Electronic supplementary information

Fine-tuning furan toxicity: fast and quantitative DNA interchain cross-link formation upon selective oxidation of a furan containing oligonucleotide

Sami Halila, Trinidad Velasco, Pierre De Clercq and Annemieke Madder*
Laboratory for Organic and Biomimetic Chemistry, Department of Organic Chemistry, Krijgslaan 281, S4, 9000 Gent, Belgium. Fax: +32-9-2644498; Tel: +32-9-2644472; E-mail: annemieke.madder@Ugent.be

Preparation of phosphoramidites 1a and 1b

General methods

Uridine and 4,4’-dimethoxytrityl chloride (DMTCl) were purchased from Acros and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, furfurylamine, furylacrylic acid from Aldrich. Other commercially available reagents and all other solvents were purchased from standard chemical suppliers and used without purification.

NMR spectra were recorded on a Bruker AM 300 spectrometer at 300 MHz for 1H-NMR, 75 MHz for 13C-NMR or 121 MHz for 31P-NMR. External standards were tetramethylsilane and 85% H3PO4 for 1H or 13C and 31P NMR, respectively. Mass spectrometry analyses were performed on a quadrupole ion trap LC mass spectrometer (Thermofinnigan, San Jose, Ca, USA) equipped with electro spray ionisation (positive mode). TLC was performed on glass plates precoated with silica gel (60F254, 0.25 mm).

2-Furylpropionic acid

2-Furylacrylic acid (5g, 36 mmol) was added to a slurry of 5% Pd/C (0.5 g) in MeOH (50 ml) under a N2 atmosphere. The nitrogen atmosphere was exchanged for hydrogen and the mixture stirred vigorously for 5 hours. The reaction was monitored by tlc (CH2Cl2:EtOAc (1:1)) using ceric sulphate dip to develop the plates. The reaction mixture was filtered through a Celite bed to remove the catalyst and the MeOH was removed under reduced pressure. The residue was recrystallised from water and the crystalline product further purified by sublimation under reduced pressure (50°C / 1 mmHg) to afford 2-furylpropionic acid as a colourless solid (4 g, 80%). This compound should be stored in the dark at –18°C to prevent decomposition. See Tetrahedron, 55, (1999), 11365-11384 for analytical data.

Pentafluorophenyl-3-(2-furyl) propanoate (5)

Solid DCC (3.16 g, 15.32 mmol) was added in portions over a period of 20 min to a solution of 2-furylpropionic acid (1.95 g, 13.93 mmol) with pentafluorophenol (2.82 g, 15.32 mmol) and anhydrous Et3N in anhydrous THF under argon atmosphere at 0°C. 15 min after the addition, the ice bath was removed and the reaction was allowed to stir at rt overnight. The reaction mixture was concentrated and diluted with CH2Cl2 (10 ml), then filtered on silica gel with elution by CH2Cl2 to afford after evaporation 5 (3.45 g, 81%) as a pale yellow solid. RF 0.75 (iOct-EtOAc, 6: 4 v/v). ESMS [M + Na]+ 329.2. 1H-NMR (300 MHz, CDCl3): 7.34 (dd, 1H, J = 0.7 Hz, J = 1.8 Hz); 6.30 (dd, 1H, J = 1.8 Hz, J = 3.2 Hz); 6.09 (dd, 1H, J = 0.7 Hz, J = 3.2 Hz) 3.10 (m, 2H, CH2); 3.01 (m, 2H, CH2). 13C-NMR (75 MHz, CDCl3): 168.5 (C=O); 152.7, 141.6, 110.3, 105.9, 32.0, 23.3 (2xCH2). Anal. Calcd for C13H7F5O3: C, 51.00; H, 2.30. Found: C, 50.76; H, 2.44.
Supplementary Material (ESI) for Chemical Communications

Scheme S2: Preparation of phosphoramidite 1a. (i) 5, DBU, DMF, 0°C to r.t., 2.5h., 75%; (ii) DMTCl, Pyr., DMAP, r.t., 18h, 92%; (iii) (iPr2N)2POCH2CH2CN,iPr2NEt, DCM, r.t., 1h, 92%.

2'-deoxy-2'-(2-furyl-2-ethoxycarbonylamino)uridine (3a)
Activated ester 5 (627 mg, 2.05 mmol) was added to a solution of 2a (610 mg, 1.18 mmol) and dry DBU (334 µl, 2.23 mmol) in dry DMF (10 ml) under argon atmosphere at 0°C. The reaction mixture was allowed to stir at rt for 2.5 h. When all starting material was consumed (checked by TLC), the solvent was evaporated to give orange syrup that was taken in DCM and purified by liquid chromatography using DCM/MeOH 10%. ESMS [M + H]+ 366.1; [M + Na]+ 388.2. 1H-NMR (300 MHz, CD3OD): 7.98 (d, 1H, J = 8.1 Hz); 7.28 (dd, 1H, J = 0.7 Hz, J = 1.7 Hz); 6.27 (dd, 1H, J = 1.7 Hz, J = 3.2 Hz); 6.06 (d, 1 H, J = 8.7 Hz); 6.01 (dd, 1H, J = 0.7 Hz, J = 3.2 Hz); 5.74 (d, 1H, J = 8.1 Hz); 4.64 (dd, 1H, J = 5.7 Hz, J = 8.7 Hz); 4.53 (bs, 1H, NH); 4.24 (dd, 1H, J = 4.4 Hz, J = 5.7 Hz); 4.08 (m, 1H); 3.79 (dd, 1H, J = 12.1 Hz); 3.73 (dd, 1H, J = 3 Hz, J = 12.1 Hz); 2.89 (m, 2H); 2.57 (m, 2H). 13C-NMR (75 MHz, CD3OD): 175.3 (C=O), 166.0, 155.7, 152.7, 142.6, 142.4, 111.2, 106.2, 103.2, 88.6, 88.0, 72.2, 63.2, 59.8, 35.1, 24.8 (2xCH2). Anal. Calcd for C16H19N3O7: C, 52.60; H, 5.24; N, 11.50. Found: C, 52.06; H, 5.45; N, 11.92.

2'-deoxy-2'-(2-furyl-2-ethoxycarbonylamino)-5'-O-(4,4'-Dimethoxytrityl)uridine (4a)
Compound 3a (0.300 g, 0.82 mmol) was dried for 2 hours under vacuum then dissolved in dry pyridine (7 ml), cooled in an ice bath, and DMTCl (0.33 g, 0.98 mmol) was added in one portion with DMAP (0.01 g, 0.08 mmol). The reaction was monitored by TLC and further portions of DMTCl (56 mg, 0.16 mmol) were added every 4 h until the starting nucleoside disappears. After completion of the reaction, the excess of DMTCl was quenched with MeOH (1 ml), and after 10 min the mixture was diluted with EtOAc (40 ml), washed with water (25 ml), sat. NaHCO3 (25 ml), and water (25 ml), then dried (Na2SO4), evaporated, coevaporated with toluene (2x10 cm³) and the residue was chromatographed on silica gel column (DCM then DCM/MeOH, 9.5 : 0.5 v/v + 1% Et3N) to afford 4a (0.5 g, 92%) as an amorphous solid. ES-MS [M + Na]+ 690.2. NMR (300 MHz, CDCl3): 7.63 (d, 1H, J = 8.1 Hz); 7.40 (m, 1H); 7.30-7.20 (m, 9H); 6.83 (m, 4H); 6.71 (d, 1H, J = 7.5 Hz); 6.23 (m, 1H); 6.08 (d, 1H, J = 8.4 Hz); 5.98 (m, 1H); 5.44 (d, 1H, J = 8.1 Hz); 4.63 (dd, 1H, J = 13.4 Hz, J = 8.4 Hz); 4.43 (m, 1H); 4.17 (m, 1H); 3.75 (s, 6H, 2x OCH3); 3.39 (m, 2H); 2.97 (m, 2H); 2.56 (m, 2H). 13C-NMR (75 MHz, CDCl3): 172.7 (NHC=O), 163.1, 158.0, 154.6, 151.8, 144.3, 141.6, 140.3, 135.4, 135.8, 130.1, 128.7, 128.1, 127.6, 113.3, 110.2, 105.7, 103.1, 87.2, 86.0, 85.4, 71.7, 63.8, 56.8, 55.3 (OCH3), 36.7, 24.0 (2xCH2). Anal. Calcd for C37H37N3O9: C, 66.56; H, 5.59; N, 6.29. Found: C, 66.66; H, 5.69; N, 6.62.
2'-deoxy-2'-(2-furyl-2-ethoxycarbonylamino)-5'-(4,4'-Dimethoxytrityl)uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (1a).

A solution of 4a (0.12 g, 0.18 mmol) in dry DCM was treated with iPr₂NEt (0.12 ml, 0.72 mmol) under Argon atmosphere then cooled (ice bath). The 2-cyanoethyl-N,N'-diisopropylchlorophosphoramidite (0.08ml, 0.72 mmol) was added dropwise by syringe over a period of 5 min with stirring. The mixture was stirred for 10 min while cooling in an ice bath, then the bath was removed, and stirring was continued at r.t. up to complete conversion of the starting material (1h). 0.1 ml of MeOH was added. The mixture was diluted 10 min later with 25 ml of DCM, washed with sat. NaHCO₃ (15 ml) and sat. NaCl (15 ml), dried over Na₂SO₄, and evaporated under vacuum. The residue was dissolved in DCM : TEA (90 : 10) to a silica column equilibrated with the same solvent. The column was eluted with this eluent and the corresponding fractions were evaporated under reduced pressure to afford 1a as a white powder (147 mg, 94%). Rf 0.58 and 0.64 (DCM-Acetone-TEA, 7 : 2 : 1 v/v/v). ESMS [M + Na] + 890.2. The broadband decoupled ³¹P-NMR (CDCl₃) gives two signals at 151.19 and 149.54 ppm with about a respectively 3:2 intensity ratio. The ¹H-NMR spectrum is complex and shows a mixture of two co-existing diastereoisomers.

Scheme S3: Preparation of phosphoramidite 1b. (i) H₂, Pd/C, MeOH.HCl, 5h, 40°C; (ii) 5, DBU, DMF, r.t., 2h, 75%; (iii) DMTCl, Pyr., DMAP, r.t., 18h; (iv) (iPr₂N)₂POCH₂CH₂CN, iPr₂NEt, DCM, r.t., 1h, 97%.

1-{2'-Amino-2'-deoxy-β-D-arabinofuranosyl} uracil hydrochloride (2b.HCl)

A suspension of 1-{2'-azido-2'-deoxy-β-D-arabinofuranosyl} uracil (0.766 g, 2.85 mmol) and 5% Pd/C (0.076 g) in partially saturated MeOH.HCl (40 ml) was hydrogenated under H₂ atm with a balloon at 40°C for 5 hrs. The solution was filtered through Celite, evaporated and dried in vacuo to give a white solid (0.8 g, 100%). The solid was pure enough to be used for the next reaction. Rf 0.15 (DCM-MeOH, 8 : 2 v/v). ESMS: [M + H] + 244.1, [M+Na] + 266.1. ¹H-NMR: see. Helv. Chim. Acta 1996, 79, 1067-1075.

1-{2-Deoxy-2-(2-furyl-2-ethoxycarbonylamino)-β-D-arabinofuranosyl} uracil (3b)

A solution of 2b.HCl (0.81 g, 2.9 mmol) in dry DMF (16 ml) was treated with DBU (0.524 ml, 3.5 mmol) and stirred for 20 min. Then pentafluorophenyl-3-(2-furyl) propanoate (5) (0.97g, 3.2 mmol) was added. The solution was kept for 2 hrs, at r.t. The solution was filtered through Celite, evaporated and dried in vacuo to give 3b as a pale yellow foam (0.98 g, 75%). Rf 0.23 (DCM-MeOH, 9 : 1 v/v). ESMS [M + Na] + 366.1. ¹H-NMR (300 MHz, CD₃OD): 7.85 (d, 1H, J = 8.1 Hz); 7.33 (d, 1H, J = 2Hz); 6.28 (dd, 1H, J = 2 Hz, J = 3.1 Hz); 6.20 (d, 1 H, J = 5.7 Hz); 6.03 (d, 1H, J = 3.1 Hz); 5.65 (d, 1H, J = 8.1 Hz); 4.53 (t, 1H, J = 5.7 Hz); 4.26 (t, J = 5.7 Hz); 3.80-3.95 (m, 3H); 2.85 (m, 2H); 2.46 (m, 2H). 

13C-NMR (75 MHz, CD₃OD): 174.6 (C=O), 166.2, 155.5, 151.8, 143.3, 142.4, 111.3, 106.2, 101.5, 86.1, 85.6, 74.2, 61.5, 59.5, 35.2, 24.7 (2xCH₂).
1-{2-Deoxy-5-((dimethoxytrityl)-2-(2-furyl-2-ethoxycarbonylamino) -β-D-arabinofuranosyl} uracil (4b)

Compound 3b (0.735 g, 2.01 mmol) was dried for 2 hours under vacuum and dissolved in dry pyridine (14 ml), cooled in an ice bath, and DMTC1 (0.82 g, 2.41 mmol) was added in one portion with DMAP (24.6 mg, 0.2 mmol). The reaction was monitored by TLC and further portions of DMTC1 (0.18 g, 0.5 mmol) were added every 4 h until the starting nucleoside disappeared. After completion of the reaction, the excess of DMTC1 was quenched with MeOH (1ml), and after 10 min the mixture was diluted with EtOAc (70 ml), washed with water (50 ml), sat. NaHCO₃ (50 ml), and water (50 ml), then dried (Na₂SO₄), evaporated, coevaporated with toluene (2x25 ml) and the residue was chromatographed on silica gel (DCM then DCM-MeOH, 9.5 : 0.5 v/v + 1% Et₃N) to afford 4b (1.3 g, 96%) as an amorphous solid. Rf 0.32 (DCM-MeOH, 9.5 : 0.5 v/v). ESMS [M + Na]⁺ 690.3. NMR (300 MHz, CDCl₃): 7.88 (d, 1H, J = 8.1 Hz); 7.4-7.2 (m, 12H); 6.66 (m, 2H); 6.18 (dd, 1H, J = 2 Hz, J = 3.0 Hz); 6.13 (d, 1 H, J = 5.7 Hz); 5.88 (dd, 1H, J = 0.6 Hz, J = 3.0 Hz); 5.34 (d, 1H, J = 8.1 Hz); 4.53-4.45 (m, 2H); 3.97 (m, 1H); 3.75 (s, 6H, 2x OCH₃); 3.57 (m, 2H); 2.82 (m, 2H); 2.31 (m, 2H). 13C-NMR (75 MHz, CDCl₃): 174.1 (NHC=O), 163.4, 158.8, 153.9, 151.0, 144.1, 141.3, 141.2, 135.1, 130.2, 128.3, 128.1, 127.2, 113.4, 110.2, 105.4, 101.9, 87.4, 85.1, 72.8, 61.7, 60.3, 55.2 (OCH₃),34.1, 23.6 (2xCH₂). Anal. Calcd for C₃₇H₃₇N₃O₉: C, 66.56; H, 5.59; N, 6.29. Found: C, 66.66; H, 5.80; N, 6.55.

1-{2-Deoxy-5-O-(4,4′-dimethoxytrityl)-2-(2-furyl-2-ethoxycarbonylamino) -β-D-arabinofuranosyl} uracil 3’-{(2-Cyanoethyl N,N-diisoproplyphosphoramidite) (1b)

A solution of 4b (0.32 g, 0.48 mmol) in dry DCM was treated with iPr₂NEt ((0.32 ml, 1.92 mmol) under Argon atmosphere and cooled (ice bath). The 2-cyanoethyl-N,N′-diisopropylchlorophosphoramidite (0.21 ml, 0.96 mmol) was added dropwise via syringe over a period of 5 min with stirring. The mixture was stirred for 10 min on an ice bath, then the bath was removed and stirring was continued at r.t. up to complete conversion of the starting material (1h). 0.2 ml of MeOH was added. The mixture was diluted 10 min later with 25 ml of DCM, washed with sat. NaHCO₃ (15 ml) and sat. NaCl (15 ml), dried over Na₂SO₄, and evaporated under vacuum. The residue was dissolved in DCM : TEA (90 : 10) to a silica column equilibrated with the same solvent. The column was eluted with this eluent and finally the desired product was eluted with DCM : EtOAc : TEA (70 : 20 : 10 v/v/v). The corresponding fractions were evaporated under reduced pressure to afford 1b as a white powder (0.4 g, 97%, 9 : 1 mixture of diastereoisomers). Rf 0.64 (DCM-MeOH-TEA, 45 : 45 : 10 v/v/v). ESMS [M + Na]⁺ 890.1. The broadband decoupled 31P-NMR (CDCl₃) gives two signals at 149.64 and 149.53 ppm with about a 1:5 intensity ratio. The 1H-NMR spectrum is complex and shows a mixture of two co-existing diastereoisomers.

Oligonucleotides

The oligonucleotides (11-mer ODNs) were synthesized by solid-phase β-cyanoethyl phosphoramidite chemistry on an ABI 392 DNA synthesizer instrument. The modified uridine phosphoramidite were prepared as described and used in automated synthesis as a 0.1M solution in acetonitrile and the coupling time were increased to 6 min. Synthesized oligonucleotides were deprotected with 32% NH₄OH for 12-15 h at 55°C. The ammonia solutions on the sample holder and interfaced to an IBM-compatible PC computer. All solutions (containing a 1:1 strand ratio of oligonucleotides, 4 µM total concentration) were prepared with a buffer containing 10 mM NaCl, adjusted to pH 7.0. Annealing of the oligonucleotides was achieved by heating at 90°C for 1 min followed by slow cooling to 25°C. Melting was performed from 20° to 65°C, with a gradient of 0.5°C/min. All experiments were done at least in duplicate, and Tₘ values were calculated by a two-state model using the hyperchromicity method from the CARY UV-Win Software; errors were in the range of ± 0.3°C. Mass spectrometry analyses were performed on a quadrupole ion trap LC mass spectrometer (Thermofinnigan, San Jose, Ca, USA) equipped with electrospray ionisation (negative mode).

Preparation of cross-linked oligonucleotides:
The purified oligonucleotides (20 µM) were treated with 1 equivalent of freshly prepared NBS solution in phosphate buffer (pH 7.0, 10 mM) plus 10 mM NaCl at 25°C. Each 15 min, 1 equivalent was added to the reaction mixture up to complete conversion. 3-4 equivalent were necessary for cross-linking of 9 and 10 and 9 and 11. Cross-linked oligonucleotides (XL-ON) were purified by reversed-phase HPLC and the cross-linking was confirmed by mass spectrometry.
Oxidation experiments on nucleoside 3a

**HPLC analysis:** The analysis and purification of oligonucleotides were performed on an Agilent 1100 series HPLC system (HPCORE ChemStation software, quaternary pump module G1311A) with a diode array UV detector (module G1315B) monitoring at 254 or 280 nm using Phenomenex Luna C18(2) columns (250 mm x 4.6 mm i.d., 1 mL/min for analysis and 250 mm x 10 mm i.d., 4 mL/min for purification) with 0.1 M aqueous triethylammonium acetate buffer, pH 7.0 containing 5% acetonitrile, and acetonitrile.

Solvent A: acetonitrile
Solvent B: TEAA 100 mM, pH 7.0, 5% acetonitrile

**Scheme S4.** Reaction products upon oxidation of nucleoside 3a.

**Figure S1.** HPLC profiles of reaction mixtures for oxidation of 3a.

*HPLC conditions:* 0% acetonitrile (2 min), 0-100% acetonitrile (15 min).

a) 3a before addition of NBS, t = t₀, b) addition of 1 eq. of NBS, t = t₀ + 15 min, c) idem coinjected with 3a, t = t₀ + 15 min, d) addition of 1 eq. of NBS followed by 2 eq. of benzylamine, t = t₀ + 30 min
Figure S2. ESI-MS spectra of oxidation products of 3a. 

a) peak at retention time 5.366 min in chromatogram S1b.
b) peak at retention time 11.432 min in chromatogram S1d.
HPLC profiles and MS spectra for oligonucleotide 8

5’-d(GCC TGT CAG-U-G)-3’

Figure S3. HPLC profile of purified oligonucleotide 8.
HPLC conditions: 0% acetonitrile (2 min), 0-30% acetonitrile (15 min).

Figure S4. ESI-MS spectrum of purified oligonucleotide 8.
C_{106}H_{134}N_{40}O_{67}P_{10}, Exact mass: 3348.57, Mol. Wt.: 3350.17
HPLC profiles and MS spectra for oligonucleotide 9

5'-d(CA CTG ACA GGC)-3'

**Figure S5.** HPLC profile of purified oligonucleotide 9.
*HPLC conditions:* 0% acetonitrile (2 min), 0-100 % acetonitrile (15 min).

**Figure S6.** ESI-MS spectrum of purified oligonucleotide 9.
*C_{106}H_{134}N_{44}O_{62}P_{10}*, Exact mass: 3324.61, Mol. Wt.: 3326.20
HPLC profiles and MS spectra for oligonucleotide 10

5'-d(GCC TGT CAG-1a-G)-3'

**Figure S7.** HPLC profile of purified oligonucleotide 10.
*HPLC conditions: 0% acetonitrile (2 min), 0-30% acetonitrile (15 min).*

**Figure S8.** ESI-MS spectrum of purified oligonucleotide 10.
*C_{113}H_{141}N_{41}O_{68}P_{10},* Exact mass: 3469.62, Mol. Wt.: 3471.30
HPLC profiles and MS spectra for oligonucleotide 11

5’-d(GCC TGT CAG-1b-G)-3’

**Figure S9.** HPLC profile of purified oligonucleotide 11.  
*HPLC conditions:* 0% acetonitrile (2 min), 0-30% acetonitrile (15 min).

**Figure S10.** ESI-MS spectrum of desalted oligonucleotide 11.  
*C_{113}H_{141}N_{41}O_{68}P_{10},* Exact mass: 3469.62, Mol. Wt.: 3471.30
HPLC profiles and MS spectra for cross-linking of oligonucleotide 9 and 10

5’-d(GCC TGT CAG-1a-G)-3’ (10)  

3’-d(CGG ACA GTC-A-C)-5’ (9)

Figure S11. HPLC profiles of cross-linking reaction mixtures.

HPLC conditions: 0% acetonitrile (2 min), 0-15 % acetonitrile (15 min).

a) before addition of NBS, t = t₀, b) addition of 2 eq. of NBS, t = t₀ + 30 min,
c) addition of 4x1eq. of NBS, t = t₀ + 60 min
Figure S12. ESI-MS spectrum of cross-linked 9-10 XL (FigS11c, peak at 14.696 min, purified). C_{219}H_{273}N_{85}O_{130}P_{20}, Exact mass: 6792.21, Mol. Wt.: 6795.48

HPLC profiles and MS spectra for cross-linking of oligonucleotide 9 and 11

5’-d(GCC TGT CAG-1b-G)-3’ (11)

Figure S13. HPLC profiles of cross-linking reaction mixtures.

HPLC conditions: 0% acetonitrile (2 min), 0-15% acetonitrile (15 min).

a) before addition of NBS, t = t_0, b) addition of 1 eq. of NBS, t = t_0 + 15 min, c) addition of 4x1 eq. of NBS, t = t_0 + 60 min
Figure S14. ESI-MS spectrum of cross-linked 9-11 XL (FigS13c, peak at 16.695 min, purified). C$_{219}$H$_{273}$N$_{66}$O$_{130}$P$_{20}$, Exact mass: 6792.21, Mol. Wt.: 6795.48