LC-MS based quantification of 2′-ribosylated nucleosides Ar(p) and Gr(p) in tRNA

David Pearsona, Antje Hienzscha, Mirko Wagnera, Daniel Globischa, Veronika Reitera, Dilek Özdena, and Thomas Carella,*

Supplementary Figure

Fig. S1. Calibration curves for d_2-Ar and d_2-Gr compared to the respective natural nucleosides. Error bars represent +/- 1 s.d. from the mean value.

Experimental

Culture methods

S. scrofa liver samples and E. coli and HeLa cells were grown/obtained and extracted as described previously, and enzymatic digests and all LC-MS experiments were performed as previously reported. 1  1L-cultures of S. cerevisiae (wild type, DMS No: 70449) and C. albicans (wild type, DMS No: 1386) were grown in rich medium (YPD, 2% glucose, 2% peptone, and 1% yeast extract) at 30 °C or 37 °C, respectively. Cells were grown to an OD_600 of 0.6 - 0.7 then harvested. Mushroom (C. nebularis and F. fomentarius) samples (~5 g) were supplied by Prof. Wolfgang Steglich (LMU Munich). tRNA was extracted from yeast samples as described previously for E. coli, and from mushroom samples as described for porcine tissue. 1

Synthetic methods

9-[2-O-(5,5-deutero-β-D-ribofuranosyl)-β-D-ribofuranosyl]-adenine 6: To compound 14 (35 mg, 0.041 mmol) was added a solution of NH₃ (7 N in MeOH, 2 mL) and the resulting solution was stirred for 5 days then concentrated. The residue was suspended in H₂O (2 mL) and washed with DCM (2 mL × 3) then concentrated. The crude product was purified by reverse-phase HPLC to give 7 mg of 6 as a white solid (43%): mp 120-123 °C, 1H NMR (400 MHz, D₂O) δ 8.36 (s, 1H), 8.25 (s, 1H), 6.17 (d, J = 6.43 Hz, 1H, NCHCH), 5.09 (s, 1H, OCH₂), 4.85-4.81 (m, 1H, NCHCH), 4.61-4.57 (m, 1H, NCHCHCH), 4.33 (d, J = 2.50 Hz, 1H, CH₂CH₂), 4.17 (d, J = 4.55 Hz, 1H,
H₂O steps) as a white foam: 1H NMR (300 MHz, CDCl₃) 80% AcOH in H₂O (100 mL) was stirred for 16 h, then concentrated and coevaporated twice with DCM. To the residue was added a solution of NH₃ (7N in MeOH, 6.5 mL) and the resulting solution was stirred for 3 days then concentrated. The residue was suspended in H₂O (2 mL) and washed with DCM (2 mL × 3) then concentrated. The crude product was purified by reverse-phase HPLC to give 9 mg of 7 as a white solid (30%). mp 120-123 °C, 1H NMR (600 MHz, D₂O) δ 8.00 (s, 1H, NCHN), 5.99 (d, J = 6.04 Hz, 1H, NCHCH), 5.09 (s, 1H, OCHO), 4.55-4.52 (m, 1H, CHCH₂), 4.24 (s, 1H, CHCH₂), 4.14 (d, J = 4.11 Hz, 1H, CHCHCHCD₂), 4.05 (dd, J = 6.91, 4.18 Hz, 1H, CHCHCD₂), 3.91-3.81 (m, 3H, CH₂ + CHCD₂), 13C NMR (151 MHz, D₂O) δ 158.9, 153.8, 151.1, 138.1 (NCHN), 116.6, 106.1 (OCHO), 86.4 (NCHCH), 86.0 (CHCH₂), 82.6 (CHCD₂), 77.8 (NCHCH), 74.3 (CHCHCHCD₂), 71.0 (CHCHCD₂), 68.8 (CHCHCH₂), 61.3 (CH₂), m/z (ES) 418.1536, calcd for C₁₅H₂₀D₂N₅O₉ (MH⁺) 418.1543.

9-[2-O-(5,5-deutero-β-D-ribofuranosyl)-β-D-ribofuranosyl]guanine 7: To 18 (76 mg, 0.073 mmol) was added TBAF (0.5 M in THF, 0.35 mL) and the resulting solution was stirred for 15 min then concentrated and co-evaporated twice with DCM. To the residue was added a solution of NH₃ (7N in MeOH, 6.5 mL) and the resulting solution was stirred for 3 days then concentrated. The residue was suspended in H₂O (2 mL) and washed with DCM (2 mL × 3) then concentrated. The crude product was purified by reverse-phase HPLC to give 9 mg of 7 as a white solid (30%). mp 120-123 °C, 1H NMR (600 MHz, D₂O) δ 8.00 (s, 1H, NCHN), 5.99 (d, J = 6.04 Hz, 1H, NCHCH), 5.09 (s, 1H, OCHO), 4.55-4.52 (m, 1H, CHCH₂), 4.24 (s, 1H, CHCH₂), 4.14 (d, J = 4.11 Hz, 1H, CHCHCHCD₂), 4.05 (dd, J = 6.91, 4.18 Hz, 1H, CHCHCD₂), 3.91-3.81 (m, 3H, CH₂ + CHCD₂), 13C NMR (151 MHz, D₂O) δ 158.9, 153.8, 151.1, 138.1 (NCHN), 116.6, 106.1 (OCHO), 86.4 (NCHCH), 86.0 (CHCH₂), 82.6 (CHCD₂), 77.8 (NCHCH), 74.3 (CHCHCHCD₂), 71.0 (CHCHCD₂), 68.8 (CHCHCH₂), 61.3 (CH₂), m/z (ES) 418.1536, calcd for C₁₅H₂₀D₂N₅O₉ (MH⁺) 418.1543.

1,2,3-O-benzoyl-5,5-deutero-5-O-(4-methoxyphenyl diphenylmethyl)-D-ribofuranose 9: A solution of 5,5′-d₂-ribose 8 (1g, 6.6 mmol) and monomethoxytrityl chloride (2.4g, 7.8 mmol) in pyridine (13 mL) was stirred at r.t for 24 h, then cooled to 0°C and a solution of benzyl chloride (3.5 mL, 4.2 g, 30 mmol) in 1,2-dichloroethane (10 mL) was added. The resulting solution was stirred for at r.t for 16 h, then cooled to 0°C and EtOH (3.5 mL) was added. The solution was stirred at r.t for 20 min, then concentrated. The residue was redissolved in chloroform, washed with H₂O, dried over NaSO₄, concentrated, then coevaporated twice with toluene. The crude material was partially purified by flash chromatography, eluting with 1:4 EtOAc/isohexane to give 9 (4.8g) as a mixture of the α and β anomers that was used in the subsequent step without further purification.

1,2,3-O-benzoyl-5,5-deutero-5-O-(4-methoxyphenyl diphenylmethyl)-D-ribofuranose 10: A mixture of 9 (3.7g, 5.0 mmol) and 80% AcOH in H₂O (100 mL) was stirred for 16 h, then concentrated and coevaporated twice with toluene. The crude material was purified twice by silica column chromatography, eluting with 1:39 EtOAc/DCM to give 10β (480 mg, 20% over two steps) as a white foam: 1H NMR (300 MHz, CDCl₃) δ 8.06 (dd, J = 8.4, 1.4 Hz, 2H), 8.04 (dd, J = 8.4, 1.3 Hz, 2H), 7.91 (dd, J = 8.4, 1.3 Hz, 2H), 7.65-7.40 (m, 7H), 7.34 (t, J = 7.7 Hz, 2H), 6.67 (s, 1H, OCHO), 5.95-5.90 (m, 2H), 4.62-4.57 (m, 1H, CHCD₂), 2.18 (s, 1H, OH). 13C NMR (75 MHz, CDCl₃) δ 165.7, 165.0, 164.7, 133.8, 133.6, 133.5, 130.0, 129.9, 129.7, 128.9, 128.8, 128.8, 128.5, 128.4, 98.9 (OCHO), 83.1 (CHCD₂), 75.5, 70.9, m/z (ES) 499.1125, calcd for C₅₀H₃₂O₅ (MCl) 499.1129, then eluting with 1:9 EtOAc/DCM to give 10α (660 mg, 28% over two steps) as a white foam: 1H NMR (300 MHz, CDCl₃) δ 8.07 (d, J = 8.4 Hz, 4H), 7.86 (dd, J = 8.33, 1.24 Hz, 2H), 7.57 (dd, J = 16.99, 7.59 Hz, 2H), 7.48 (t, J = 7.46 Hz, 1H), 7.34-7.15 (m, 6H), 6.95 (d, J = 4.38 Hz, 1H, OCHO), 5.84 (dd, J = 6.61, 2.28 Hz, 1H, (CHCHCD₂)), 5.65 (dd, J = 6.61, 4.40 Hz, 1H, (CHCHCHCD₂)), 4.65 (d, J = 2.28 Hz, 1H, (CHCD₂)), 2.53 (s, 1H, OH), 13C NMR (75 MHz, CDCl₃) δ 165.9, 165.3, 165.0, 133.5, 133.4, 133.4, 129.9, 129.
1,2,3-O-benzoyl-5,5-deutero-5-O-phenoxyacetyl-β-D-ribofuranose 11: To a solution of 10β (450 mg, 0.97 mmol) in pyridine (1 mL) and 1,2-dichloroethane (4 mL) was added phenoxyacetic anhydride (360 mg, 1.3 mmol) and the resulting solution was stirred at r.t for 1 h, then MeOH (0.2 mL) was added. The solution was stirred for 30 min, then diluted with DCM, washed with brine, dried over Na2SO4 and concentrated. The crude material was purified by silica column chromatography, eluting with DCM to obtain 11 (350 mg, 60%) as a white foam: 1H NMR (400 MHz, CDCl3) δ 8.09 (d, J = 7.23 Hz, 2H), 8.03 (d, J = 7.24 Hz, 2H), 7.90 (d, J = 7.26 Hz, 2H), 7.59 (t, J = 7.46 Hz, 2H), 7.52 (t, J = 7.47 Hz, 1H), 7.47-7.41 (m, 4H), 7.33 (t, J = 7.88 Hz, 2H), 7.25-7.20 (m, 2H), 6.95 (t, J = 7.38 Hz, 1H), 6.77 (d, J = 7.80 Hz, 2H), 6.64 (s, 1H, OCHO), 5.94-5.92 (m, 1H, CHCH2CH2), 5.89 (dd, J = 6.89, 4.88 Hz, 1H, CHCH2), 4.74 (d, J = 6.89 Hz, 1H, CHCD2), 4.51 (d, J = 16.04 Hz, 1H, CH/H), 4.40 (d, J = 16.07 Hz, 1H, CH/H). 13C NMR (101 MHz, CDCl3) δ 168.4, 165.4, 165.0, 164.5, 157.5, 133.8, 133.7, 133.7, 129.9, 129.9, 129.8, 129.5, 129.0, 128.8, 128.7, 128.6, 128.5, 121.7, 114.6, 99.0 (OCHO), 79.6 (CHCD2), 75.0 (CHCH2CH2), 71.3 (CHCH2), 64.9 (CH2), m/z (ES) 616.2155, calcd for C34H30D2NO10 (M.NH4+) 616.2152.

N6-benzoyl-9-[2-O-2,3,5-deutero-5-O-phenoxyacetyl-β-D-ribofuranosyl]-3,5-O-(1,1,3,3-tetraisopropylsiloxane-1,3-diy1)-β-D-ribofuranosyl-adenine 13: A solution of 11 (100 mg, 0.17 mmol) [note: reactants 11 and 12 were each dried under vacuum (<0.5 mmHg) at 45°C for 4 h prior to reaction] in 1,2-dichloroethane (2 mL) was cooled to 0°C and SnCl4 (30 μL, 66 mg, 0.26 mmol) was added. The resultant solution was stirred at 0°C for 10 min, then 12 (120 mg, 0.20 mmol) was added. The solution was stirred at 0°C for 16 h, then DCM (2 mL) and sat. NaHCO3 (2 mL) were added. The mixture was stirred for 20 min, then filtered through celite, washed with H2O, dried over NaSO4 and concentrated. The crude material was purified by silica column chromatography, eluting with 1:99 MeOH/DCM to give 13 (160 mg, 86%) as a white foam: 1H NMR (600 MHz, CDCl3) δ 9.04 (s, 1H, NH), 8.75 (s, 1H), 8.29 (s, 1H), 8.02 (dd, J = 8.35, 1.22 Hz, 2H), 7.98 (dd, J = 8.43, 1.30 Hz, 2H), 7.87 (dd, J = 8.41, 1.27 Hz, 2H), 7.62-7.57 (m, 2H), 7.54-7.50 (m, 3H), 7.43 (dd, J = 8.20, 7.52 Hz, 2H), 7.32 (dd, J = 8.25, 7.53 Hz, 2H), 7.21 (dd, J = 8.75, 7.37 Hz, 2H), 6.92 (t, J = 7.36 Hz, 1H), 6.85 (dd, J = 8.76, 0.99 Hz, 2H), 6.19 (s, 1H), 5.81 (dd, J = 4.98, 0.82 Hz, 1H), 5.80-5.77 (m, 2H), 4.97 (dd, J = 9.27, 4.64 Hz, 1H), 4.77 (d, J = 4.61 Hz, 1H), 4.66 (d, J = 16.16 Hz, 1H, OCH/HCOO), 4.61 (d, J = 16.17 Hz, 1H, OCH/HCOO), 4.61 (d, J = 6.31 Hz, 1H), 4.21 (dd, J = 13.35, 1.43 Hz, 1H, CHC/H), 4.16 (td, J = 9.22, 2.18 Hz, 1H), 4.02 (dd, J = 13.30, 2.62 Hz, 1H, CHCH/H), 1.11-0.94 (m, 28H, 4×CH(CH3)2), 13C NMR (151 MHz, CDCl3) δ 168.7, 165.3, 164.9, 164.5, 157.6, 152.7, 150.8, 149.4, 141.9, 133.8, 133.49, 133.48, 132.7, 129.7, 129.5, 129.0, 128.83, 128.78, 128.5, 128.4, 127.8, 123.5, 121.7, 114.6, 105.8, 88.8, 81.4, 79.6, 78.9, 75.5, 72.3, 69.8, 65.2 (OCH3/COO), 59.8 (CHCH2), 17.4, 17.33, 17.28, 17.26, 17.2, 17.1, 16.9, 16.8, 13.3, 12.9, 12.8, 12.6, m/z (ES) 1090.4301, calcd for C56H64D4N16O14Si2 (MH+) 1090.4270.

N6-benzoyl-9-[2-O-2,3,5-deutero-5-O-phenoxyacetyl-β-D-ribofuranosyl]-β-D-ribofuranosyl-adenine 14: To 13 (130 mg, 0.12 mmol) was added TBAF (0.5 M in THF, 1 mL) and the resulting solution was stirred for 15 min then
concentrated. The crude material was purified twice by silica column chromatography, eluting with 1:24 MeOH/DCM to give 14 (65 mg, 64%) as a white foam: $^1$H NMR (600 MHz, CDCl$_3$) δ 9.27 (s, 1H), 8.80 (s, 1H), 8.21 (s, 1H), 8.01 (d, J = 7.17 Hz, 2H), 7.89-7.84 (m, 4H), 7.58 (t, J = 7.44 Hz, 1H), 7.54-7.50 (m, 2H), 7.49 (t, J = 7.78 Hz, 2H), 7.34 (dt, J = 8.27, 1.88 Hz, 4H), 7.26 (t, J = 8.05 Hz, 2H), 6.96 (t, J = 7.38 Hz, 1H), 6.92 (dd, J = 8.75, 0.93 Hz, 2H), 6.08 (d, J = 7.42 Hz, 1H, NCHCH), 6.02 (d, J = 11.06 Hz, 1H, CH$_2$OH), 5.58 (dd, J = 5.37, 2.13 Hz, 1H, CHCHCHCD$_2$), 5.55 (t, J = 5.28 Hz, 1H, CHCHCD$_2$), 5.19 (dd, J = 7.39, 4.70 Hz, 1H, NCHCH), 5.15 (d, J = 2.11 Hz, 1H, OCHO), 4.74 (d, J = 0.85 Hz, 2H, OCH$_2$COO), 4.63 (d, J = 4.70 Hz, 1H, CHCHCH$_2$), 4.34 (d, J = 5.23 Hz, 1H, CHCD$_2$), 4.30 (s, 1H, CHCH$_2$), 3.95 (d, J = 12.97 Hz, 1H, CHCH/H), 3.73 (t, J = 11.85 Hz, 1H, CHCH/H), 3.39 (s, 1H, CHO/H), $^{13}$C NMR (151 MHz, CDCl$_3$) δ 168.8, 165.4, 165.3, 164.53, 157.53, 152.2, 150.5, 150.3, 144.2, 133.8, 133.7, 133.5, 132.8, 129.8, 129.7, 129.6, 128.8, 128.5, 128.5, 128.4, 127.9, 124.3, 121.9, 114.6, 106.7 (OCHO), 89.2 (NCHCH), 87.2 (CHCH$_2$), 81.1 (NCHCH), 79.9 (CHCD$_2$), 75.7 (CHCHCHCD$_2$), 71.7 (CHCHCH$_2$), 71.6 (CHCHCD$_2$), 65.3 (OCH$_2$COO), 63.2 (CHCH$_2$), m/z (ES) 848.2763, calcd for C$_{44}$H$_{38}$D$_2$N$_2$O$_{13}$ (MH$^+$) 848.2748.

1-O-methyl-2,3,5-O-benzoyl-5,5-deutero-β-D-ribofuranose 15: A solution of 5,5′-d$_2$-ribose 8 (200 mg, 1.3 mmol) in MeOH (4 mL) was cooled to 0°C and Dowex 50 W-8 100 cation exchange resin (300 mg, vacuum dried) was added. The resulting mixture was kept at 4°C for 24 h then filtered through celite and concentrated. The residue was dried by evaporation of pyridine (3 × 5 mL), then redissolved in DCM (1 mL) and pyridine (2.4 mL), cooled to 0°C and BzCl (1 g, 7.1 mmol) was added. The resulting mixture was kept at 4°C for 24 h, then H$_2$O (0°C, 5 mL) was added and the organic layer was separated. The aqueous layer was washed with DCM (3 × 10 mL) then the organic layers were combined then co-evaporated with toluene (3 × 10 mL). The crude material was purified by column chromatography, eluting with 1:9 to 1:14 EtOAc/isohexane to give 15 (513 mg, 82%) as a colourless oil. $^1$H NMR (600 MHz, CDCl$_3$) δ 8.08 (dd, J = 8.41, 1.30 Hz, 2H), 8.02 (dd, J = 8.40, 1.30 Hz, 2H), 7.89 (dd, J = 8.42, 1.29 Hz, 2H), 7.59-7.49 (m, 3H), 7.44-7.38 (m, 4H), 7.32 (dd, J = 8.32, 7.51 Hz, 2H), 5.87 (ddd, J = 7.04, 4.86, 0.35 Hz, 1H), 5.68 (d, J = 4.87 Hz, 1H), 5.16 (s, 1H), 4.72 (d, J = 7.10 Hz, 1H), 3.42 (s, 3H, CH$_3$), $^{13}$C NMR (151 MHz, CDCl$_3$) δ 166.2, 165.4, 165.2, 133.4, 133.3, 133.1, 129.8, 129.7, 129.7, 129.2, 128.9, 128.5, 128.3, 128.3, 128.3, 128.3, 128.3, 80.6, 78.9, 75.4, 72.4, 55.4 (CH$_3$), m/z (ES) 496.1933, calcd for C$_{27}$H$_{35}$D$_2$NO$_8$ (M,NH$_4^+$) 496.1940.

1-O-acetyl-2,3,5-O-benzoyl-5,5-deutero-β-D-ribofuranose 16: A solution of 15 (350 mg, 0.73 mmol) in AcOH (1.6 mL) and Ac$_2$O (3.9 mL) was cooled to 0°C and H$_2$SO$_4$ (98%, 0.55 mL) was added. The resulting solution was kept at 4°C for 26 h, then H$_2$O (4°C, 10 mL) was added and the organic layer was separated. The aqueous layer was washed with DCM (3 × 20 mL) then the organic layers were combined and concentrated. The crude material was purified by column chromatography, eluting with 1:4 EtOAc/isohexane, then further purified by recrystallisation from isopropanol to give 16 (180 mg, 49%) as a white solid. m.p. 113-116°C, $^1$H NMR (600 MHz, CDCl$_3$) δ 8.08 (dd, J = 8.40, 1.28 Hz, 2H), 8.00 (dd, J = 8.41, 1.28 Hz, 2H), 7.89 (dd, J = 8.42, 1.27 Hz, 2H), 7.60-7.51 (m, 3H), 7.45-7.39 (m, 4H), 7.33 (dd, J = 8.32, 7.51 Hz, 2H), 6.43 (s, 1H), 5.91 (dd, J = 7.12, 4.89 Hz, 1H), 5.79 (d, J = 4.89 Hz, 1H), 4.78 (d, J = 7.16 Hz, 1H), 2.00 (s, 3H, CH$_3$), $^{13}$C NMR (151 MHz, CDCl$_3$) δ 169.0, 166.0, 165.3, 165.0, 133.6, 133.5, 133.2, 129.8, 129.7, 129.6, 128.8, 128.6, 128.5, 128.4, 128.4, 98.4, 79.8, 75.0, 71.3, 20.9, m/z (ES) 529.1436, calcd for C$_{28}$H$_{32}$D$_2$NaO$_9$ (M,Na$^+$) 519.1444.
$N^2$-isobutyryl-9-$[2-O-[2,3,5-O-benzoyl-5,5-deutero-\beta-D-ribofuranosyl]-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-\beta-D-ribofuranosyl]$guanine 18: A solution of 16 (100 mg, 0.17 mmol) [note: reactants were dried under vacuum (<0.5 mmHg) at 45ºC for 4 h prior to reaction] in 1,2-dichloroethane (2 mL) was cooled to 0ºC and SnCl$_4$ (30 µL, 66 mg, 0.26 mmol) was added. The resultant solution was stirred at 0ºC for 10 min, then 17 (120 mg, 0.20 mmol) was added. The solution was stirred at 0ºC for 16 h, then sat. NaHCO$_3$ (1 mL) was added. The mixture was stirred for 10 min, then filtered through celite, washed with H$_2$O, dried over NaSO$_4$ and concentrated. The crude material was purified by silica column chromatography, eluting with 2:98 MeOH/DCM to give 18 (93 mg, 52%) as a white foam:

$^1$H NMR (600 MHz,CDCl$_3$) δ 8.16 (s, 1H, NC$_2$H$_4$N), 8.00-7.96 (m, 4H), 7.91 (dd, $J = 8.31$, 1.20 Hz, 2H), 7.59 (t, $J = 7.45$ Hz, 1H), 7.55 (t, $J = 7.46$ Hz, 2H), 7.43 (t, $J = 7.88$ Hz, 2H), 7.37-7.32 (m, 4H), 6.13 (t, $J = 5.47$ Hz, 1H), 5.89 (d, $J = 5.52$ Hz, 1H), 5.86 (s, 1H), 5.75 (s, 1H), 4.77 (d, $J = 5.83$ Hz, 1H), 4.57 (dd, $J = 9.40$, 3.81 Hz, 1H), 4.29 (d, $J = 3.79$ Hz, 1H), 4.27 (d, $J = 13.59$ Hz, 1H, CHCH$_3$), 4.18 (dd, $J = 9.42$, 2.05 Hz, 1H), 4.01 (dd, $J = 13.56$, 2.60 Hz, 1H, CHCHH), 2.89 (sept., $J = 6.88$ Hz, 1H, COCHCH$_3$CH$_3$), 1.35 (d, $J = 6.86$ Hz, 3H, COCHCH$_3$CH$_3$), 1.25 (d, $J = 6.83$ Hz, 3H, COCHCH$_3$CH$_3$), 1.14-0.90 (m, 28H, 4×CH(CH$_3$)$_2$), $^{13}$C NMR (151 MHz,CDCl$_3$) δ 179.4, 167.9, 165.3, 165.0, 154.8, 148.6, 146.9, 135.7, 134.0, 133.6, 129.71, 129.68, 129.0, 128.9, 128.8, 128.7, 128.5, 128.4, 120.5, 105.5, 87.5, 81.3, 79.3, 78.7, 75.8, 73.2, 69.3, 59.4 (CH$_2$), 36.1, 19.2 (COCHCH$_3$CH$_3$), 18.9 (COCHCH$_3$CH$_3$), 17.5, 17.4, 17.29, 17.27, 17.1, 17.0, 16.8, 16.7, 13.3, 13.0, 12.8, 12.6, m/z (ES) 1042.4273, calcd for C$_{52}$H$_{64}$D$_2$N$_5$O$_{14}$Si$_2$ (MH$^+$) 1042.4270.
NMR Spectra