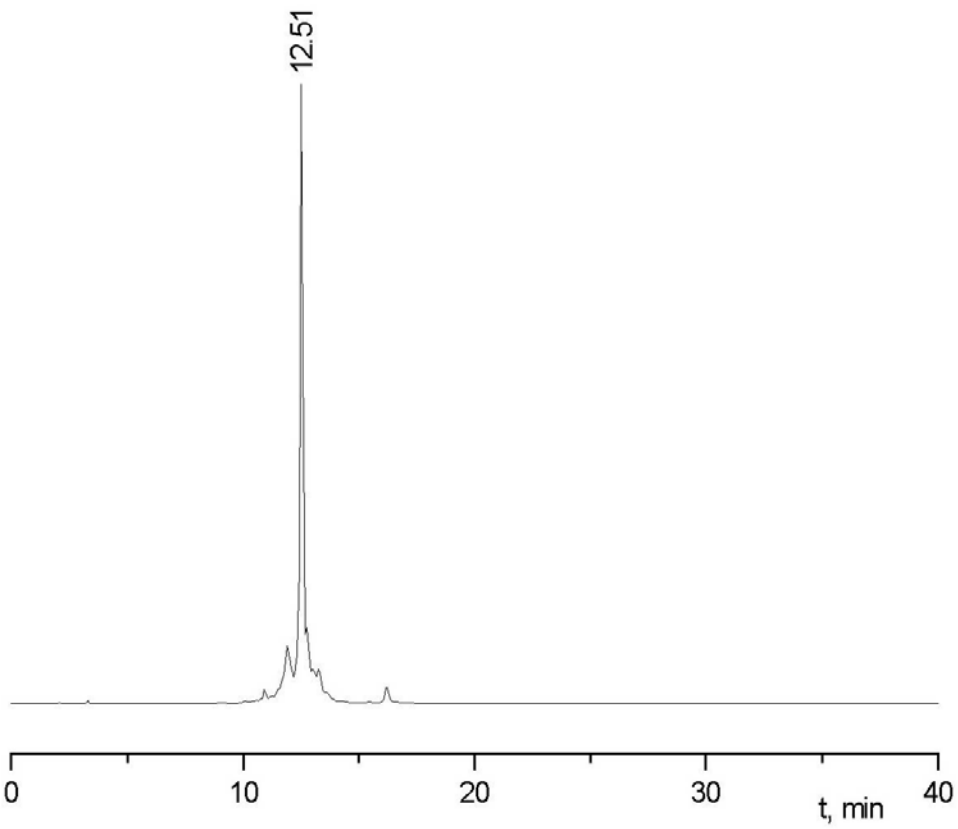

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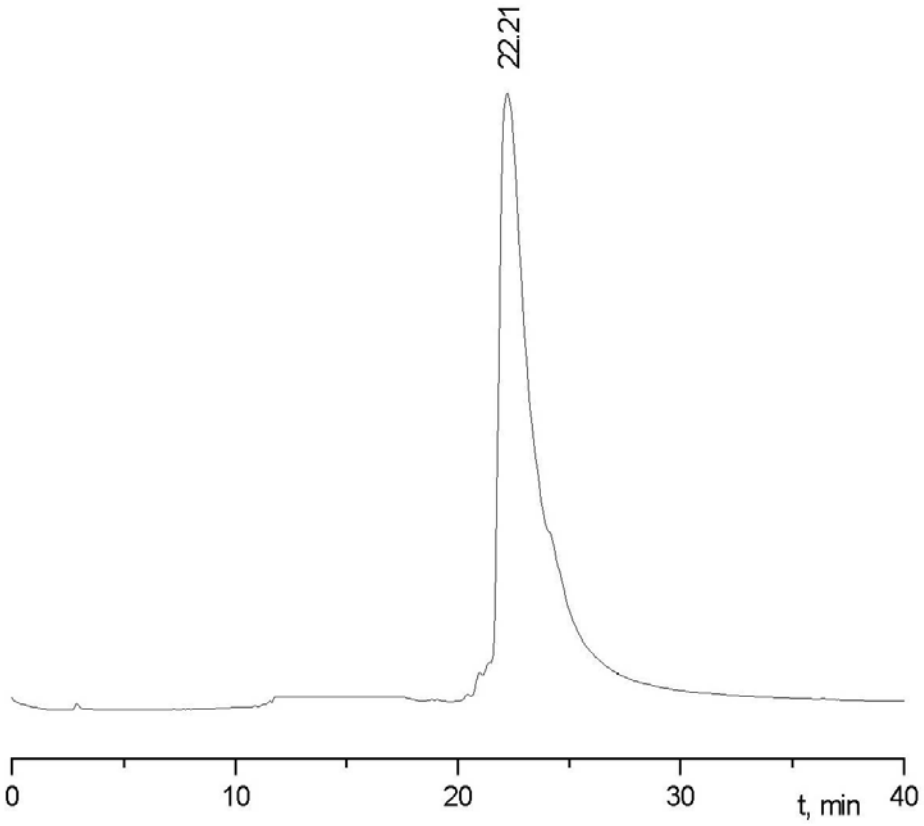

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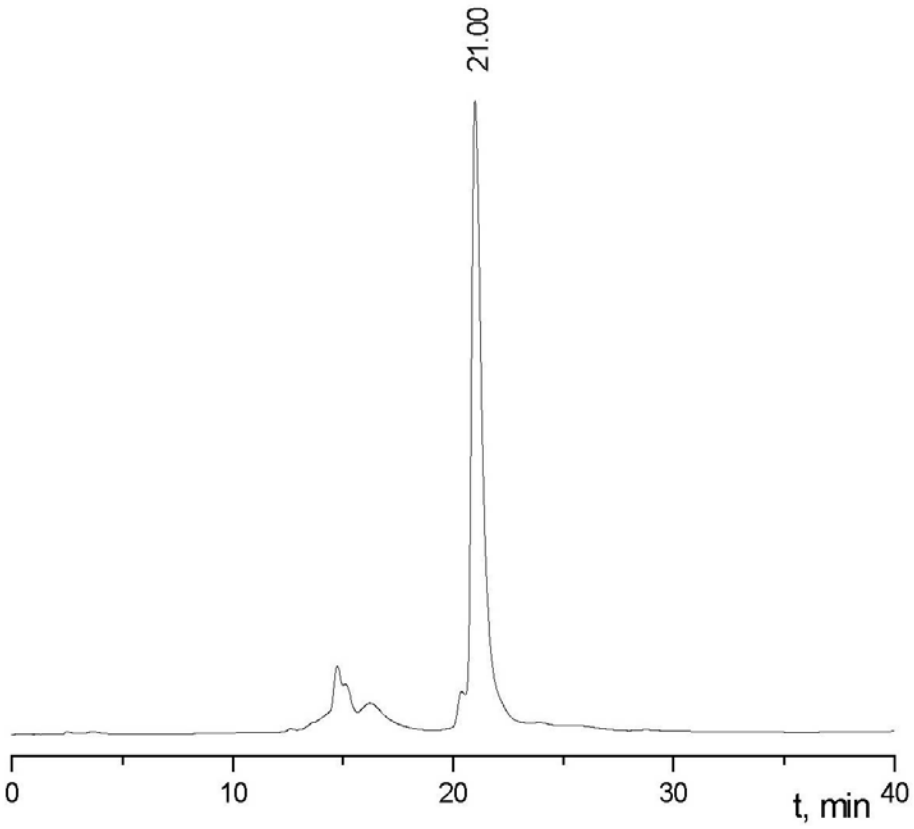

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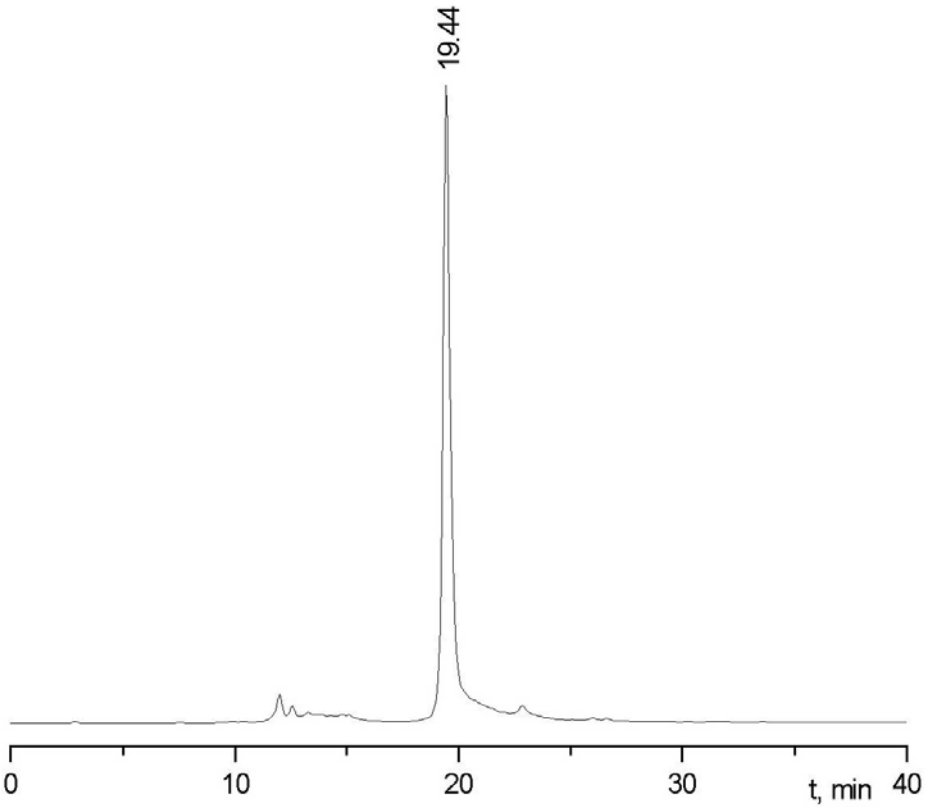

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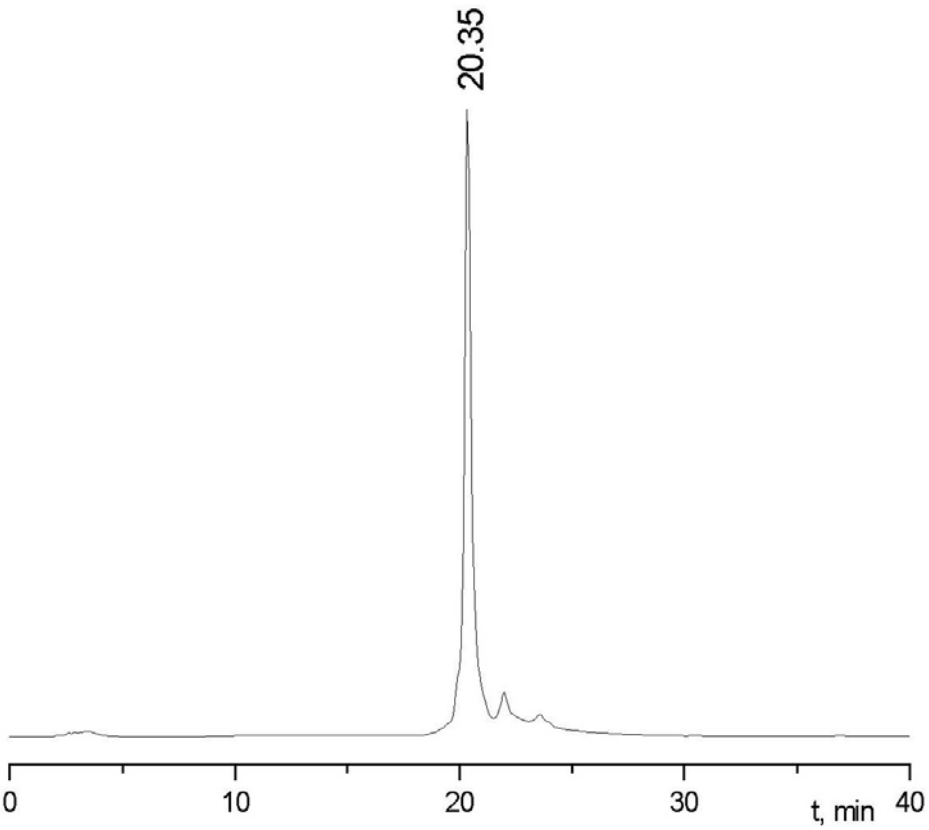

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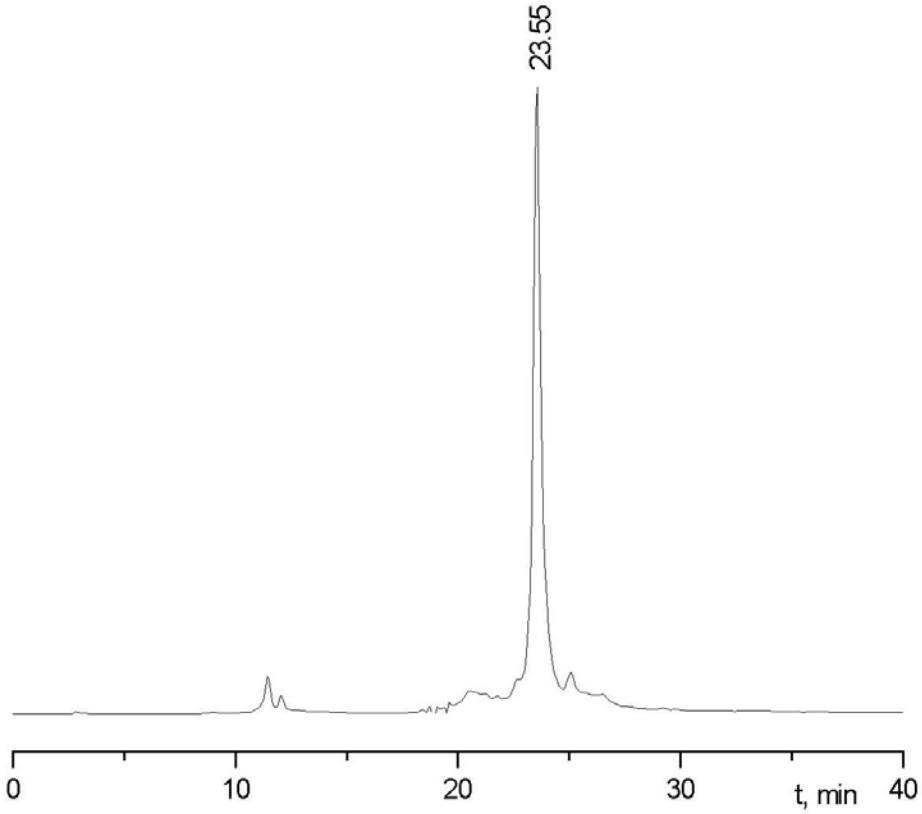

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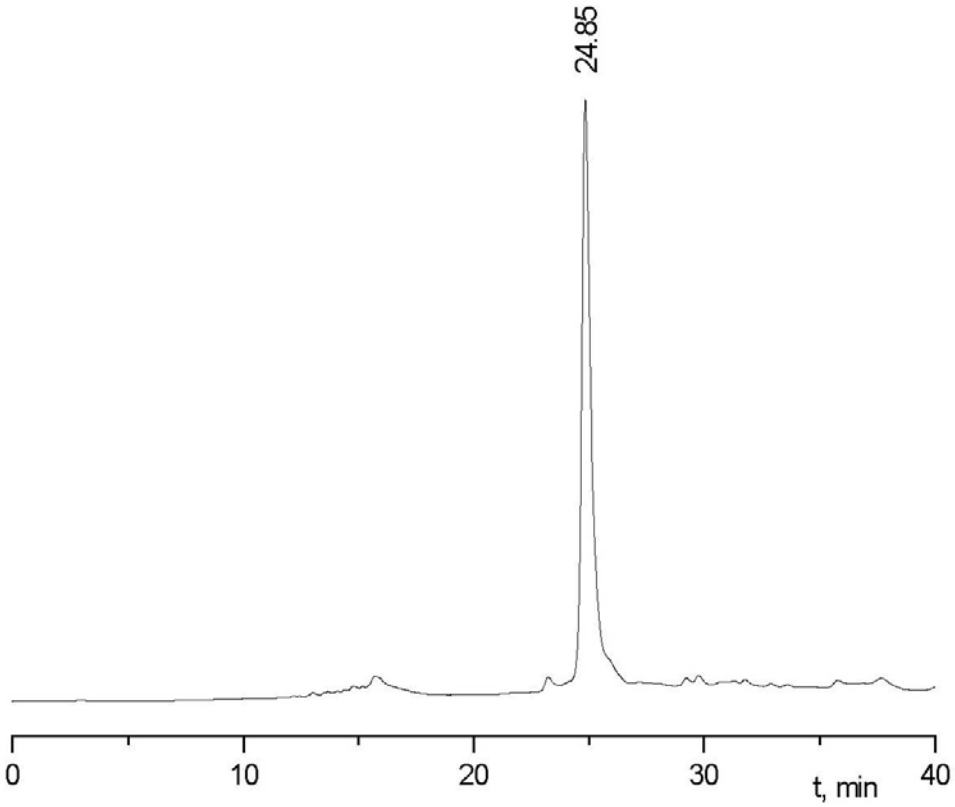
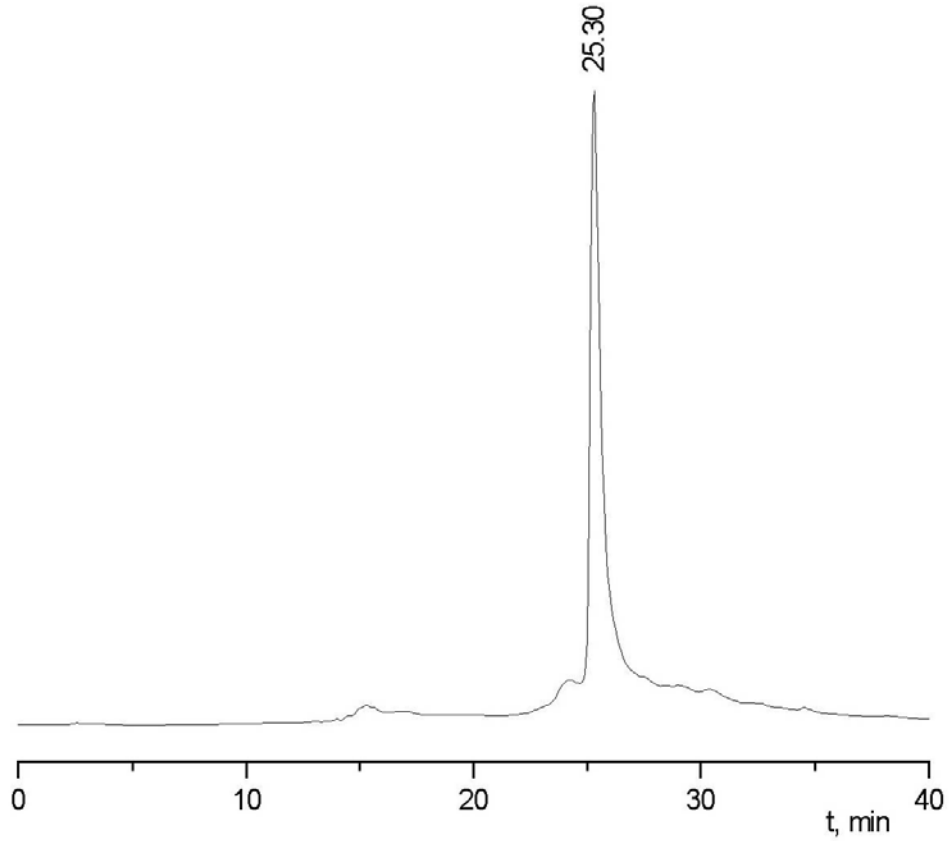


ATTTGAGCCTGGGAG(**7d**) (**XV**)



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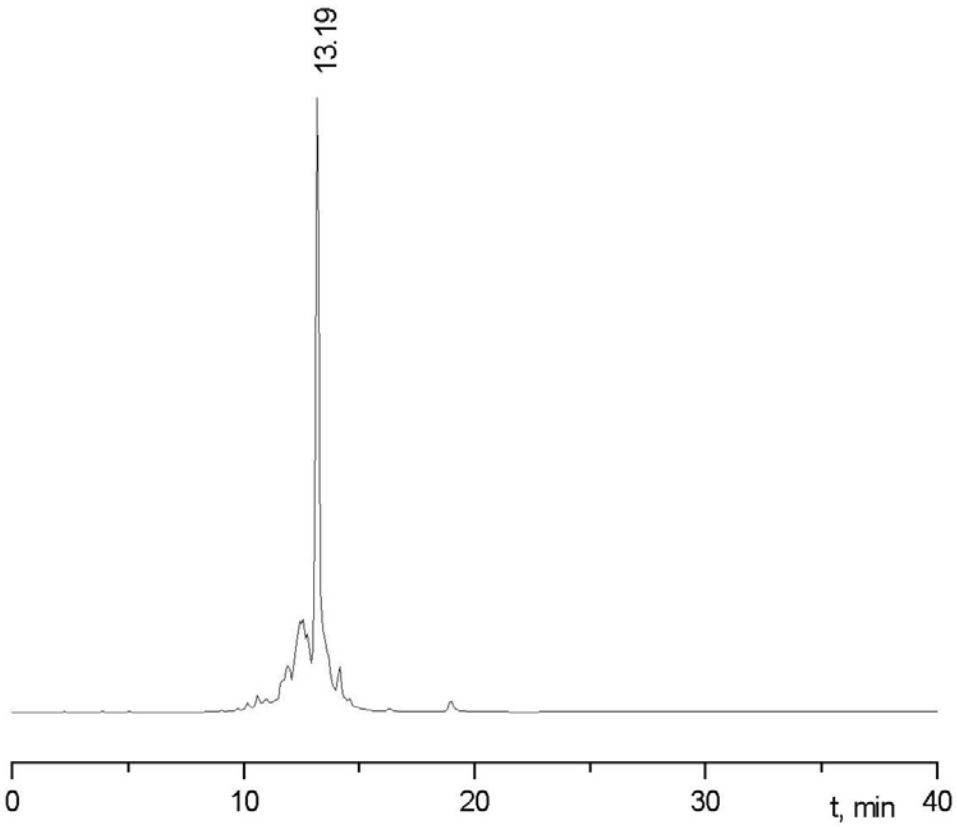
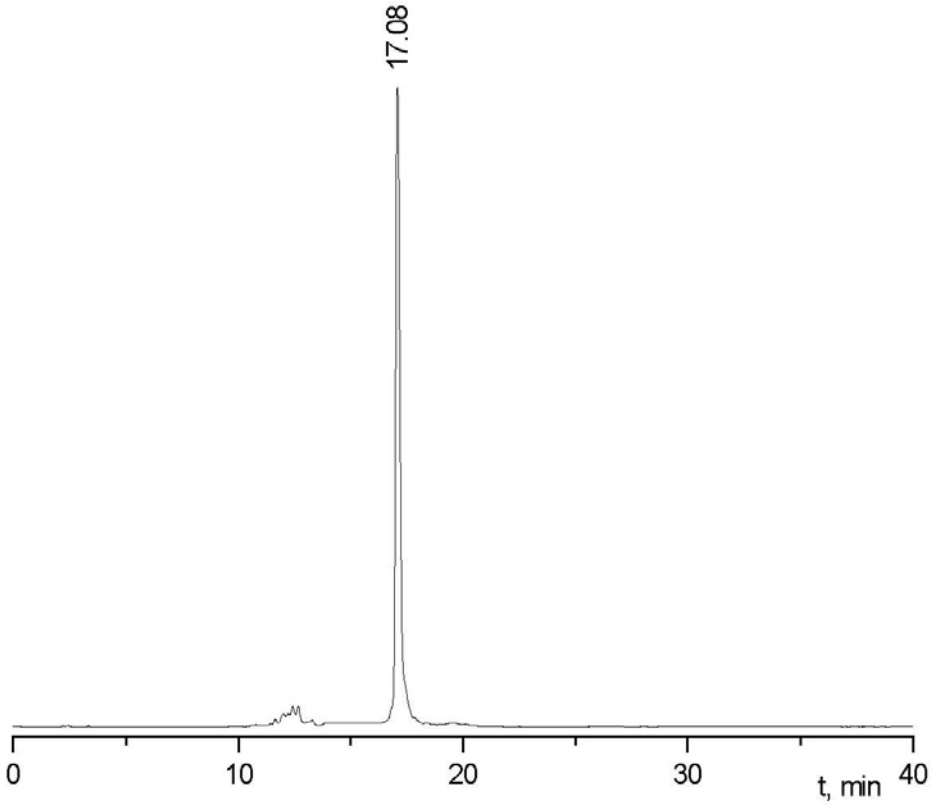


CTCCCAGGCTCAAAT(**7f**) (**XVII**)



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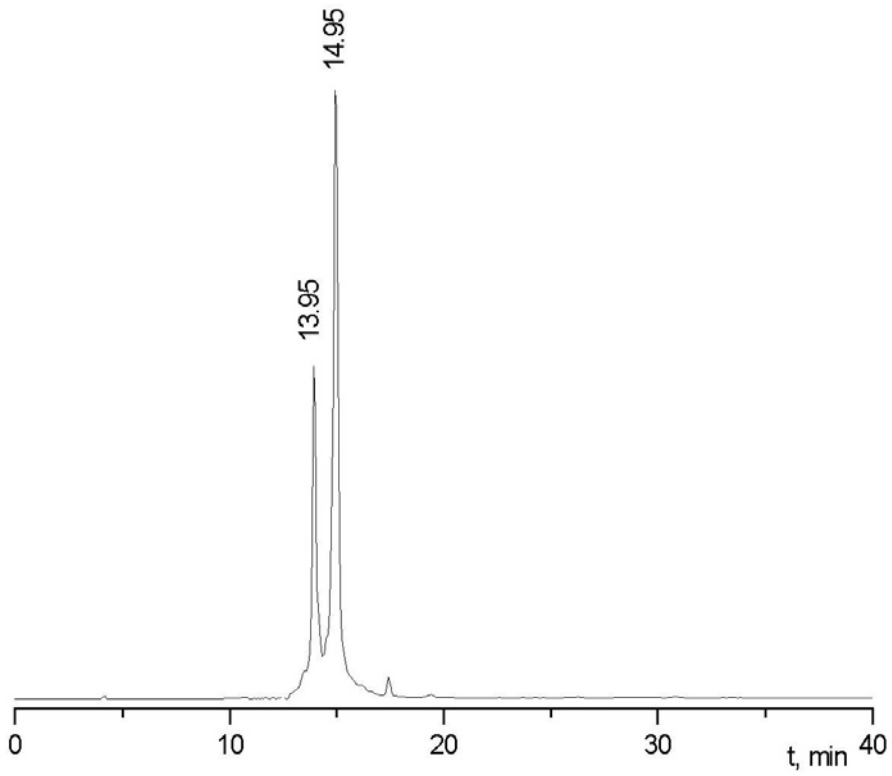
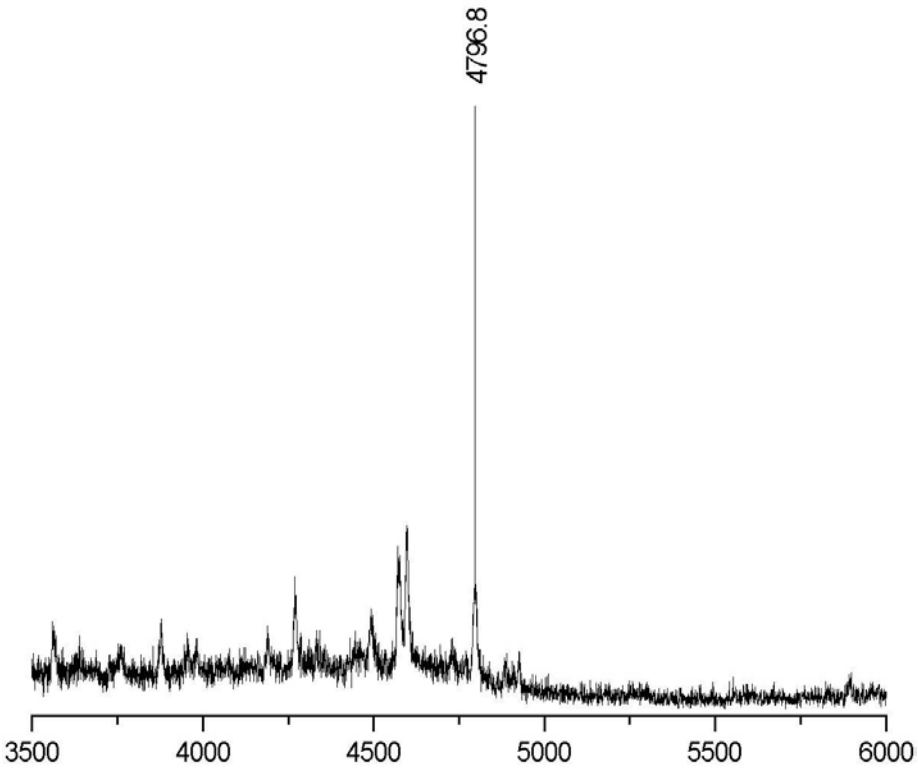
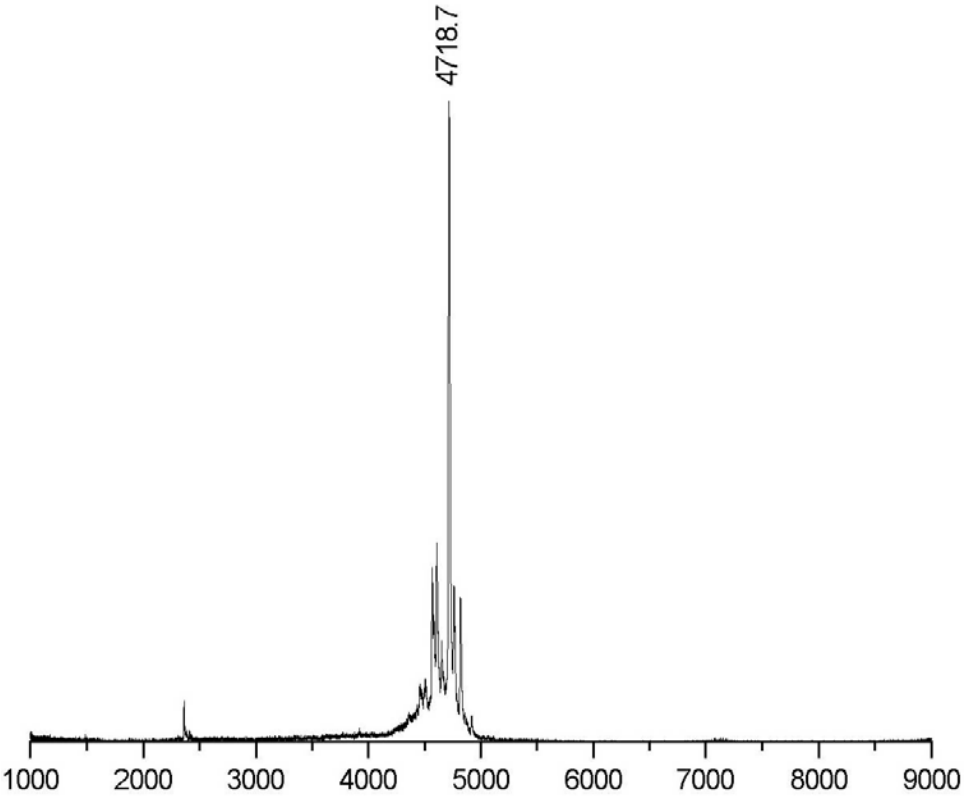


MALDI-TOF spectra of crude oligonucleotides containing (*R*)-2,4-dihydroxybutyramide modifications (Table 2).

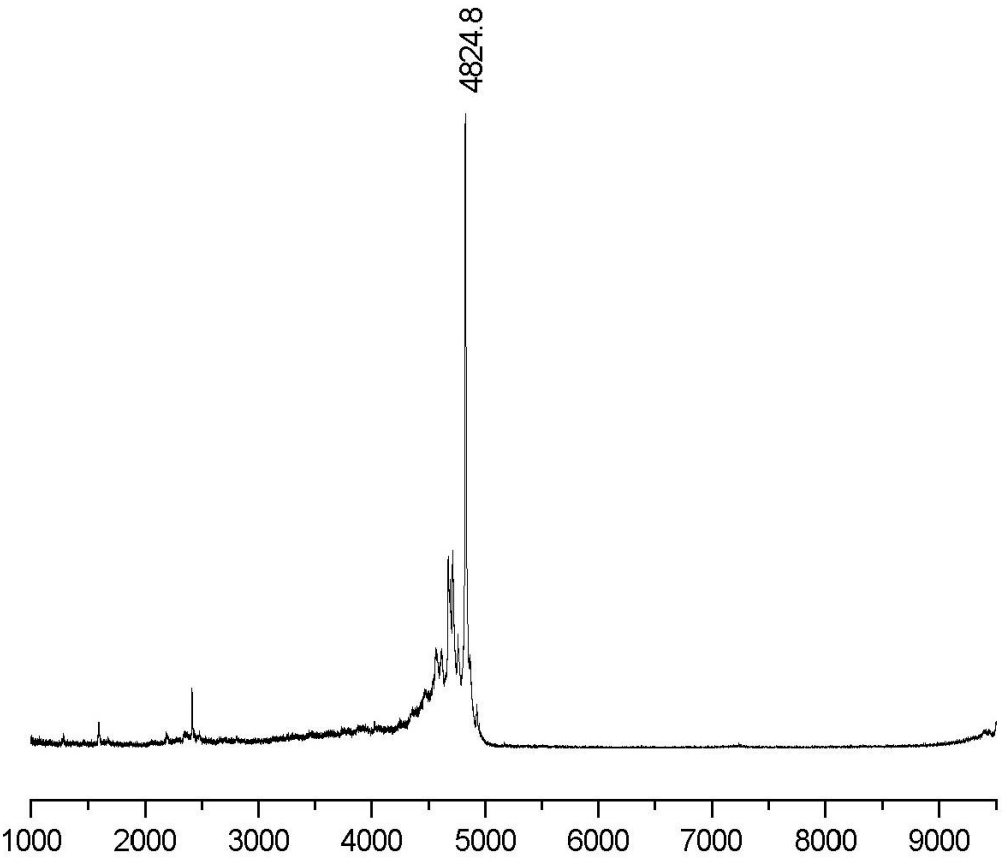
CTCCCAGGCTCAAAT(**6a**)p (**III**)



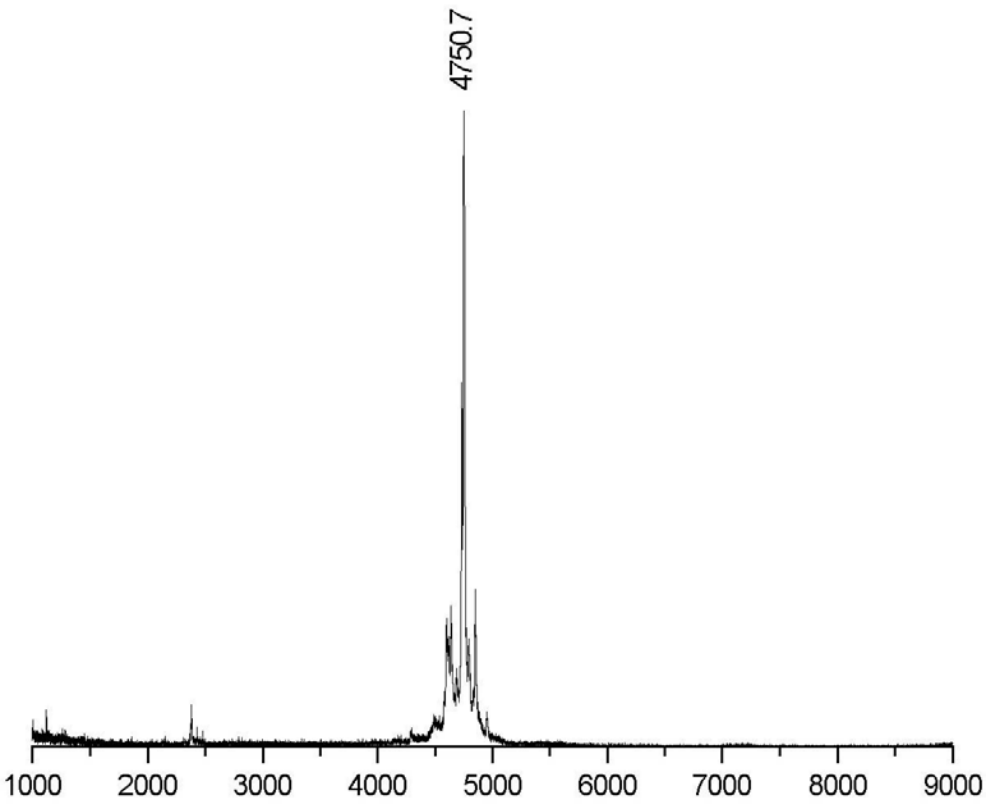
CTCCCAGGCTCAAAT(**7a**) (**IV**)



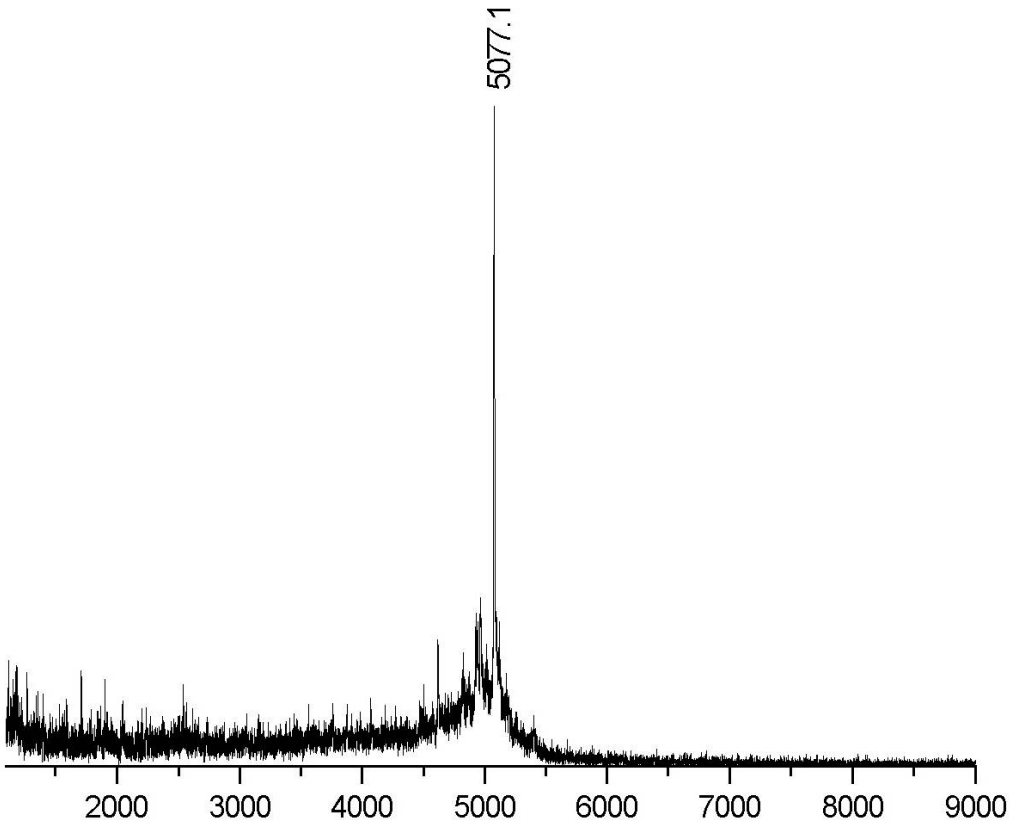
CTCCCAGGCTCAAAT(**6b**)p (**V**)



(**6b**)CTCCCAGGCTCAAAT (**VI**)



CTCCCAGGCTCAAAT(**6b**)(**6b**)p (**VII**)



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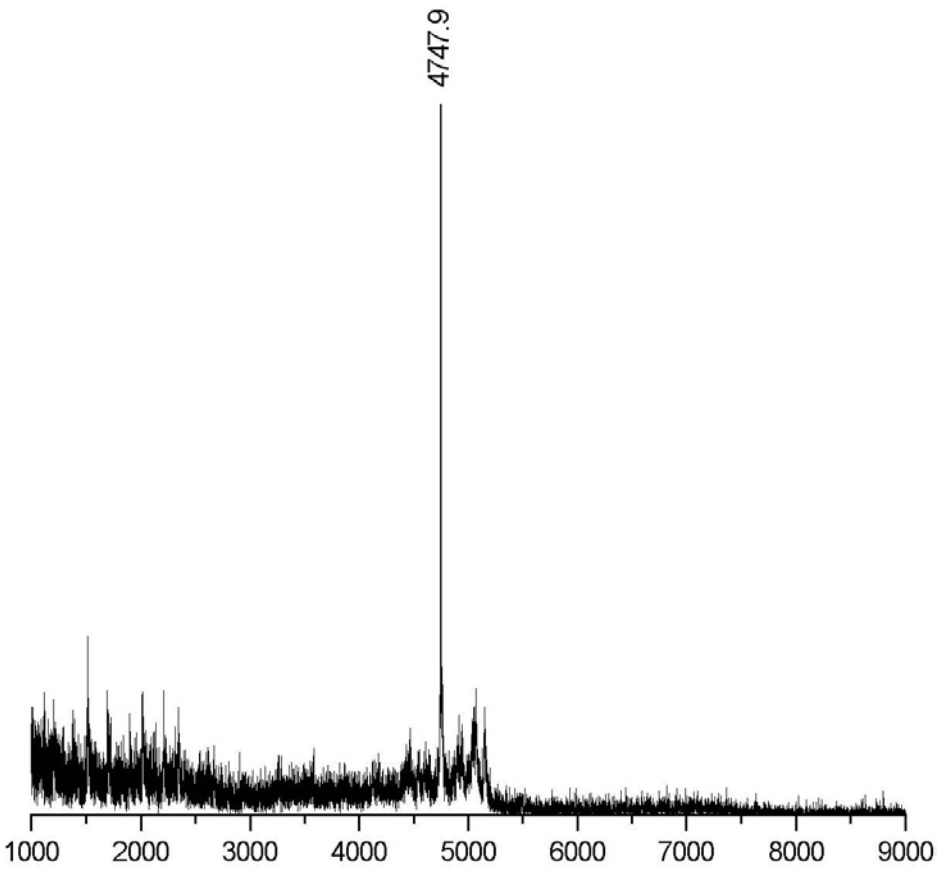

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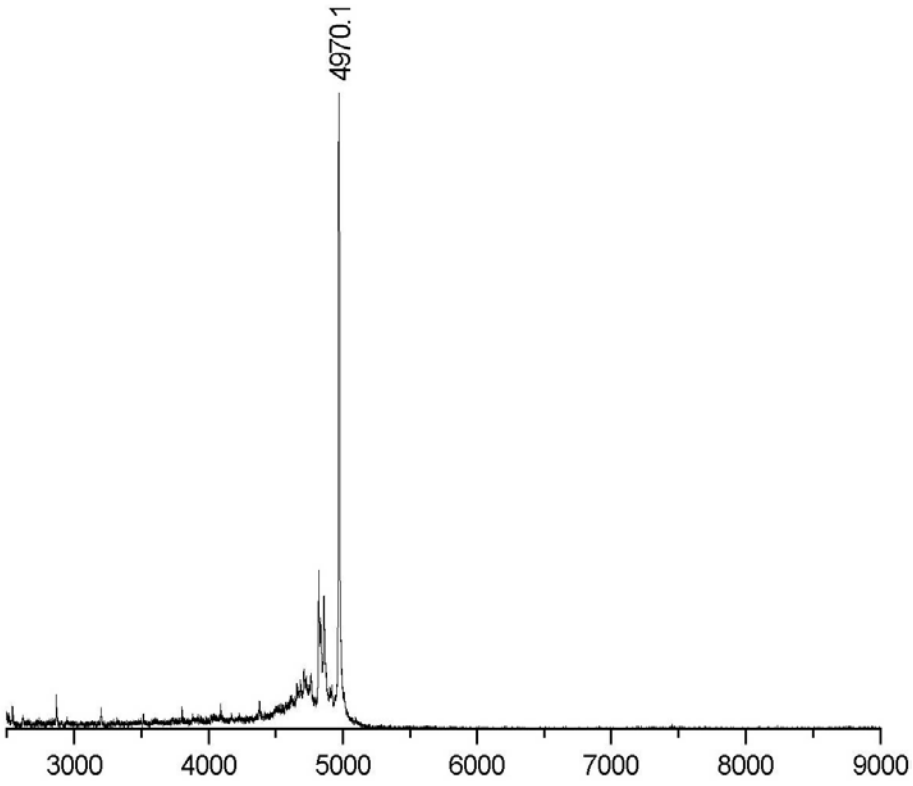

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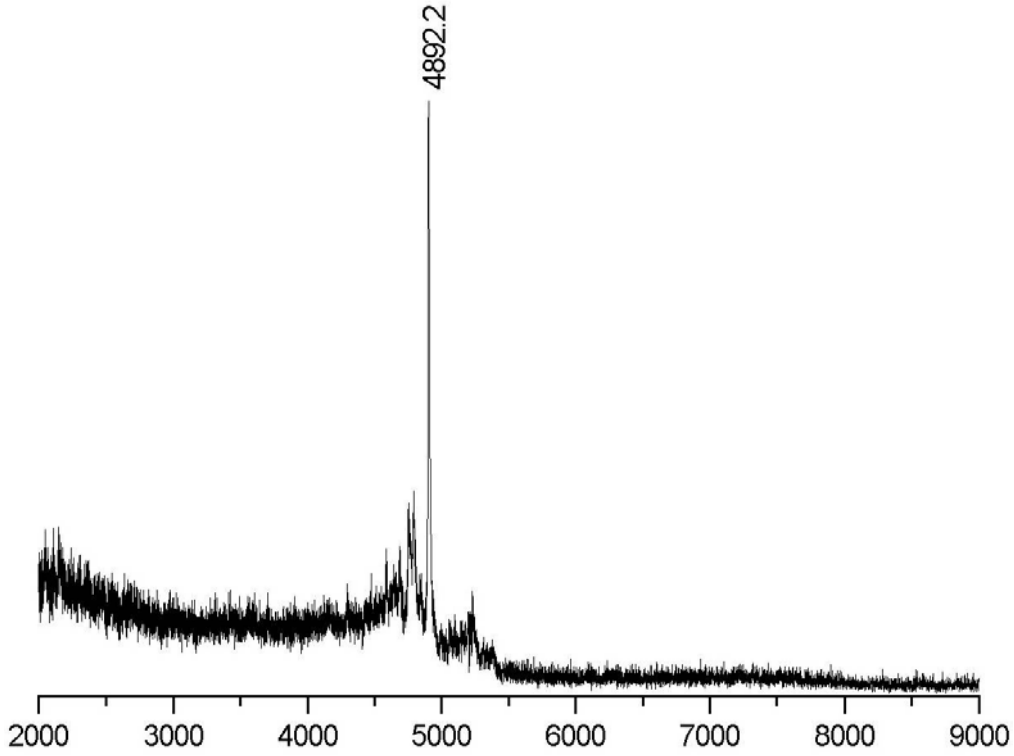
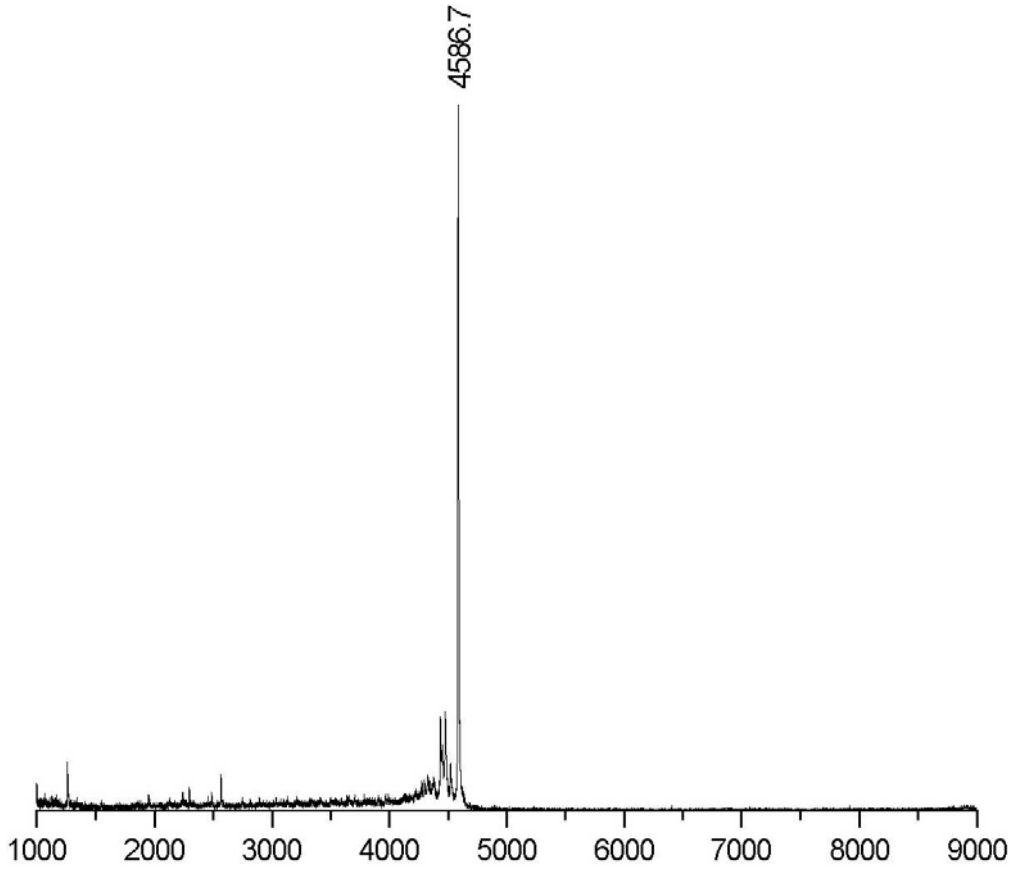


CTCCCAGGC(**6c**)CAAAT (**XI**)



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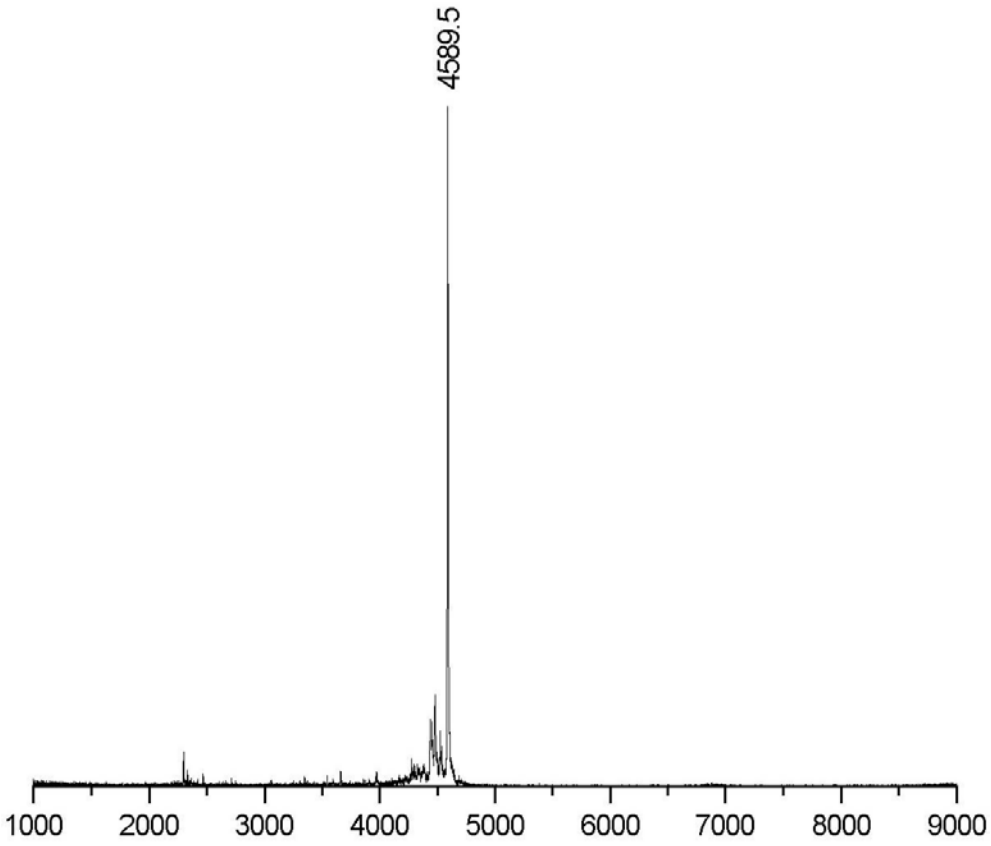

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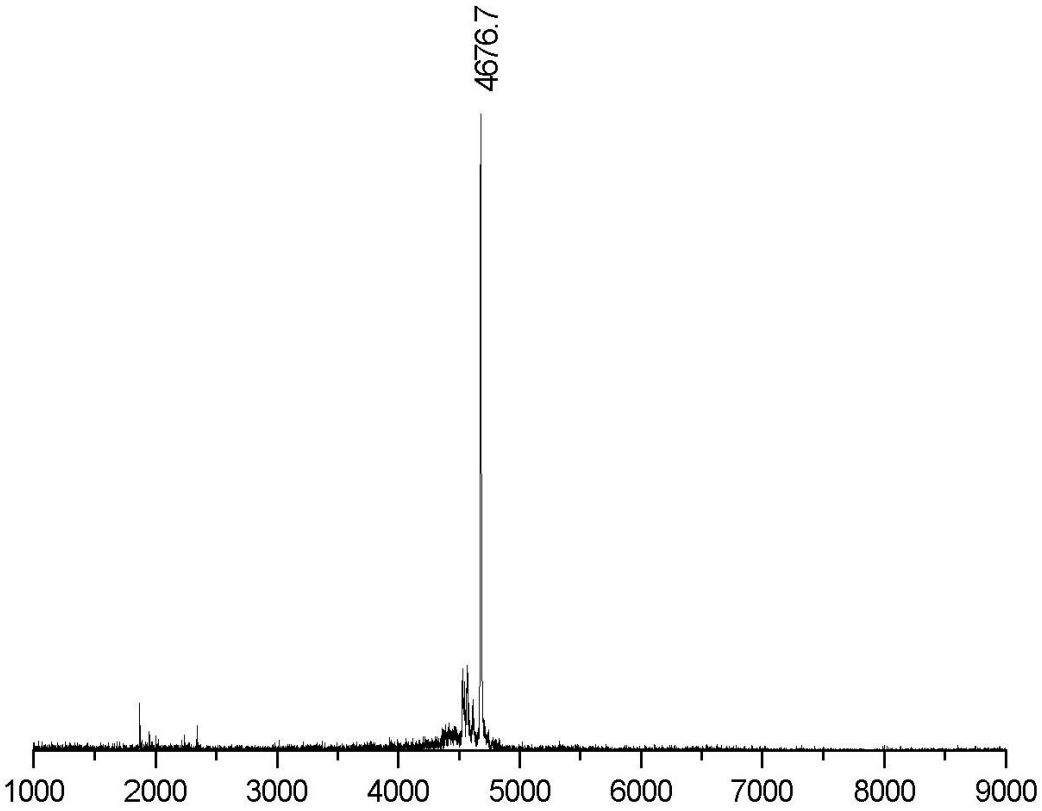

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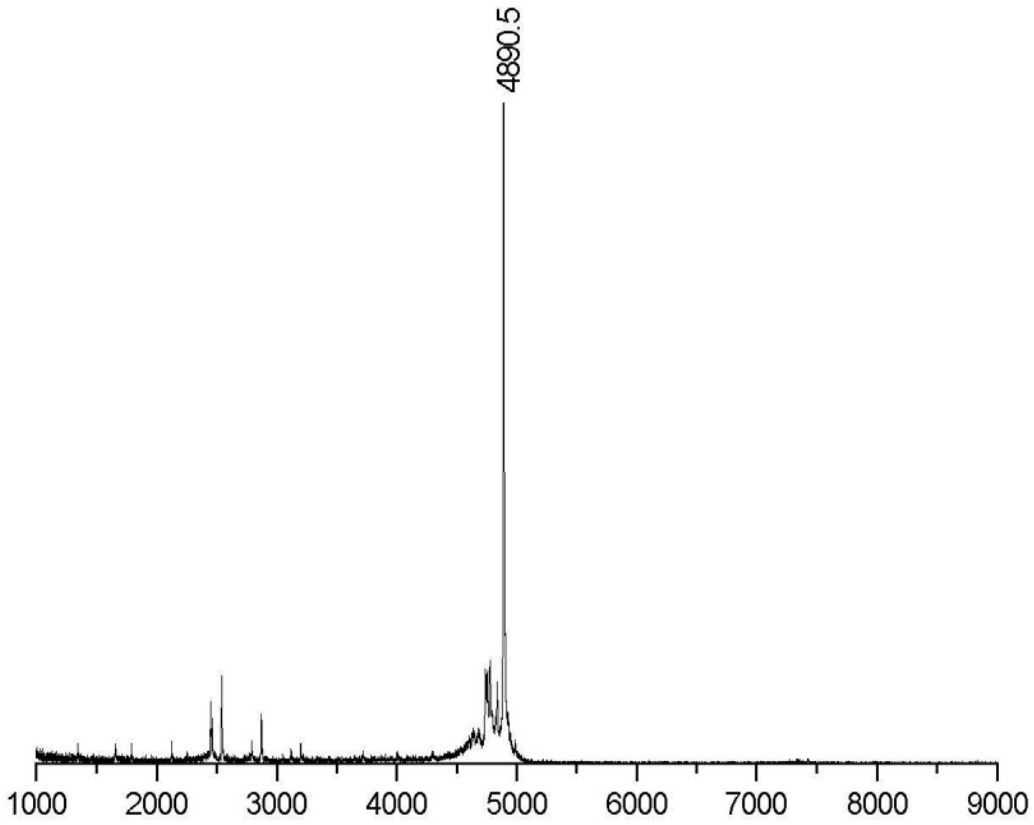
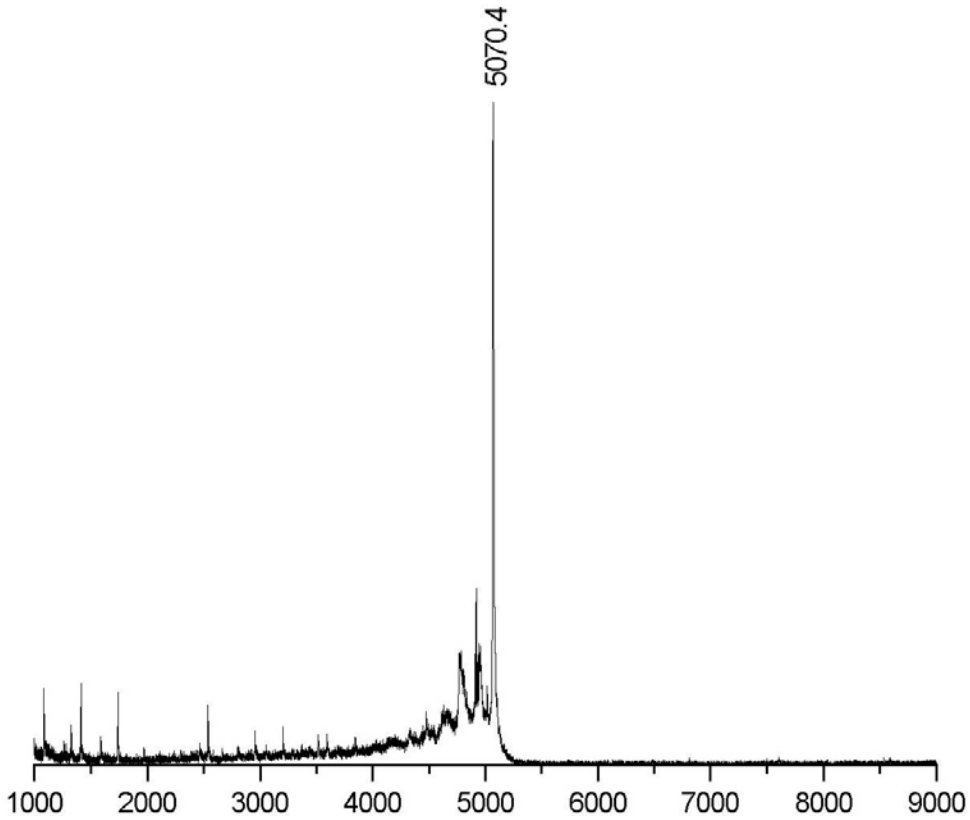


ATTTGAGCCTGGGAG(**7d**) (**XV**)



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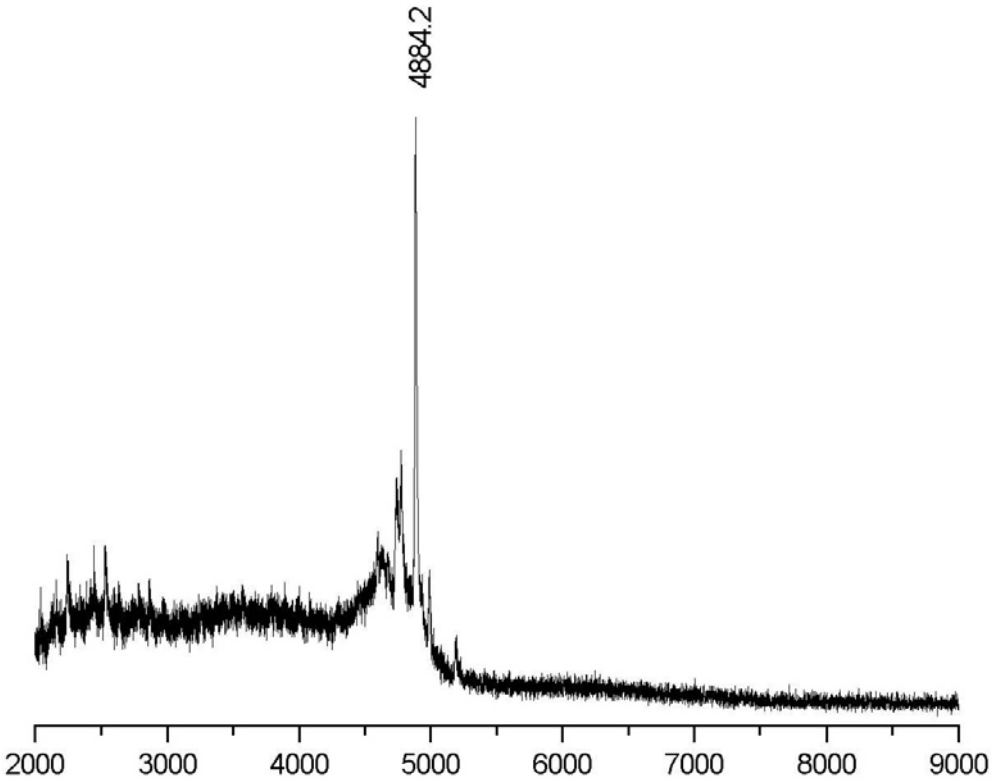
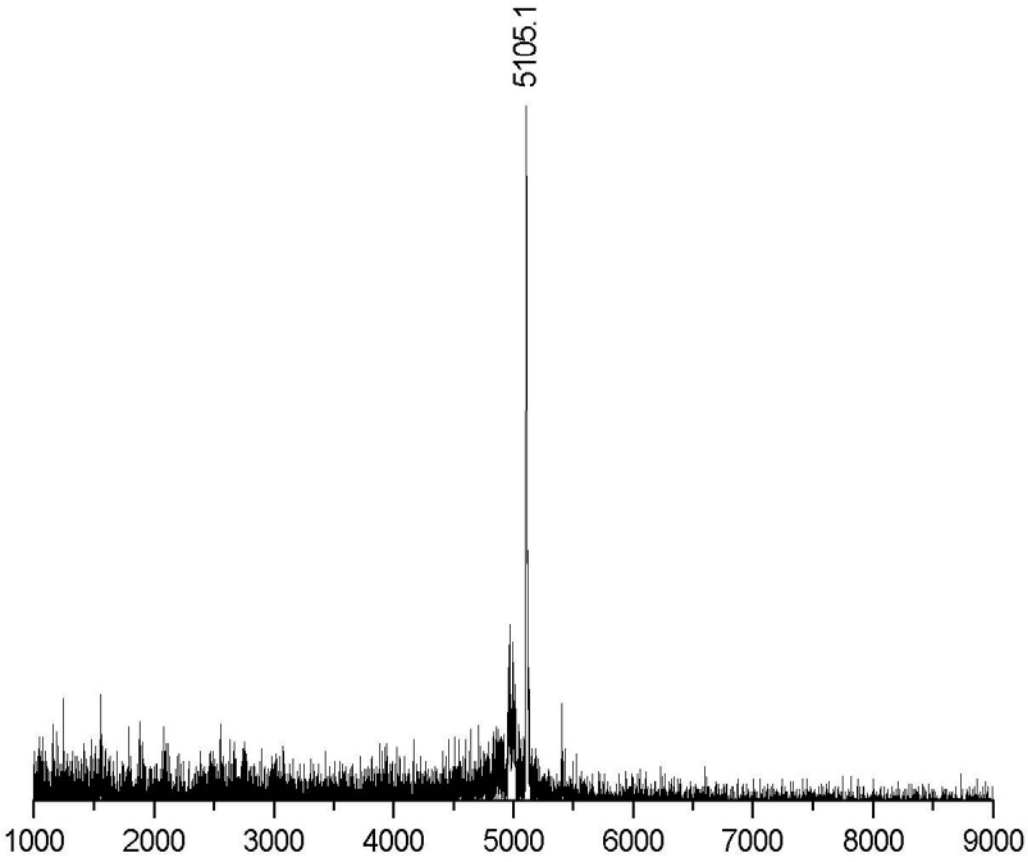
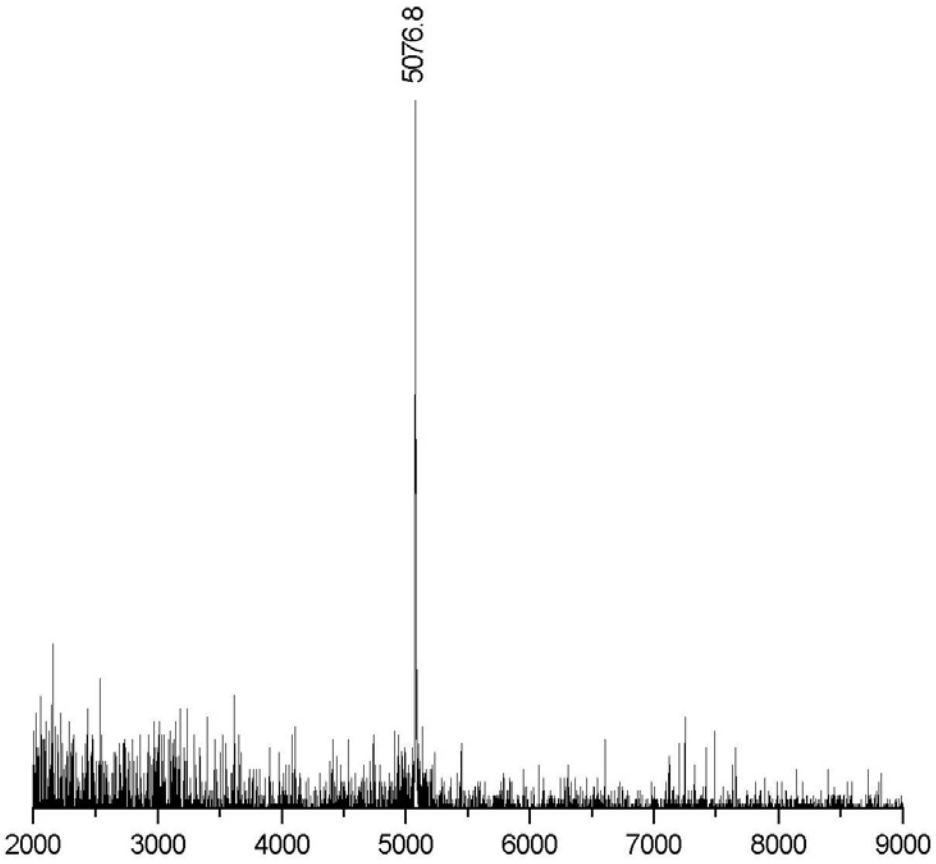


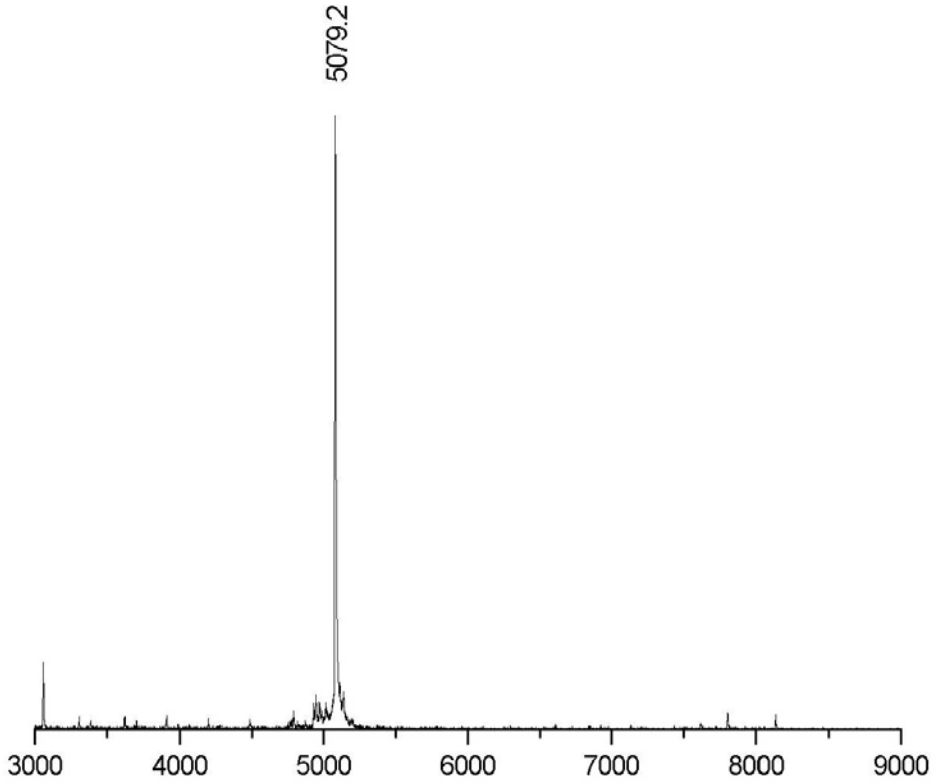
CTCCCAGGCTCAAAT(**7f**) (**XVII**)



CTCCCAGGCTCAAAT(**7g**) (**XVIII**), faster isomer



CTCCCAGGCTCAAAT(**7g**) (**XVIII**), slower isomer



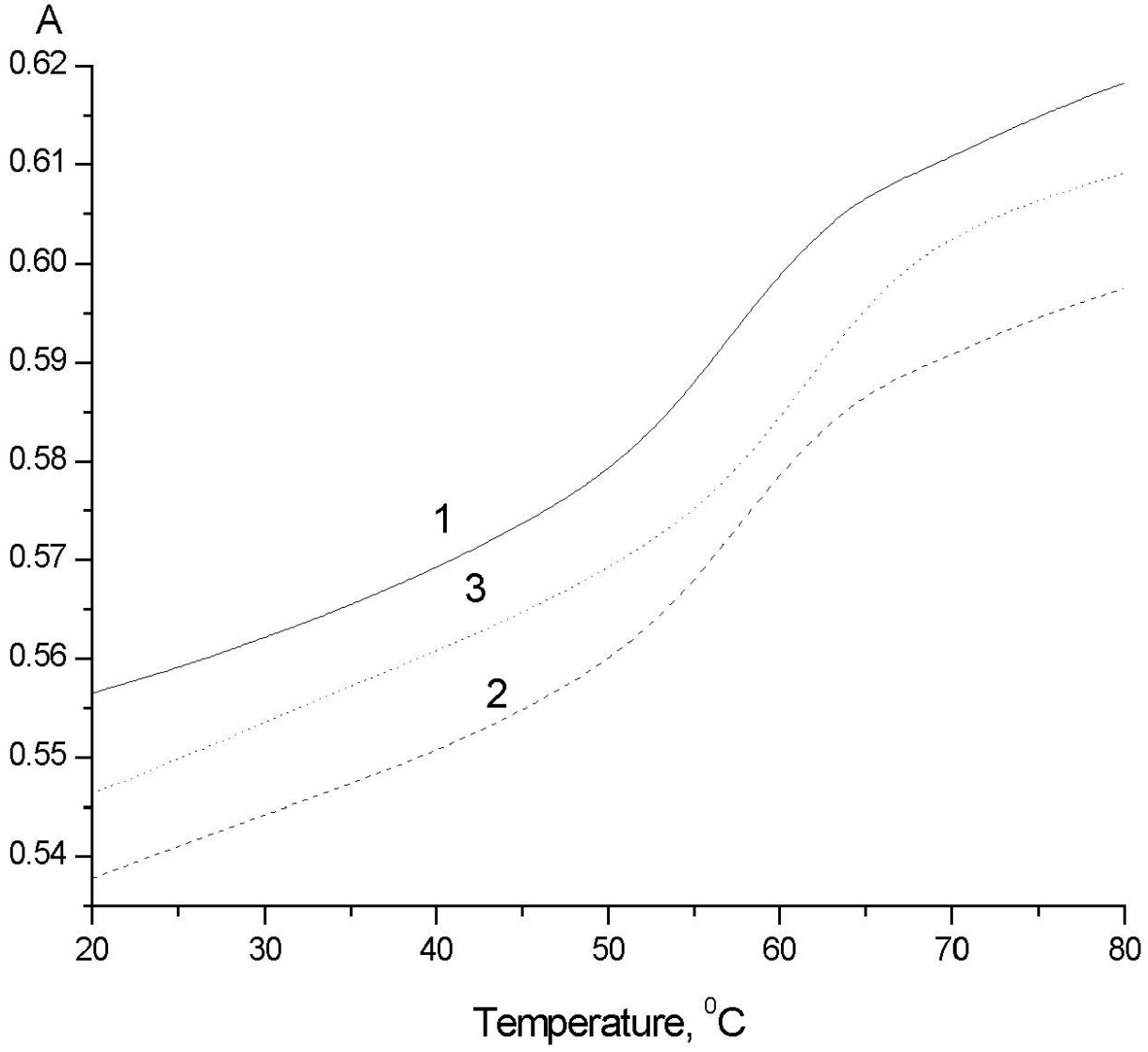


Figure 1. Examples of the melting curves of the oligonucleotides modified with 2,4-dihydroxybutyrolactone units: (**1**) biotin (**XVII**); (**2**) long chain amine (**XVI**); (**3**) 1-pyrenemethyl (**VIII**).