Vibrational Stark Effect Probes for Nucleic Acids

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Supporting Information

EXPERIMENTAL

Synthetic Chemistry

General

All reagents were ACS reagent quality, purchased from Aldrich or Acros, and used without further purification unless otherwise noted. Nucleosides were purchased from ChemGenes or Chem-Impex International. The following compounds were prepared according to literature procedures: 5-cyano-2'-deoxyuridine,[1] 3',5'-Bis-*O*-(*tert*-butyldiphenylsilyl)-5-bromomethyl-2'-deoxyuridine,[2] 5'-*O*-(4,4'-dimethoxytrityl)-2'-azido-2'-deoxyuridine (**6**),[3] N^3-Benzyol-2'-*O*-(2-cyanoethyl)-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1.3-yl)-uridine (**8**),[4] 2'-*O*-(2-cyanoethyl)-3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1.3-yl)-adenosine (**9**).[4]

All reactions were stirred with a magnetic stir bar and conducted under a dry nitrogen or argon atmosphere. Analytical thin layer chromatography (TLC) was performed on 0.2 mm silica plastic coated sheets (Selecto Scientific) with F_{254} indicator. Preparative TLC was performed on 1.0 mm silica coated glass plates or 0.3-1.7 mm silica Tapered plates with F_{254} indicator. Flash column chromatography was performed on 230-400 mesh silica gel.

NMR spectra were obtained at the following frequencies: ^{1}H (500 MHz) and ^{13}C (125 MHz) unless noted otherwise. Spectra were obtained in chloroform-*d* ($CDCl_3$) unless otherwise noted. Chemical shifts are reported in parts per million (ppm) and coupling constants are reported in hertz (Hz). ^{1}H spectra in $CDCl_3$ were referenced to tetramethylsilane (TMS = 0.0 ppm) as an internal standard. ^{13}C NMR spectra in $CDCl_3$ were referenced to the solvent peak at 77.0 ppm. ^{1}H NMR spectra in methanol-*d4* were referenced to the residual water peak at 4.87 ppm. ^{13}C NMR spectra in DMSO-*d6* were referenced to the solvent peak at 39.52 ppm. IR spectra were obtained as ATR spectra of a thin film and the absorptions are reported in cm^{-1}. Mass spectrometry was performed at the University of Illinois School of Chemical Sciences, Urbana, IL on a PE Biosystems Voyager, Micromass 70-VSE-B or a Micromass ZAB-SE spectrometer. Electrospray ionization was used unless otherwise noted. Mass spectra are reported in Daltons with a relative intensity to a base peak (base = 100). Melting points were measured on a Mel–Temp melting point apparatus and are uncorrected.

Abbreviations: ATR (attenuated total reflectance); d.i. H_2O (deionized water); DMAP (4-dimethylaminopyridine); DMF (dimethyl formamide); dmf (dimethylforamidine); EtOAc (ethyl acetate); MeOH (methanol); PE (low boiling petroleum ether).

Procedures

3′,5′-Bis-*O*-(*tert*-butyldiphenylsilyl)-5-Cyano-2′-deoxyuridine (1). 5-Cyano-2'-deoxyuridine[1] (47 mg, 0.19 mmol) was dissolved in DMF (1.1 mL) and *tert*-butyldiphenylchlorosilane (0.11 mL, 0.42 mmol) and imidazole (59 mg, 0.88 mmol) were added. The mixture was stirred at ambient temperature for 18 h. The reaction mixture was diluted with d.i. water (3 mL) and extracted with diethyl ether (2 x 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by preparatory TLC (4% MeOH/CH_2Cl_2) to give 62.3 mg (46%) of **1** as a colorless oil: IR 2235.4, 1702.4, 1625.3, 1461.6, 1427.3, 1281.9, 1112.1, 733.7, 700; ^{1}H NMR δ 8.31 (1H, s), 8.22 (br s, 1H), 7.58-7.25 (20H, m), 6.31 (dd, *J* = 8.5, *J* = 5.3, 1H), 4.43 (d, *J* = 4.9, 1H), 4.01 (s, 1H), 3.66 (dd, *J* = 11.8, *J* = 2.1, 1H), 3.26 (dd, *J* = 11.8, *J* = 2.6, 1H), 2.54 (dd, *J* = 13.1, *J* = 5.3, 1H), 1.95 (m, 1H), 1.06 (s, 9H), 0.95 (s, 9H); ^{13}C NMR δ 158.38, 148.02, 147.63, 135.62, 135.55, 135.47, 135.35, 134.78, 132.88, 132.76, 131.93, 131.89, 130.20, 130.15, 130.12, 128.05, 127.93, 127.92, 127.71, 112.08, 90.31, 89.03, 87.43, 74.29, 63.81, 42.61, 26.90, 26.81, 19.10, 18.92; MS 752.1 (M + Na^+, 18), 747.2 (45), 133.1 (100).

3′,5′-Bis-*O*-(*tert*-butyldiphenylsilyl)-5-cyanomethyl-2'-deoxyuridine (2). 3′,5′-bis-*O*-(*tert*-butyldiphenylsilyl)-5-bromomethyl-deoxyuridine[2] (398 mg, 0.50 mmol) was dissolved in CH_2Cl_2 (4 mL) and tetraethylammonium cyanide (125 mg, 0.80 mmol) was then added. The solution was stirred at ambient temperature overnight and then evaporated onto silica gel, and the residue was loaded onto a chromatography column for purification (1:5 → 2:5 ethyl acetate/hexane), affording 77 mg (21%) of the title compound as a white powder: IR 2253.8, 1689.5, 1470.6, 1427.2, 1103.3, 700.1; ^{1}H NMR δ 8.78 (1H, s), 7.67-7.26 (20H, m), 6.46 (1H, dd, J=8.78 & J=5.37), 4.52 (1H, d, J=5.61), 4.04 (1H, m), 3.75 (1H, m), 3.36 (1H, m), 2.63 (2H, d, J=1.46) 2.44 (1H, m), 1.95 (1H, m), 1.06 (9H, s), 0.95 (9H, s); ^{13}C NMR δ 161.47, 149.59, 138.23, 135.68, 135.64, 135.30, 135.03, 133.33, 133.06, 132.95, 131.91, 130.31, 130.21, 130.10, 130.06, 128.08, 128.05, 127.94, 127.91, 116.06, 104.84, 104.73, 88.66, 85.65, 74.03, 64.02, 41.71, 26.92, 26.85, 19.36, 18.99, 15.06; MS 766.23 (M + Na^+, 100), 615.29 (8).

3′,5′-Bis-*O*-(*tert*-butyldiphenylsilyl)-5-thiocyanatomethyl-2′-deoxyuridine ($S^{12}CN$-derivative) (3).[5] To a solution of bis-*O*-(*tert*-butyldiphenylsilyl)-5-bromomethyl-deoxyuridine[2] (100 mg, 0.125 mmol) in acetonitrile (1.7 mL) was added potassium thiocyanate (42 mg, 0.47 mmol) and the mixture was stirred at ambient temperature for 4.5 h. The acetonitirile was removed under reduced pressure and the residual solid was partitioned between d.i. water and ethyl acetate. The organic layer was dried (Na_2SO_4), and concentrated under reduced pressure. The product was purified by preparatory TLC (1:3 ethyl acetate/hexane) yielding 84 mg (87%) of the title compound as a white solid after hexane coevaporation: IR 2152.2, 1693.0, 1470.8, 1427.5, 1112.3, 702.0; ^{1}H NMR δ 8.49 (s, 1H), 7.66 (s, 1H), 7.63-7.30 (m, 20H), 6.48 (dd, *J* = 8.5, *J* = 5.4, 1H), 4.54 (d, *J* = 5.6, 1H), 4.03 (m, 1H), 3.76 (dd, *J* = 11.5, *J* = 2.6, 1H), 3.35 (dd, *J* = 11.5, *J* = 2.9, 1H), 3.17 (m, 2H), 2.45 (m, 1H), 2.01 (m, 1H), 1.08 (s, 9H), 0.94 (s, 9H); ^{13}C NMR δ 161.20, 149.50, 139.19, 135.69, 135.67, 135.43, 135.12, 133.39, 133.13, 132.97, 132.09, 130.14, 130.08, 130.05, 128.06, 128.04, 127.94, 127.92, 111.92 ($S^{12}CN$ peak), 108.18, 88.16, 85.57, 73.84, 63.91, 41.40, 30.45, 26.99, 26.88, 19.39, 19.03; MS 717.4 (M – SCN, 64), 199.2 (100).

3′,5′-Bis-*O*-(*tert*-butyldiphenylsilyl)-5-thiocyanatomethyl-2′-deoxyuridine (S^{13}CN-derivative) (3c).[5] To a solution of bis-*O*-(*tert*-butyldiphenylsilyl)-5-bromomethyl-deoxyuridine[2] (100 mg, 0.125 mmol) in acetonitrile (2.0 mL) was added C-13 labeled potassium thiocyanate (46.1 mg, 0.470 mmol) and the mixture was stirred at ambient temperature for 2 h. The acetonitrile was removed under reduced pressure and the residual solid was partitioned between d.i. water and ethyl acetate. The organic layer was dried (Na_2SO_4), and concentrated under reduced pressure followed by hexane coevaporation. The product was purified by preparatory TLC (1:3 ethyl acetate/hexane) yielding 59 mg (61%) of the title compound as a white solid: IR 2102.7, 1687.7, 1470.6, 1427.3, 1112.2, 701.5; ^{1}H NMR δ 8.88 (br s, 1H), 7.67 (s, 1H), 7.64-7.30 (m, 20H), 6.48 (dd, *J* = 8.5, *J* = 5.6, 1H), 4.54 (d, *J* = 5.6, 1H), 4.04 (apparent d, *J* = 2.0, 1H), 3.77 (dd, *J* = 11.5, *J* = 2.4, 1H), 3.35 (dd, *J* = 11.5, *J* = 2.9, 1H), 3.19 (m, 2H), 2.45 (m, 1H), 2.02 (m, 1H), 1.08 (s, 9H), 0.94 (s, 9H); ^{13}C NMR δ 161.50, 149.65, 139.17, 135.67, 135.65, 135.41, 135.10, 133.47, 133.36, 133.11, 132.95, 132.07, 130.11, 130.06, 130.02, 128.03, 128.01, 127.92, 127.89, 111.95 (S^{13}CN peak), 108.18, 88.13, 85.55, 73.80, 63.88, 41.39, 30.44, 26.97, 26.86, 19.37, 19.00; MS 748.9 (M^+ - CN).

3′,5′-Bis-*O*-(*tert*-butyldiphenylsilyl)-5-azidomethyl-2′-deoxyuridine (4).[6] 3',5'-bis-*O*-(*tert*-butyldiphenylsilyl)-5-bromomethyl-2'-deoxyuridine was prepared from 3',5'-bis-*O*-(*tert*-butyldiphenylsilyl)-5-methyl-2'-deoxyuridine (251 mg, 0.35 mmol) according to the method of Hong and Greenberg[2] and the crude product was dissolved in dry acetonitrile (15 mL) and sodium azide (75 mg, 1.15 mmol) was added. The heterogeneous mixture was stirred at ambient temperature for 3 days. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine. The combined aqueous layers were extracted with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and concentrated. Purification via preparatory TLC (2:5 EtOAc/PE) provided 94 mg (36%, 2 steps) of the title compound as a white foam: IR 2106.1, 1686.4, 1470.8, 1427.5, 1112.2, 701.2; ^{1}H NMR δ 8.21 (s, 1H), 7.64-7.28 (m, 20H), 6.48 (dd, *J* = 8.9, *J* = 5.2, 1H), 4.54 (d, *J* = 5.4, 1H), 4.01 (d, *J* = 1.7, 1H), 3.75 (dd, *J* = 11.6, *J* = 2.4, 1H), 3.54 (d, *J* = 13.7, 1H), 3.51 (d, *J* = 13.7, 1H), 3.32 (dd, *J* = 11.6, *J* = 2.4, 1H), 2.39 (m, 1), 1.95 (m, 1H), 1.08 (s, 9H), 0.93 (s, 9H); ^{13}C NMR δ 162.05, 149.64, 138.60, 135.69, 135.64, 135.41, 135.07, 133.11, 133.09, 132.99, 131.96, 130.11, 130.08, 130.05, 127.98, 127.95, 127.92, 127.91, 127.84, 109.61, 88.01, 85.25, 73.95, 63.94, 46.61, 41.55, 26.90, 26.86, 19.31, 18.99; MS 782.2 (M + Na^+, 38), 760.2 (M^+ + 1, 72), 451.4 (100).

3′,5′-Bis-*O*-(*tert*-butyldiphenylsilyl)-5-cyanomethyl-2'-deoxycytidine (5). Compound **2** (77 mg, 0.10 mmol) was dissolved in acetonitrile (1 mL) and DMAP (31.4 mg, 0.258 mmol), triethylamine (36 μL, 0.26 mmol), and triphenylchlorosilane (78 mg, 0.258 mmol) were added. The mixture was stirred for 4 h, at which time aqueous ammonia (3 mL, 28-30 wt %) was added. Stirring was continued for 2 h and then the mixture was concentrated under reduced pressure. The residue was partitioned between methylene chloride and d.i. water, and the organic layer was dried (Na_2SO_4), concentrated, and purified via column chromatography (2 → 5% methanol/methylene chloride) and again by preparative TLC (1:10:10 MeOH/CH_2Cl_2/EtOAc) to yield 15 mg (20%) of **9** as a yellow liquid. IR 2254.2, 1726.3, 1665.2, 1487.8, 1471.0, 1427.5, 1112.1, 1105.5, 701.7; ^{1}H NMR δ 7.69-7.30 (20H, m), 6.49 (1H, dd, *J*=7.81, 5.62), 4.51 (1H, d, J=5.62), 4.10 (1H, m), 3.74 (1H, m), 3.36 (1H, m), 2.55 (2H, d, J=3.42) 1.90 (1H, m), 1.08 (9H, s), 0.93 (9H, s); MS 743.4 (M + Na^+, 100), 151.3 (33).

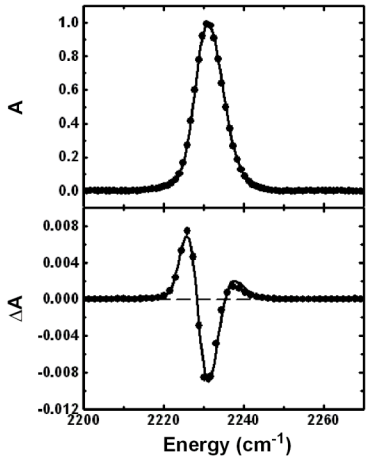
3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)–N2-dmf-2′-deoxyguanosine (10). N2-dmf-2'-deoxyguanosine (0.166 g, 0.513 mmol) was dissolved in anhydrous DMF (0.5 mL) and imidazole (0.288 g, 4.31 mmol) and *tert*-butyldimethylchlorosilane (0.382 mL, 2.05 mmol) were added. The mixture was stirred at ambient temperature for 24 h and was subsequently diluted with d.i. water and extracted with ethyl acetate. The organic layer was washed with d.i. water, dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography (3→10% methanol/ methylene chloride) afforded 0.1732 g (61%) of **11** as a yellow oil. ^{1}H NMR δ 9.87 (s, 1H), 8.51 (s, 1H), 6.25 (t, 1H, *J* = 6.6 Hz), 4.47 (m, 1H), 3.86 (m, 1H), 3.66 (m, 2H), 3.08 (s, 3H), 3.01 (s, 3H), 2.32 (m, 1H), 2.78 (m, 1H), 0.81 (s, 18 H), 0.00 (s, 6H), -0.026 (s, 6H).

3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)–N2-nitrile-2′-deoxyguanosine (7). N2-dmf-2′-deoxyguanosine (0.50 g, 1.55 mmol) was dissolved in anhydrous DMF (1.5 mL) and imidazole (0.838 g, 12.50 mmol) and *tert*-butyldimethylchlorosilane (1.15 mL, 6.20 mmol) were added. The mixture was stirred at ambient temperature for 39 h and was subsequently diluted with d.i. water and extracted with ethyl acetate. The organic layer was washed with d.i. water, dried (Na_2SO_4), and concentrated under reduced pressure to give the crude **10**. The crude product was then dissolved in ammonia in methanol (7*N*, 250 mL) and I_2 (0.76 g, 3.0 mmol) was added. The yellow mixture was stirred at room temperature under argon for 23 h, after which time it was colorless. The solvent was removed under reduced pressure and the residue was partitioned between EtOAc and d.i. water. The organic layer washed with aqueous $Na_2S_2O_3$, dried (Na_2SO_4) and concentrated under reduced pressure. Column chromatography (5→15% methanol/methylene chloride) afforded 0.600 g (74%, 2 steps) of **7** as a white powder: mp 225 ºC (dec.); IR 3405.0, 2954.7, 2928.0, 2857.4, 2169.3, 1687.1, 1583.1, 1378.2, 1256.4, 1118.0, 837.2, 780.4; ^{1}H NMR (methanol-d_4) δ 7.94 (s, 1H), 6.32 (t, *J* = 6.6, 1H), 4.67 (m, 1H), 3.97 (m, 1H), 3.87 (m, 2H), 2.72 (m, 1H), 2.41 (m, 1H), 0.95 (s, 9H), 0.93 (s, 9H) 0.15 (s, 6H), 0.10 (s, 3H), 0.098 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 159.86, 158.05, 151.72, 134.56, 120.84, 116.51, 87.13, 82.09, 72.54, 63.15, 62.80, 48.60, 25.83, 25.78, 17.99, 17.78, -4.71, -4.87, -5.40, -5.47; MS 543.5 (M + Na^+, 30), 521.4 (M^+ + 1, 70), 177.3 (100).

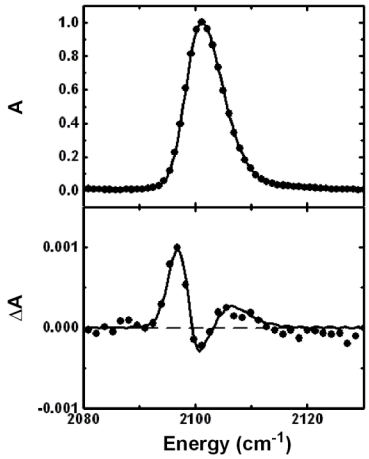
Stark Spectroscopy

Spectra: Absorption and Stark spectra of a number of compounds are shown below. For each compound, the top panel contains the absorption data (dots) and fit using a Savitsky-Golay polynomial smoothing filter (solid line), and the bottom panel contains the field-on minus field-off Stark data (dots) and fit to a weighted sum of zeroth, first, and second derivatives of the smoothed absorption line shape (solid line). Absorption spectra are scaled to 1, and Stark spectra are scaled to the absorption spectra and to an applied electric field of 1 MV/cm to facilitate comparison. Actual absorbance values and electric field strengths obtained in the spectra are listed in Table S1 below.

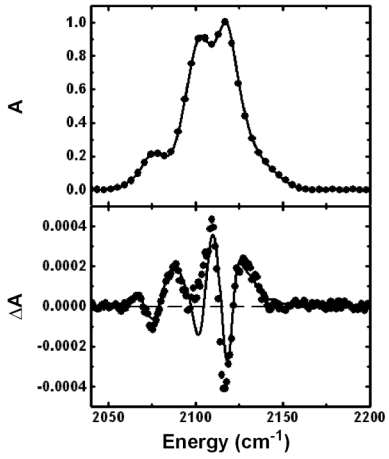
Compound 1



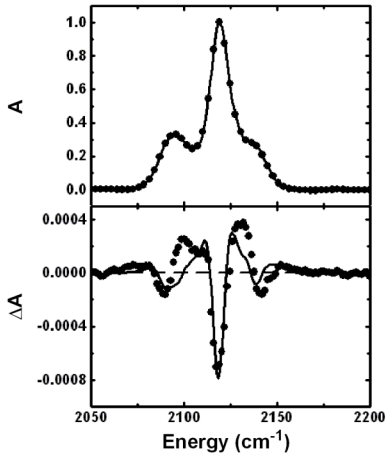
Compound 3



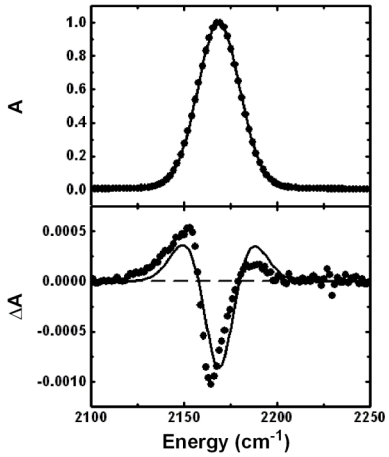
Compound 4



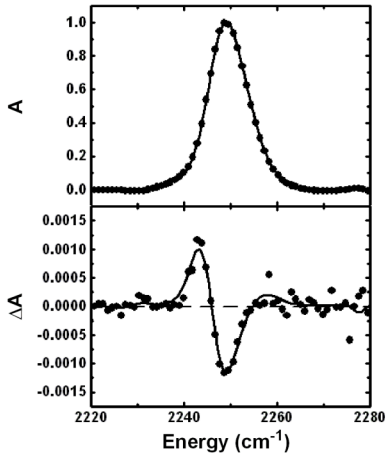
Compound 6



Compound 7



Compound 8



Compound 9

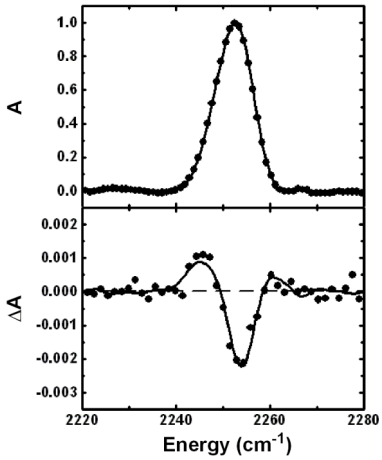


Table S1. Actual Absorptions and Fields Values from the Stark Experiments.

Compound	Absorbance[1]	Field[2]
1	0.10	1.6
3c	0.12	1.2
4	0.57	1.2
6	0.90	1.1
7	0.32	1.2
8	0.036	1.3
9	0.021	1.4

[1]Actual absorbance obtained in the spectra above before scaling to A = 1.
[2]Actual external electric field applied in the spectra above, in units of MV/cm, before scaling to F = 1 MV/cm.

References and Notes

(1) (a) Torrence, P. F.; Bhooshan, B.; Descamps, J.; De Clercq, E. *J. Med. Chem.* **1977**, 20, 974-976. (b) Markley, J. C.; Chirakul, P.; Sologub, D.; Sigurdsson, S. Th. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2453-2455.
(2) Hong, S.; Greenberg, M. *Org. Lett.* **2004**, *6*, 5011-5013.
(3) (a) Verheyden, J. P. H.; Wagner, D.; Moffatt, J. G. *J. Org. Chem.* **1971**, *36*, 250-254. (b) Vasil'eva, S. V.; Abramova, T. V.; Ivanova, T. M.; Shishkin, G. V; Sil'nikov, V. N. *Russian J. Bioorg. Chem.* **2004**, *30*, 234-241. (c) Greiner, B.; Pfleiderer, W. *Helv. Chim. Acta* **1998**, *81*, 1528-1544.
(4) Saneyoshi, H.; Seio, K.; Sekine, M. *J. Org. Chem.* **2005**, *70*, 10453-10460.
(5) The assignment of the product as the thiocyanate (R-SCN) rather than the isothiocyanate (R-NCS) is based upon the spectral properties, especially the ^{13}C peak at 111.9 ppm, see: Ando, T.; Clark, J. H.; Cork, D. G.; Fujita, M.; Kimura, T. *J. Org. Chem.* **1987**, *52*, 681-685.
(6) For the 3',5'-Bis-*O*-(*tert*-butyldimethylsilyl) analogue of this compound, see: Miyata, K.; Tamamushi, R.; Ohkubo, A.; Taguchi, H.; Seio, K.; Santa, T.; Sekine, M. *Org. Lett.* **2006**, *8*, 1545-1548.