Published in final edited form as:

Curr Protoc Nucleic Acid Chem. 2011 December ; 0 1: Unit-1.2516. doi:
10.1002/0471142700.nc0125s47.

Synthesis of the Tellurium-Derivatized Phosphoramidites and their Incorporation into DNA Oligonucleotides

Sibo Jiang, Jia Sheng, and Zhen Huang

Department of Chemistry, Georgia State University, Atlanta, GA, 30303, USA

Zhen Huang: huang@gsu.edu

Introduction

In this unit, an efficient method for the synthesis of 2'-tellurium modified phosphoramidite and its incorporation into oligonucleotide are presented. We choose 5'-O-DMTr-2',2'-anhydro-uridine and -thymidine nucleosides (S.1, S.2) as starting materials due to their easy preparation. The 5'-O-DMTr-2',2'-anhydro-uridine and -thymidine can be converted to corresponding the 2'-tellurium-derivatized nucleosides by treating with the telluride nucleophiles. Subsequently, the 2'-Te-nucleosides can be transformed into 3'-phosphoramidites, which are the building blocks for DNA/RNA synthesis. The DNA synthesis, purification and applications of oligonucleotides containing 2'-Te-U or 2'-Te-T are described in this protocol.

Keywords

tellurium nucleic acid; nucleic acid modification; Te-derivatized DNA; nucleic acid X-ray crystallography; DNA damaging; conductive DNA; DNA imaging

This protocol is based on the previously published work (Sheng, et al. 2009), and it contains the detailed procedures for the synthesis of 2'-tellurium-modified uridine (2'-Te-U) and thymidine (2'-Te-T) phosphoramidites. They and oligonucleotides containing 2'-Te-U or 2'-Te-T are illustrated in Figure 1. Basic Protocol 1 describes the synthesis of 2'-phenyltelluride-modified U and T phosphoramidites. Basic Protocol 2 describes the synthesis of 2'-methyltelluride-modified U and T phosphoramidites. The products from Basic Protocol 1 or Basic Protocol 2 can be applied to solid-phase DNA synthesis. In Basic Protocol 3, synthesis and purification of oligonucleotide containing 2'-Te-T or 2'-Te-U are further explained. The starting material 5'-O-DMTr-2',2'-anhydro-U and -T (S.1, S.2) can be prepared from commercially available uridine and ribothymidine, respectively, by two steps synthesis with high yields (Carrasco, et al. 2004 and Sheng, et al. 2007). The organotellurium reagent (dimethylditelluride or diphenylditelluride) is reduced first, and then the tellurium functionality is introduced to 2'-alpha position by a S_N2 reaction (phenyltelluride as nucleophile in Basic Protocol 1, methyltelluride as nucleophile in Basic Protocol 2, respectively). The resulted key intermediates (S.3a, S.4a, S.3b, and S.4b) are converted to corresponding phosphoramidites (S.5a, S.6a, S.5b, and S.6b) by standard procedures. The Te-phosphoramidite monomer is then incorporated into oligonucleotides by

solid-phase synthesis, followed by deprotection, purification and analysis. (Basic Protocol 3).

BASIC PROTOCOL 1 PREPARATION OF THE 2'-PHENYLTELLANYL PHOSPHORAMIDITE

The introduction of the phenyl-telluride functionality to nucleoside is conducted by attack of phenyltelluride nucleophile at the alpha-face of 2, 2'-anhydro-uridine or -thymidine nucleoside. Instead of using elemental tellurium, diphenylditelluride is used as a source of tellurium to avoid rapid oxidation of tellurol. Phenyltelluride nucleophile is generated by reduction of the organotellurium reagent with NaBH₄ in THF. The starting material, 5'-DMTr-2, 2'-anhydrouridine **S.1** or 5'-DMTr-2, 2'-anhydrothymidine **S.2**, can be easily prepared by following the literatures or purchased commercially, respectively. The resulting 2'-PhTe nucleosides can be converted to corresponding phosphoramidites by treating with 2-cyanoethyl N,N-diisopropyl-chlorophosphoramidite in the presence of Hünig's base (N,N-diisopropylethylamine).

Materials

Dry argon (Ar)
5'-DMTr-2, 2'-anhydrouridine **S.1** (SeNA Research)
5'-DMTr-2, 2'-anhydrothymidine **S.2** (SeNA Research)
Diphenylditelluride (PhTeTePh) (TCI America)
NaBH₄ (sodium borohydride) (Sigma)
Anhydrous THF (tetrahydrofuran)
Anhydrous EtOH (ethanol)
Anhydrous DCM (methylene chloride)
Anhydrous Na₂SO₄
N, N-diisopropylethylamine (Chemgene)
2-cyanoethyl N,N-diisopropyl-chlorophosphoramidite (Chemgene)
Ethyl acetate
Hexane
Silica gel 60 (230 – 400 mesh)
Al₂O₃
Saturated NaHCO₃ aqueous solution
Vacuum pump
250 mL separatory funnel
250-, 100-, 50-, 25-, 5-mL round bottom flasks

1, 3, 5 and 10 mL disposable syringes

Introduction of Phenyltelluride Functionality—All reactions are performed in Ar atmosphere. An Ar atmosphere can be carried out through a rubber septum that seals the flask and connects to balloon filled with Ar. A slightly positive pressure is maintained in the system to provide dry and oxygen-free atmosphere. Liquid chemical is added using syringe through rubber septum.

- 1** Weigh sodium borohydride (NaBH_4 , 6.2 mg, 0.15 mmol), place it in an oven-dried 25 mL round-bottom flask containing an 8 x 1.5 mm magnetic stir bar. Dry under high vacuum for 1 hour then purge with argon 2–3 times. Inject 5 mL anhydrous tetrahydrofuran (THF) into the flask, place the flask in an ice bath on top of a magnetic stirring plate.
- 2** Weigh diphenylditelluride (200 mg, 0.5 mmol) in a 5 mL round-bottom flask. Dry under high vacuum for 1 hour, purge the flask with argon 2–3 times. Inject 5 mL anhydrous tetrahydrofuran (THF) into the flask, shake it gently until the compound completely dissolved.
- 3** Transfer the diphenylditelluride solution to a 5-mL syringe through an 18-G needle and inject it to the cooled and stirred NaBH_4 suspension in THF. (To balance pressure during the transfer, attach an Ar balloon when necessary.)
- 4** Slowly inject several drops of anhydrous ethanol. Allow the reaction proceed at 0°C until the solution turns colorless. (Add a few more drops of ethanol when necessary.)

For uridine nucleoside

- 5a** Dissolve starting material **S.1** (0.285g, 0.5 mmol) in anhydrous tetrahydrofuran (THF, 5 mL) and inject the prepared Te-solution slowly into reaction mixture.

For thymidine nucleoside

- 5b** Dissolve starting material **S.2** (0.292 g, 0.5 mmol) in anhydrous tetrahydrofuran (THF, 5 mL) and inject the Te-solution slowly into reaction mixture.
- 6** Warm up the reaction flask to room temperature naturally and heat it to 50°C (oil bath) for three hours.
- 7** Monitor reaction by TLC using 4% MeOH in CH_2Cl_2 .

Work up procedure

- 8** After the reaction is complete, concentrate the reaction mixture to dryness on a rotary evaporator.
- 9** Dissolve the residue in 50 mL of CH_2Cl_2 and 20 mL of H_2O . Transfer the mixture in to a 125 mL separatory funnel. Wash the organic layer (bottom) sequentially with 20 mL of H_2O twice, 20 mL of brine twice.

- 10** Collect organic layer in a 100 mL oven-dried beaker. Add 1g anhydrous MgSO_4 , wait for 15 min, filter off the drying agent. Evaporate solvent by a rotary evaporator to afford crude product.
- 11** Dissolve crude compound with 1 mL CH_2Cl_2 and load it on a silica gel column which is pre-equilibrated with CH_2Cl_2 . Elute column with pure CH_2Cl_2 and then a gradient elution with 0.5%, 1.0%, 1.5%, 2% and 3% MeOH in CH_2Cl_2 . Collect fractions and monitor by TLC (4% MeOH in CH_2Cl_2).
- 12** Combine desired fractions, evaporate solvent on a rotary evaporator and dry under high vacuum. Obtain compound **S.3a** and **S.4a** as light yellow color solid (**S.3a**: 310 mg, 80% yield; **S.4b**: 292 mg, 78% yield)
- 13** Characterize products by ^1H NMR, ^{13}C NMR and HRMS.
- S.3a:** ^1H NMR (400 MHz, CDCl_3 , 25 °C): δ =3.45-3.46 (m, 2H), 3.82 (s, 3H), 3.92-3.95 (m, 1H), 4.24 (m, 1H), 4.54-4.57 (m, 1H), 5.12 (d, J = 8 Hz, 1H), 6.63 (d, J = 9.2 Hz, 1H), 6.81-6.86 (m, 4H), 7.19-7.37 (m, 12H), 7.45 (d, J = 8 Hz, 1H), 7.82 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3 , 25 °C): δ =36.9, 55.3, 63.9, 85.5, 87.2, 91.6, 102.5, 109.6, 113.3, 127.2, 127.8, 128.1, 128.1, 128.7, 129.5, 130.1, 135.2, 140.2, 144.2, 150.2, 158.7, 162.7; HRMS: m/z : calcd for $\text{C}_{36}\text{H}_{34}\text{N}_2\text{O}_7\text{TeNa}$: 759.1326, found: 759.1316 $[\text{M}+\text{Na}]^+$.
- S.4a:** ^1H NMR (400 MHz, CDCl_3 , 25 °C): δ =1.21 (s, 3H), 3.35-3.52 (m, 2H), 3.81 (s, 6H), 3.98-4.01 (m, 1H), 4.22 (m, 1H), 4.55 (m, 1H), 6.69 (d, J = 10 Hz, 1H), 6.81-6.84 (m, 4H), 7.16-7.35 (m, 15H), 7.83 (m, 2H), 8.17 (b, 1H); ^{13}C NMR (100 MHz, CDCl_3 , 25 °C): δ =11.5, 36.8, 55.3, 63.9, 85.2, 87.2, 91.0, 109.5, 111.3, 113.3, 127.2, 128.0, 128.1, 128.6, 129.6, 130.1, 135.0, 135.2, 140.3, 144.2, 150.4, 158.8, 163.1; HRMS: m/z : calcd for $\text{C}_{37}\text{H}_{36}\text{N}_2\text{O}_7\text{TeNa}$: 773.1477, found: 773.1475 $[\text{M}+\text{Na}]^+$.

Prepare phosphoramidite

- 14** Dry a 25 mL flask containing **S.3a** (500 mg, 0.68 mmol) or **S.4a** (510 mg, 0.68 mmol) and a stir bar with vacuum pump for 1 hour, then purge the flask with argon.
- 15** Inject 2.5 mL methylene chloride and keep flask under ice bath.
- 16** Inject N, N-diisopropylethylamine (0.17 mL, 1.03 mmol, 1.5 eq) and 2-cyanoethyl N,N-diisopropyl-chlorophosphoramidite (195 mg, 0.83 mmol, 1.2 eq). Stir it for 10 min under an ice bath.
- 17** Remove the ice bath and continue stirring it for 45 min at room temperature. Monitor reaction by TLC using 4% MeOH in CH_2Cl_2 .
- At room temperature, the reaction is completed in 45 minutes, generating a mixture of two diastereomers (product R_f = 0.63 and 0.68).
- 18** Quench the reaction by adding 2 mL saturated aqueous NaHCO_3 , and stir for 5 min.

- 19** Extract product with methylene chloride (3x5 mL). Combine the organic layers and wash it with saturated aqueous NaCl.
- 20** Dry the organic layer with anhydrous Na₂SO₄ and filter off drying agent.
- 21** Concentrate CH₂Cl₂ layer to about 2 mL by a rotary evaporator under reduced pressure.
- 22** Prepare 100 mL anhydrous petroleum ether in a 125 mL erlenmeyer flask, add the concentrated CH₂Cl₂ solution into petroleum ether dropwise with vigorous stirring. White precipitate will form during addition.
- 23** Carefully decant the petroleum ether solution, dissolve the precipitate again with 2 mL CH₂Cl₂ then load it onto Al₂O₃ column that is pre-equilibrated by CH₂Cl₂/Hexane (1:1)
- 24** Elute the column with CH₂Cl₂/EtOAc (7:3). Collect fractions, remove the solvents by rotary evaporator. Dry the product under high vacuum, offering product **S.5a** or **S.6a** (570 mg) as a white foamy solid.
- 25** Characterize product by ¹H NMR, ¹³C NMR and HRMS.

S.5a: ¹H NMR (400 MHz, CDCl₃, 25 °C): δ=1.13-1.35 (m, 24H), 2.37 and 2.68 (2x t, *J*=6.4Hz, 4H), 3.38-3.69 (m, 12H), 3.83 (s, 12H), 3.90-4.03 (m, 2H), 4.32 and 4.38 (2x m, 2H), 4.67-4.82 (m, 2H), 4.95 and 5.00 (2x d, *J*=8 Hz, 2H), 6.73 and 6.75 (2x d, *J*=5.8 Hz, 2H), 6.83-6.86 (m, 8H), 7.16-7.37 (m, 26H), 7.76 (br, 2H), 7.81-7.85 (m, 4H); ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ=20.13, 20.20, 20.44, 20.51, 24.50, 24.61, 24.69, 25.23, 33.85, 34.43, 43.36, 43.40, 43.49, 43.52, 55.29, 57.68, 57.87, 59.04, 59.23, 63.58, 63.73, 74.91 and 75.09, 84.97, 87.29, 89.38, 111.69, 113.46, 117.29, 126.99, 127.81, 128.54, 130.21, 130.42, 135.22, 135.40, 135.44, 144.10, 150.37, 158.85, 163.34; ³¹P NMR (160 MHz, CDCl₃, 25 °C): δ=148.6, 149.2; HRMS: *m/z* : calcd for C₄₅H₅₂N₄O₈PTe: 937.2579, found: 937.2578 [M+H]⁺.

S.6a: ¹H NMR (400 MHz, CDCl₃, 25 °C): δ=1.10-1.42 (m, 24H), 2.34 and 2.66 (2x t, *J*=6.4Hz, 4H), 3.35-3.69 (m, 12H), 3.83 (s, 12H), 3.92-4.05 (m, 2H), 4.31 and 4.37 (2x m, 2H), 4.65-4.82 (m, 2H), 6.71 and 6.74 (2x d, *J*=5.8 Hz, 2H), 6.84-6.86 (m, 8H), 7.16-7.37 (m, 26H), 7.77 (br, 2H), 7.80-7.84 (m, 4H); ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ=11.69, 20.12, 20.22, 20.43, 20.53, 24.49, 24.60, 24.67, 25.24, 33.85, 34.43, 43.37, 43.40, 43.49, 43.53, 55.27, 57.67, 57.88, 59.05, 59.25, 63.57, 63.74, 74.92 and 75.09, 84.97, 87.27, 89.38, 111.68, 113.45, 117.28, 126.98, 127.81, 128.54, 130.20, 130.39, 135.24, 135.38, 135.42, 144.11, 150.35, 158.87, 163.36; ³¹P NMR (160 MHz, CDCl₃, 25 °C): δ=148.4, 149.3; HRMS: *m/z* : calcd for C₄₆H₅₃N₄O₈PTeNa: 973.2561, found: 973.2560 [M+Na]⁺.

BASIC PROTOCOL 2: PREPARATION OF THE 2'-METHYLTELLANYL PHOSPHORAMIDITE

In the case of introducing methyltelluride to nucleoside, based on our previous observation, the reducing power of NaBH_4 is not sufficient for dimethyl ditelluride reagent. In order to generate methyltelluride nucleophile efficiently, as shown in Figure 1, we apply LiAlH_4 as reducing agent. The reaction yield is further optimized by lowering temperature to 0°C . Moreover, crown ether (12-crown-4) is applied to chelate lithium cation for the purpose of enhancing methyltelluride's nucleophilicity. The desired product can be identified by tellurium isotope distribution in high resolution mass spectrum or a characteristic peak at -21.2ppm in ^{13}C NMR spectrum. Phosphoramidite synthesis procedures are the same as described in Basic Protocol 1.

Additional Materials (Also see Basic Protocol 1)

Dimethyl ditelluride ($\text{CH}_3\text{TeTeCH}_3$, Organometallics)

1M LiAlH_4 Solution in THF,

12-crown-4 (1,4,7,10-tetraoxacyclododecane)

Introduction of Methyltelluride functionality

- 1 Place a stir bar in an oven-dried 25 mL round bottom flask, dry by vacuum pump, and purge it with argon 2–3 times to deplete oxygen.
- 2 Inject 10 mL anhydrous THF into flask, followed by injection of dimethyl ditelluride ($\text{CH}_3\text{TeTeCH}_3$, 0.2 mL, 1.1 mmol)
- 3 Keep the reaction flask under an ice bath; slowly inject 1 M LiAlH_4 Solution in THF (0.55 mL, 0.55 mmol); the addition may take 5 min for the optimal result.
- 4 After the solution turns slightly yellow (from a dark red), add 12-crown-4 (0.6 mmol, 0.1 mL) and stir the reaction mixture for 20 min under an ice bath.

For uridine derivative

- 5a Dissolve the starting material (**S.1** 0.58 g, 1.0 mmol) in 2 mL THF, add the THF solution into reaction mixture dropwise under an ice bath.

For thymidine derivative

- 5b Dissolve the starting material (**S.2** 0.59 g, 1.0 mmol) in 2 mL THF, add the THF solution into reaction mixture dropwise under an ice bath.
- 6 Monitor reaction by TLC (5% CH_3OH in CH_2Cl_2) (R_f approximately 0.4). The reaction yield will reach maximum in 4–5 hours (approximately 50–60% yield). Before too much byproduct forms, quench the reaction by adding 10 mL saturated sodium chloride aqueous solution.

- 7 Transfer the mixture to a 125 mL separatory funnel, add 30 mL CH₂Cl₂, and shake gently to allow layers separation. Wash the organic layer (bottom) sequentially with 20 mL of H₂O twice and 20 mL of brine twice.
- 8 Collect organic layer in a 100 mL oven-dried beaker. Add 1g anhydrous MgSO₄, wait for 15 min, filter off the drying agent. Evaporate solvent by a rotary evaporator to afford crude product.
- 9 Purify the residue by column chromatography (silica gel column equilibrated by CH₂Cl₂, elute column by gradient elution (CH₃OH in CH₂Cl₂, 0–3% v/v)
- 10 Combine desired fractions and evaporate the solvents under reduced pressure to give compound **S.3b** or **S.4b** as a color foamy product in 40%–47% yield.
- 11 Characterize products by ¹H NMR, ¹³C NMR and HRMS.

S.3b: ¹H NMR (400 MHz, CDCl₃, 25 °C): δ=2.0 (s, 3H), 2.5 (s, 1H), 3.52 (dd, *J*₁=2.8 Hz, *J*₂=7.2Hz, 2H), 3.69 (t, *J*= 4 Hz, 1H), 3.82 (s, 6H), 4.21–4.23 (m, 1H), 4.31–4.36 (m, 1H), 5.40 (d, *J*=8.4 Hz, 1H), 6.33 (d, *J*= 8.4Hz, 1H), 6.86–6.88 (m, 4H), 7.26–7.39 (m, 9H), 7.77 (d, *J*=8.4 Hz, 1H), 8.38 (br, 1H); ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ= –21.2, 33.35, 55.29, 63.37, 73.75, 84.51, 87.29, 89.90, 102.73, 113.35, 127.28, 128.07, 130.08, 135.12, 139.77, 144.20, 150.23, 158.79, 162.64; HRMS: *m/z* : calcd for C₃₁H₃₁N₂O₇Te: 673.1194, found: 673.1204 [M-H][–].

S.4b: ¹H NMR (400 MHz, CDCl₃, 25 °C): δ=1.43 (s, 3H), 1.99 (s, 3H), 3.47 (dd, *J*₁=1.6 Hz, *J*₂= 9.6Hz, 2H), 3.70–3.74 (m, 1H), 3.82 (s, 6H), 4.24–4.25 (m, 1H), 4.36–4.37 (m, 1H), 6.37 (d, *J*=9.2 Hz, 1H), 6.85–6.87 (m, 4H), 7.26–7.40 (m, 9H), 7.60 (s, 1H), 8.03 (br, 1H); ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ= –20.8, 11.71, 33.11, 55.29, 63.86, 74.62, 84.64, 87.19, 89.90, 111.75, 113.32, 127.29, 128.09, 129.14, 135.20, 135.16, 144.21, 150.48, 158.82, 163.41; HRMS: *m/z*: calcd for C₃₂H₃₃N₂O₇Te: 687.1350, found: 687.1354 [M-H]⁺.

Prepare phosphoramidite

For uridine derivative

- 12a Apply step 14 to 24 in Basic Protocol 1 by using **S.3b** (460 mg, 0.68 mmol) to obtain **S.5b** as the product.

For thymidine derivative

- 12b Apply step 14 to 24 in Basic Protocol 1 by using **S.4b** (468 mg, 0.68 mmol) to obtain **S.6b** as the product.
- 13 Characterize product by ¹H NMR, ¹³C NMR and HRMS.
S.5b: ¹H NMR (400 MHz, CDCl₃, 25 °C): δ=0.85–1.38 (m, 24H), 1.98 and 1.99 (2x s, 6H), 2.41 and 2.67 (2x t, *J*=7.6Hz, 4H), 3.42–3.69 (m, 12H), 3.83 (s, 12H), 3.89–4.05 (m, 4H), 4.22 and 4.30 (2x m, 2H), 4.57–4.77 (m, 2H), 6.43 and 6.55 (2x d, *J*=8.6 Hz, 2H), 6.80–6.92 (m, 8H), 7.16–7.45 (m, 18), 7.78 and 7.79 (s, 2H); ¹³C NMR (CDCl₃, 25 °C): δ= –21.32, 19.16, 19.66, 20.88, 24.81, 24.44,

43.30, 43.46, 46.34, 47.53, 51.47 and 51.85, 55.62, 57.03, 58.39, 62.52, 73.36, 73.67, 84.57, 87.35, 88.12, 103.39, 113.42, 117.28, 127.21, 127.94, 128.36, 130.19, 130.25, 135.17, 135.38, 139.53, 144.20, 150.42, 158.80, 163.19. ^{31}P NMR (160 MHz, CDCl_3 , 25 °C): δ =148.5, 149.2; ESI-TOF: m/z calcd for $\text{C}_{40}\text{H}_{48}\text{N}_4\text{O}_8\text{PTe}$: 873.2272, found 873.2264.

S.6b: ^1H NMR (400 MHz, CDCl_3 , 25 °C): δ = 0.89-1.42 (m, 24H), 1.40 (2x s, 6H), 2.10 (2x s, 6H), 2.42 and 2.69 (2x t, J =7.8 Hz, 4H), 3.48 -3.72 (m, 12H), 3.82 (s, 12H), 3.90-4.05 (m, 2H), 4.22 and 4.31 (2x m, 2H), 4.58-4.72 (m, 2H), 6.42 (d, J =8.6 Hz, 2H), 6.79-6.92 (m, 8H), 7.20-7.45 (m, 18H), 7.62 (2x s, 2H), 8.25 (br, 2H); ^{13}C NMR (100 MHz, CDCl_3 , 25 °C): δ = -21.32, 4.58, 4.72, 20.13, 20.16, 20.48, 20.55, 22.52, 24.39, 24.47, 24.59, 24.68, 24.76, 24.83, 43.21, 43.32, 43.34, 43.41, 46.35, 46.42, 47.37, 47.42, 50.36, 55.32, 63.29, 63.33, 74.90, 75.09, 84.99, 87.28, 89.36, 111.70, 113.44, 117.29, 126.99, 127.83, 128.55, 130.20, 130.41, 135.23, 135.41, 135.44, 144.11, 150.38, 158.86, 163.35. ^{31}P NMR (160 MHz, CDCl_3 , 25 °C): δ =148.6, 149.5; ESI-TOF: m/z calcd for $\text{C}_{41}\text{H}_{52}\text{N}_4\text{O}_8\text{PTe}$: 889.2585, found 889.2587.

BASIC PROTOCOL 3: SYNTHESIS, PURIFICATION, AND CHARACTERIZATION OF OLIGONUCLEOTIDES CONTAINING 2'-TELLURIUM FUNCTIONALITY

The Te-U/T phosphoramidite synthesized from Basic Protocol 1 or Basic Protocol 2 is diluted to desired concentration then attached to DNA synthesizer. Apply 2'-PhTe-U/T phosphoramidite from Basic Protocol 1 for acquiring oligonucleotides contains 2'-PhTe functionality to create a bulky 2'-substituent. Or accordingly, use 2'-MeTe-U/T phosphoramidite from Basic Protocol 2 when DNA contains 2'-MeTe groups is needed. All oligonucleotides synthesis is carried out using ABI3400 DNA/RNA synthesizer on 1.0- μmol scale. All native monomers are ultra-mild phosphoramidites with 0.1 M concentration in anhydrous acetonitrile; the Te-modified phosphoramidites are prepared with 0.06 M concentration in anhydrous acetonitrile. The oligonucleotide synthesis works from 3' to 5' end; the first nucleotide is attached to controlled-pored glass (CPG) through a succinyl linkage. The deblocking step uses 3% trichloroacetic acid in methylene chloride to remove trityl group. The coupling step uses 0.3 M (benzylmercapto)-1*H*-tetrazole (5-BMT) solution in acetonitrile as activator. The coupling time for the Te-modified phosphoramidites is 60 seconds. The capping step uses phenoxyacetic anhydride in pyridine/THF to block the failure oligonucleotides. The oxidation step is carried out by treating with 0.02 M iodine in THF/Pyridine/ H_2O . All oligonucleotides are prepared in 5'-DMTr-on form. In order to cleave oligonucleotides from CPG beads and fully deprotect them, after oligonucleotide synthesis, the CPG beads are dried by argon stream and treated with 1 mL 0.05 M K_2CO_3 in methanol solution for 8 hours at room temperature. After centrifugation, the supernant is separated and neutralized with 0.1 M HOAc solution and treated with 5 μL of 1 M borane in THF before HPLC analysis and purification. The 5'-DMTr deprotection of DNAs is performed in a 3% trichloroacetic acid solution for 3 min, followed by neutralization and petroleum ether extraction to remove DMTr-OH.

Materials

2'-Te-modified U and T phosphoramidites (Basic Protocol 1, Basic Protocol 2)

Acetonitrile (CH₃CN), anhydrous (Glen Research)

Ultra mild DNA phosphoramidite & reagent: (Glen research; diluted to 0.1 M in anhydrous acetonitrile attaching to the synthesizer)

Pac-dA-CEPhosphoramidite: 5'-dimethoxytrityl-N-phenoxyacetyl-2'-deoxyadenosine,

3'-[(2-cyanoethyl)-(N, N-diisopropyl)]-phosphoramidite

iPr-Pac-dG-CE phosphoramidite:

5'-Dimethoxytrityl-N-p-isopropyl-phenoxyacetyl-2'-deoxyguanosine,

3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Ac-dC-CE Phosphoramidite: 5'-dimethoxytrityl-N-acetyl-2'-deoxycytidine,3'-[(2-cyanoethyl)- (N,N-diisopropyl)]-phosphoramidite

dT-CE Phosphoramidite: 5'-dimethoxytrityl-2'-deoxythymidine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Deblocking Mix: 3% TCA (trichloroacetic acid) in DCM (dichloromethane)

Activator: 0.25 M 5-benzylthio-1H-tetrazole in acetonitrile

CapA: THF/Pyridine/PacO (Tetrahydrofuran 85%, Pyridine 10%, Phenoxyacetic anhydride 5%)

CapB: 16% MeIm (1-methylimidazole) in THF

Oxidizing Solution: 0.02 M I₂ in THF/Pyridine/H₂O (Iodine 2.5%, Tetrahydrofuran 75.5%,

Pyridine 20%, Water 2%)

0.05 M K₂CO₃ in methanol

2 M triethylammonium acetate (TEAA) buffer, pH 7.0

Acetonitrile (CH₃CN), HPLC grade

30% aqueous trichloroacetic acid (TCA)

Argon

2- mL eppendorf tube

HPLC system (optional) with detector at 260 and/or 369 nm

HPLC column: 21.2 × 250-mm Zorbax RX-C8 (Agilent Technology) or 21 × 250-mm XB-C18 (Welch Materials)

Lyophilizer

Te modified Oligonucleotide synthesis

1. Dissolve the Te-modified phosphoramidite in anhydrous CH_3CN at 0.06 M concentration and attach the solution bottle to synthesizer.
2. Load a DNA synthesis column with 1 μmol (i.e., 25 mg for 40 $\mu\text{mol/g}$ beads) of DNA CPG (controlled pore glass) support corresponding to the first nucleotide at 3'-end of DNA.
3. Perform automated solid-phase oligonucleotide synthesis on DMTr-on mode.
4. When the synthesis is done, remove the column from synthesizer and dry it by an argon stream.
5. Transfer the CPG support into a 2-mL eppendorf tube, and add 1 mL of 0.05 M K_2CO_3 in methanol. The beads are incubated for 6–8 hours at room temperature to cleave oligonucleotides from CPG support. The deprotecting solution also removes all the protecting groups on nucleobases and phosphate backbones, while keeping the 5'-DMTr group intact.
6. Transfer the supernatant to a clean 2-mL eppendorf tube. Add 200 μL water to wash the CPG support and vortex the tube before centrifugation for 2 min. Transfer the supernatant to combine with the deprotected oligonucleotide solution. Repeat this washing procedure twice.
7. Evaporate methanol from the combined supernatant from step 6 in a Speedvac (i.e., 30 min at the room temperature)
8. Purify the sample by RP-HPLC using the following recommended conditions:
 - Column: 21.2 $\times$ 250-mm Zorbax RX-C8
 - Buffer A: 20 mM TEAA buffer, pH 7.0
 - Buffer B: 50% CH_3CN in buffer A
 - Gradient: 0% to 100% buffer B over 20 min
 - Flow rate: 6 mL/min
 - Detection wavelength: 260 nm.
9. Collect the DMTr-On fraction (retention time: approximately 21 min) and lyophilize it. A typical profile of RP-HPLC analysis for DMTr-on Te-DNA is present in Figure 2.
10. Dissolve the residue in 0.2 mL water, add 20 μL of 30% TCA aqueous solution to it, and then vortex it for 5 min
11. Neutralize the solution to pH 7.0 by adding 20 μL of 2.0 M TEAA buffer.
12. Centrifuge the tube at 10,000 rpm (10,600 g) for 2 min and transfer the supernatant to another tube.
13. Purify the sample again by RP-HPLC using the same buffer condition but change gradient to 0% to 70% buffer B over 20 min.

14. Collect the fraction with a retention time around 17 min and lyophilize it to dryness.
15. Fill the tube with argon and store it at -80°C .
16. Analyze the oligonucleotide by RP-HPLC
17. The Te-oligonucleotides are analyzed by MALDI-TOF-MS using 3-hydroxypicolinic acid (3-HPA)/diammonium citrate (9:1) as a matrix.
18. UV-melting temperatures of the Te-DNAs are analyzed with the recommended buffer system [50 mM NaCl, 10 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ (pH 6.5), 0.1 mM EDTA, 10 mM MgCl_2].

COMMENTARY

Background information

From SeNA to TeNA—Selenium derivatization of protein has been widely used for protein X-ray crystallography, where selenium serves as a scattering center for multi-wavelength anomalous diffraction (MAD). (Hendrickson, et al. 1990, Hendrickson 1991, Hendrickson 2000) Inspired by this revolutionary breakthrough in protein structure determination, our research group has pioneered and developed selenium-derivatized nucleic acids (SeNA) for nucleic acid structure determination. (Sheng and Huang 2008, Caton-Williams and Huang 2008) After more than 12 years of systematic study, selenium modification on different positions of DNAs and RNAs have been achieved by us and other research groups, including the 2'-position (Du, et al. 2002, Carrasco, et al. 2004, Hobartner and Micura 2004, Moroder, et al. 2006, Jiang, et al. 2007, Sheng, et al. 2007), 4'-position (Jeong, et al. 2008, Watts, et al. 2008, Alexander, et al. 2010), 5' (Carrasco, et al. 2001, Zhang and Huang 2011), phosphate backbone (Wilds, et al. 2002, Carrasco, et al. 2004, Carrasco, et al. 2006, Brandt, et al. 2006) and the nucleobases (Salon, et al. 2007, Hassan, et al. 2009, Hassan, et al. 2010). Driven by these encouraging results, the tellurium-modified oligonucleotides were first synthesized by our research group for studying the impact of tellurium in nucleic acid X-ray crystallography. Tellurium, a chalcogen element following selenium in group VI in periodic table, has not been well studied in biomolecules due to its synthetic difficulty, caused by tellurium's metallic property. However, tellurium was successfully incorporated into proteins in 1989 through Te-resistant fungi grown in the presence of tellurite on a sulphur-free medium. (Ramadan, et al. 1989) Later, Boles and co-workers reported the expression of telluromethionine (TeMet) dihydrofolate reductase in 1994, (Boles, et al. 1994) and the Te-incorporation technique was further optimized to efficiently introduce TeMet into several proteins. (Besse, et al. 1997) Furthermore, the Te-protein stability under X-ray radiation and the isomorphism of the Te-proteins were confirmed, and more tellurium chemistry has also been developed. (Budisa, et al. 1995, Farina, et al. 2004, Karnbrock, et al. 1996)

Application of TeNA—Tellurium has a larger atomic radius ($1.35 \text{ \AA}$), compared with selenium ($1.17 \text{ \AA}$), sulfur ($1.04 \text{ \AA}$), and oxygen ($0.73 \text{ \AA}$). Te can generate clear isomorphous signals by just using the normal in-house X-ray source. This property makes tellurium modification a potential methodology for structure determination without using a

synchrotron facility. Besides being a scattering center in X-ray crystallography, tellurium has highest electron delocalization ability among O, S, Se and Te, due to its strong metallicity. This metallic property can facilitate electron delocalization in a DNA duplex with relative electron deficiency. A higher visibility and conductivity of Te-DNAs have also been observed under STM imaging. (Sheng, et al. 2011) Moreover, Te-modified DNA can be used for studying DNA damaging, especially for DNA fragmentation and base damaging. (Sheng, et al. 2009) Under an elevated temperature (such as 50 °C), a site-specific cleavage of the 2'-TePh DNA happened at the modification site in the presence of either B₂H₆ or I₂. The cleavage of 2'-TePh-modified DNA was detected by mass spectrometry analysis (Figure 3). This result is consistent with the previous observation of the 2',3' elimination of the 2'-Te-nucleosides. (Sheng, et al. 2008) This site-specific fragmentation provides a good model for DNA damage study. Furthermore, in a 2'-TeMe-modified DNA 9-mer [5'-ATGG(2'-TeMe-dU)GCTC-3'], the base elimination reaction (1',2' elimination) can be observed (Figure 4). It is surprising that the 2'-Te-Ph functionality generates the fragmented product, while the 2'-Te-Me functionality creates the abasic product. These two Te-DNA modification strategies can be useful in investigation of the site-specific DNA cleavage and base damage (Chen and Stubbe 2004, Usui, et al. 2008, Maul, et al. 2008).

It's also note worthy that a small portion of the Te-DNA can be oxidized to telluroxide-DNA during the solid phase synthesis and purification (Figure 5). The redox property of Te-modified DNA can be revealed by HPLC analysis. The tellurium functionality in Te-DNA can be oxidized to telluoxide by treating with I₂, and the telluoxide-DNA can be reduced back to Te-DNA by adding B₂H₆. The oxidizing and reducing process can be conveniently monitored by analytical HPLC (Figure 6). This provides an efficient approach for recovering oxidized Te-DNA during DNA synthesis and purification and also allows further investigation of the redox property of the Te-DNA. The fragmentation, base elimination and redox properties are clearly illustrated in Figure 7.

Thermo-denaturation experiment of the Te-DNA can also be done to test the thermo stability of the Te-DNA duplex (Figure 8). Due to the bulkiness of 2'-PhTe functionality, we expect more perturbation in 2'-PhTe-DNA than that in 2'-MeTe or native DNA. In the self-complementary duplex [5'-G(2'-TeX-dU)GTACAC-3', X=Ph or Me]₂, where double Te-modifications were introduced to this short DNA duplex, the melting temperatures dropped by 15 °C (7.5 °C per modification) in 2'-TePh-DNA duplex, 10 °C (5 °C per modification) in 2'-TeMe-DNA duplex compared with the native duplex (Sheng, et al. 2009). When introducing the Te-functionalities to a non-self-complementary duplex [5'-C(2'-TeX-dU)TCTTGTCCG-3' and 5'-CGGACAAGA-AG-3', X=Me or Ph], it was observed that the melting temperatures of the native, 2'-TeMe and 2'-TePh duplexes were 44.0, 40.7 and 36.8 °C, respectively. Obviously, the perturbation caused by the 2'-Te-functionality leads to a decrease of duplex stability and this duplex destabilization property may be useful in investigating the formation of DNA or RNA loop, bulge, and other secondary structure.

Critical Parameters and Trouble Shooting

Routine techniques for organic lab operation are required, such as extraction, solvent evaporation, TLC analysis and chromatography purification. General protocol for organic

lab safety has to be followed properly. The critical step during the synthesis process is introduction of the tellurium functionality. Keeping a dry and oxygen-free atmosphere is very critical. When performing silica gel column purification for any DMTr-on nucleosides, adding 0.5–1% triethylamine in solvent mixture is highly recommended in order to prevent detritylation during the purification. For making the Te-phosphoramidites, the N,N,N,N-tetraisopropylphosphoramidite may also be used as an alternative reagent in the presence of 5-(benzylmercapto)-1H-tetrazole (5-BMT).

Anticipated Results

2'-Te-modified phosphoramidites can be synthesized with good to moderate yield. The incorporation of the 2'-Te modified nucleotides into oligonucleotide can be achieved by solid-phase synthesis with high yields, even when the concentration of the modified phosphoramidites is adjusted to 0.06 M instead of 0.1 M. After deprotection and purification, a Te-DNA solution can be obtained and stored at –80 °C in argon atmosphere. The quality of the Te-DNA can be analyzed by RP-HPLC and MALDI-Mass (Table 1). The redox property of Te-DNA can be examined by treating with I₂/BH₃ followed by RP-HPLC analysis. The thermo-stability of Te-DNA duplex can be tested by UV-melting experiment.

Time Considerations

With the basic synthetic skills, the synthesis of a 2'-Te-phosphoramidite can be accomplished in one week. The time for oligonucleotides synthesis, purification, and analysis is also approximately one week.

Acknowledgments

This work was supported by the following grants:

- A. Distinguished GCC Cancer Clinicians and Scientists Award
- B. National Institute of General Medical Sciences (NIH, GM095086)
- C. National Science Foundation (NSF, MCB-0824837)

Literature Cited

- Alexander V, Choi WJ, Chun J, Kim HO, Jeon JH, Tosh DK, Lee HW, Chandra G, Choi J, Jeong LS. A New DNA Building Block, 4'-Selenothymidine: Synthesis and Modification to 4'-Seleno-AZT as a Potential Anti-HIV Agent. *Organic Letters*. 2010; 12:2242–2245. [PubMed: 20405850]
- Besse D, Budisa N, Karnbrock W, Minks C, Musiol HJ, Pegoraro S, Siedler F, Weyher E, Moroder L. Chalcogen-analogs of amino acids. Their use in X-ray crystallographic and folding studies of peptides and proteins. *Biological Chemistry*. 1997; 378:211–218. [PubMed: 9165073]
- Boles JO, Lewinski K, Kunkle M, Odom JD, Dunlap B, Lebiada L, Hatada M. Bio-incorporation of telluromethionine into buried residues of dihydrofolate reductase. *Nature structural biology*. 1994; 1:283–284. [PubMed: 7664033]
- Brandt G, Carrasco N, Huang Z. Efficient substrate cleavage catalyzed by hammerhead ribozymes derivatized with selenium for X-ray crystallography. *Biochemistry*. 2006; 45:8972–8977. [PubMed: 16846240]
- Budisa N, Steipe B, Demange P, Eckerskorn C, Kellermann J, Huber R. High-level biosynthetic substitution of methionine in proteins by its analogs 2-aminohexanoic acid, selenomethionine, telluromethionine and ethionine in *Escherichia coli*. *European Journal of Biochemistry*. 1995; 230:788–796. [PubMed: 7607253]

- Carrasco N, Ginsburg D, Du Q, Huang Z. Synthesis of selenium-derivatized nucleosides and oligonucleotides for X-ray crystallography. *Nucleosides Nucleotides & Nucleic Acids*. 2001; 20:1723–1734.
- Carrasco N, Huang Z. Enzymatic synthesis of phosphoroselenoate DNA using thymidine 5'-(alpha-P-seleno)triphosphate and DNA polymerase for X-ray crystallography via MAD. *Journal of the American Chemical Society*. 2004; 126:448–449. [PubMed: 14719925]
- Carrasco N, Buzin Y, Tyson E, Halpert E, Huang Z. Selenium derivatization and crystallization of DNA and RNA oligonucleotides for X-ray crystallography using multiple anomalous dispersion. *Nucleic Acids Research*. 2004; 32:1638–1646. [PubMed: 15007109]
- Carrasco N, Caton-Williams J, Brandt G, Wang SM, Huang Z. Efficient enzymatic synthesis of phosphoroselenoate RNA by using adenosine 5'-(alpha-P-seleno)triphosphate. *Angewandte Chemie (International ed in English)*. 2006; 45:94–97. [PubMed: 16304655]
- Caton-Williams J, Huang Z. Biochemistry of Selenium-Derivatized Naturally Occurring and Unnatural Nucleic Acids. *Chemistry & Biodiversity*. 2008; 5:396–407. [PubMed: 18357549]
- Chen J, Stubbe J. Synthesis and characterization of oligonucleotides containing a 4'-keto abasic site. *Biochemistry*. 2004; 43:5278–5286. [PubMed: 15122893]
- Du Q, Carrasco N, Teplova M, Wilds CJ, Egli M, Huang Z. Internal derivatization of oligonucleotides with selenium for X-ray crystallography using MAD. *Journal of the American Chemical Society*. 2002; 124:24–25. [PubMed: 11772055]
- Farina M, Soares FA, Zeni G, Souza DO, Rocha JB. Additive pro-oxidative effects of methylmercury and ebselen in liver from suckling rat pups. *Toxicology Letters*. 2004; 146:227–235. [PubMed: 14687760]
- Hassan AEA, Sheng J, Jiang JS, Zhang W, Huang Z. Synthesis and Crystallographic Analysis of 5-Se-Thymidine DNAs. *Organic Letters*. 2009; 11:2503–2506. [PubMed: 19469515]
- Hassan AEA, Sheng J, Zhang W, Huang Z. High Fidelity of Base Pairing by 2-Selenothymidine in DNA. *Journal of the American Chemical Society*. 2010; 132:2120–2121. [PubMed: 20108896]
- Hendrickson WA, Horton JR, LeMaster DM. Selenomethionyl proteins produced for analysis by multiwavelength anomalous diffraction (MAD): a vehicle for direct determination of three-dimensional structure. *The EMBO journal*. 1990; 9:1665–1672. [PubMed: 2184035]
- Hendrickson WA. Determination of macromolecular structures from anomalous diffraction of synchrotron radiation. *Science*. 1991; 254:51–58. [PubMed: 1925561]
- Hendrickson WA. Synchrotron crystallography. *Trends in biochemical sciences*. 2000; 25:637–643. [PubMed: 11116192]
- Hobartner C, Micura R. Chemical synthesis of selenium-modified Oligoribonucleotides and their enzymatic ligation leading to an U6SnRNA stem-loop segment. *Journal of the American Chemical Society*. 2004; 126:1141–1149. [PubMed: 14746483]
- Jeong LS, Tosh DK, Kim HO, Wang T, Hou X, Yun HS, Kwon Y, Lee SK, Choi J, Zhao LX. First synthesis of 4'-selenonucleosides showing unusual Southern conformation. *Organic Letters*. 2008; 10:209–212. [PubMed: 18088134]
- Jiang JS, Sheng J, Carrasco N, Huang Z. Selenium derivatization of nucleic acids for crystallography. *Nucleic Acids Research*. 2007; 35:477–485. [PubMed: 17169989]
- Karnbrock W, Weyher E, Budisa N, Huber R, Moroder L. A New Efficient Synthesis of Acetyltelluro- and Acetylselenomethionine and Their Use in the Biosynthesis of Heavy-Atom Protein Analogs. *Journal of the American Chemical Society*. 1996; 118:913–914.
- Maul MJ, Barends TR, Glas AF, Cryle MJ, Domratcheva T, Schneider S, Schlichting I, Carell T. Crystal structure and mechanism of a DNA (6-4) photolyase. *Angewandte Chemie (International ed in English)*. 2008; 47:10076–10080. [PubMed: 18956392]
- Moroder H, Kreutz C, Lang K, Serganov A, Micura R. Synthesis, Oxidation Behavior, Crystallization and Structure of 2'-Methylseleno Guanosine Containing RNAs. *Journal of the American Chemical Society*. 2006; 128:9909–9918. [PubMed: 16866550]
- Ramadan SE, Razak AA, Ragab AM, el-Meigy M. Incorporation of tellurium into amino acids and proteins in a tellurium-tolerant fungi. *Biological Trace Element Research*. 1989; 20:225–232. [PubMed: 2484755]

- Salon J, Sheng J, Jiang JS, Chen GX, Caton-Williams J, Huang Z. Oxygen replacement with selenium at the thymidine 4-position for the Se base pairing and crystal structure studies. *Journal of the American Chemical Society*. 2007; 129:4862–4863. [PubMed: 17388591]
- Sheng J, Jiang JS, Salon J, Huang Z. Synthesis of a 2'-Se-thymidine phosphoramidite and its incorporation into oligonucleotides for crystal structure study. *Organic Letters*. 2007; 9:749–752. [PubMed: 17263541]
- Sheng J, Huang Z. Selenium derivatization of nucleic acids for phase and structure determination in nucleic acid x-ray crystallography. *International Journal of Molecular Sciences*. 2008; 9:258–271. [PubMed: 19325748]
- Sheng J, Hassan AEA, Huang Z. New telluride-mediated elimination for novel synthesis of 2'-3'-dideoxy-2',3'-dideoxynucleosides. *Journal of Organic Chemistry*. 2008; 73:3725–3729. [PubMed: 18410145]
- Sheng J, Hassan AEA, Huang Z. Synthesis of the First Tellurium-Derivatized Oligonucleotides for Structural and Functional Studies. *Chemistry-a European Journal*. 2009; 15:10210–10216.
- Sheng J, Hassan AE, Zhang W, Zhou J, Xu B, Soares AS, Huang Z. Synthesis, structure and imaging of oligodeoxyribonucleotides with tellurium-nucleobase derivatization. *Nucleic Acids Research*. 2011; 39:3962–3971. [PubMed: 21245037]
- Usui K, Aso M, Fukuda M, Suemune H. Photochemical generation of oligodeoxynucleotide containing a C4'-oxidized abasic site and its efficient amine modification: dependence on structure and microenvironment. *Journal of Organic Chemistry*. 2008; 73:241–248. [PubMed: 18062702]
- Watts JK, Johnston BD, Jayakanthan K, Wahba AS, Pinto BM, Damha MJ. Synthesis and biophysical characterization of oligonucleotides containing a 4'-selenonucleotide. *Journal of the American Chemical Society*. 2008; 130:8578–8579. [PubMed: 18543920]
- Wilds CJ, Pattanayek R, Pan C, Wawrzak Z, Egli M. Selenium-assisted nucleic acid crystallography: use of phosphoroselenoates for MAD phasing of a DNA structure. *Journal of the American Chemical Society*. 2002; 124:14910–14916. [PubMed: 12475332]
- Zhang W, Huang Z. Synthesis of the 5'-Se-Thymidine Phosphoramidite and Convenient Labeling of DNA Oligonucleotide. *Organic Letters*. 2011; 13:2000–2003. [PubMed: 21434607]

Figure 1.

General scheme of synthesizing 2'-TePh-T, 2'-TePh-U, 2'-TeMe-T and 2'-TeMe-U phosphoramidites from 5'-O-DMTr-2',2'-anhydro-T and 5'-O-DMTr-2',2'-anhydro-U (Basic Protocol 1 and Basic Protocol 2).

Figure 2.

HPLC analysis of the Te-DNA: 5'-DMTr-G(2'-TePh-dU)GTACAC-3'. This analysis was performed on a Zorbax SB-C18 column (4.6 x 250 mm) with a linear gradient from buffer A (20 mM TEAAc: triethylammonium acetate, pH 7.1) to 100% buffer B (50% acetonitrile in 20 mM TEAAc) in 20 min.; the retention time of the full-length Te-DNA is 20.7 min.

Figure 3.

Cleavage of the 2'-TePh-modified DNA 8-mer [5'-G(2'-TePh-dU)GT ACAC-3']. (A) before heating, the molecular formula: $C_{83}H_{101}N_{30}O_{46}P_7Te$, $[M-H^+]^-$: 2599.3; (B) after fragmentation with either I_2 or B_2H_6 at 50 °C, the fragmented 6-mer (5'-p-GTACAC-3'); the 6-mer molecular formula: $C_{58}H_{74}N_{23}O_{36}P_6$, $[M-H^+]^-$: 1856.2 calculated; its observed molecular mass peak: 1856.3.

Figure 4. MALDI-TOF analysis of the reductive debase of the 2'-TeMe modified sequence 5'-ATGG(2'-TeMe-U)GCTC-3'. Full length product formula: $C_{88}H_{112}N_{32}O_{54}P_8Te$, $[M-H^+]^-$: 2857.4, observed: 2857.2. Debased product formula: $C_{83}H_{108}N_{30}O_{52}P_8$, $[M-H^+]^-$: 2603.5, observed 2603.4.

Figure 5. MALDI-TOF MS analysis of the Te-DNA [5'-G(2'-TePh-dU)GTACAC-3']. The peak with a mass of M+16 indicates formation of the telluoxide.

Figure 6.

Redox property of 2'-TePh-DNA 8-mer [5'-G(2'-TePh-dU)GTACAC] with the I_2 and B_2H_6 treatments. 1): the non-oxidized Te-DNA; 2): the Te-DNA after I_2 treatment; 3): the co-injection of the non-oxidized and oxidized Te-DNAs; 4): the oxidized Te-DNA after B_2H_6 treatment.

Figure 7.

This scheme shows the fragmentation, base elimination and redox properties of Te-DNA

Figure 8.

Normalized thermo-denaturation curves of the DNA duplexes [5'-C(2'-TeX-dU)TCTTGTC CG-3' & 3'-CGGACAAGAAG-5', X=Me or Ph]. The samples (1 mM duplex) were heated from 6 to 75 °C with a rate of 0.5 °C/min. The red, blue and green curves represent the native, 2'-TeMe and 2'-TePh duplex, respectively. The average T_m (four measurements): 44.0 ± 0.1 °C, 40.7 ± 0.1 °C and 36.8 ± 0.2 °C, respectively.

Table 1

MALDI-TOF analysis of 2'-Te modified oligonucleotides.

DNA sequences	Formula	MS [M-H] ⁺ (Calcd.)
G(2'-TeMe-dU)GTACAC	C ₇₈ H ₉₉ N ₃₀ O ₄₆ P ₇ Te	2536.1 (2536.2)
ATGG(2'-TeMe-dU)GCTC	C ₈₈ H ₁₁₂ N ₃₂ O ₅₄ P ₈ Te	2856.3 (2856.4)
G(2'-TePh-dU)GTACAC	C ₈₃ H ₁₀₁ N ₃₀ O ₄₆ P ₇ Te	2599.4 (2599.3)
ATGG(2'-TePh-dU)GCTC	C ₉₃ H ₁₁₄ N ₃₂ O ₅₄ P ₈ Te	2919.0 (2918.5)
GCG(2'-TePh-dU)ATACGC	C ₁₀₂ H ₁₂₅ N ₃₈ O ₅₈ P ₉ Te	3218.0 (3216.7)
G(2'-TePh-dU)GTACAC	C ₈₄ H ₁₀₃ N ₃₀ O ₄₆ P ₇ Te	2611.8 (2612.3)
ATGG(2'-TePh-dU)GCTC	C ₉₄ H ₁₁₆ N ₃₂ O ₅₄ P ₈ Te	2933.3 (2932.5)
CT(2'-TePh-dU)CTTGTCCG	C ₁₁₁ H ₁₄₀ N ₃₂ O ₆₉ P ₁₀ Te	3464.5 (3465.4)
CT(2'-TeMe-dU)CTTGTCCG	C ₁₀₆ H ₁₃₈ N ₃₂ O ₆₉ P ₁₀ Te	3400.5 (3401.8)