Supplementary Material (ESI) for Chemical Communications


**Supporting Information**

# A Smart Magnetic Resonance Imaging Contrast Agent Responsive to Adenosine Based on DNA Aptamer-conjugated Gadolinium Complex

**Weichen Xu and Yi Lu***

**General:** All DNA samples were purchased from Integrated DNA Technologies, Inc. (Coralville, IA) and desalted. NHS-activated DOTA was purchased from Macrocyclics, Inc. (Dallas, TX). Streptavidin and other chemicals were purchased from Sigma-Aldrich, Inc.

**Conjugation of DOTA-Gd to DNA**: In a typical conjugation, 50 μL of 0.5 mM amine modified DNA strand to be conjugated to DOTA-Gd (called Gd-strand, whose sequence is presented in Fig. 1), 100 μL carbonate buffer (0.5 M, pH 10) and 850 μL water were mixed. NHS-activated DOTA was dissolved in anhydrous acetonitrile with a concentration of 0.5 mg/mL. To conjugate the DOTA-Gd to DNA, 4 μL of NHS-DOTA solution was added into 1 mL of DNA solution every hour for five hours, and this mixture was stirred at 4°C. The mixture was stirred for another three to five hours after the final addition. The solution was then desalted with a C-18 cartridge (Sep-Pak, Waters), lyophilized and rehydrated in 180 μL acetate buffer (50 mM, pH 5.5) with an approximate concentration of 150 μM. $GdCl_3$ solution (10 mM) was then added and mixed slowly until the concentration of $Gd^{3+}$ reached 180 μM. The solution was then heated at 75°C for 30 minutes and the final product was purified by size exclusion chromatography (PD10, GE Health Care Life Sciences) and lyophilized.

The MALDI-MS of the DOTA conjugated DNA and DOTA-Gd conjugated DNA are presented in Fig. S1. As shown in Fig. S1A, after the conjugation of DOTA to the Gd-strand (sequence in Figure 1A), the specie's mass increased by 386.1 Da. This mass is consistent with the theoretical value of 386 Da. As shown in Fig. S1B, the mass of the Gd-strand increased by 154 Da after incorporation, indicating that $Gd^{3+}$ was incorporated into the DOTA coupled Gd-strand.

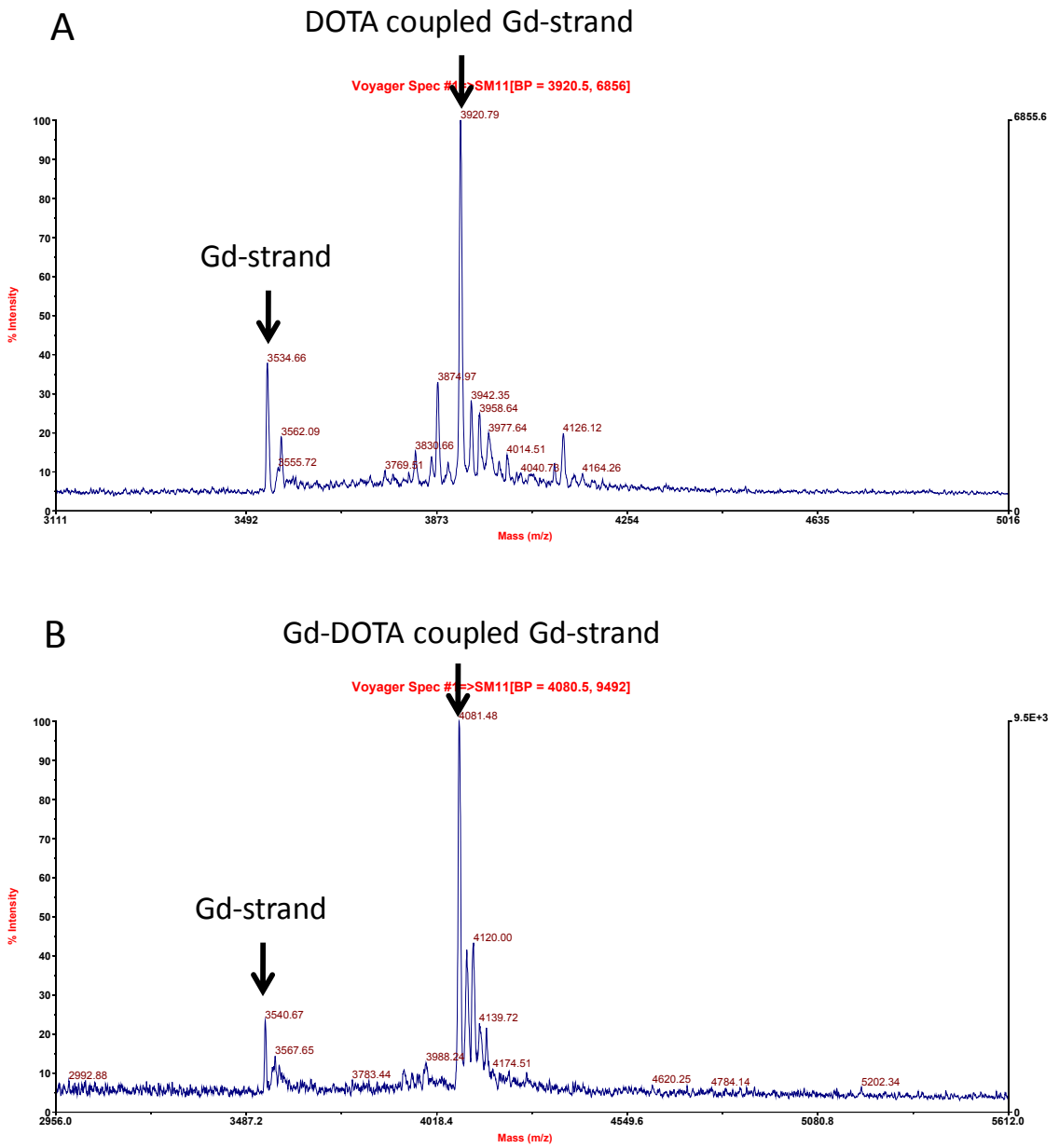


Fig. S1 MALDI-MS of the Gd-strand after conjugation of DOTA (A) and incorporation of $Gd^{3+}$ (B).

**DNA Sample preparation:** The adenosine aptamer-based contrast agent was prepared by dissolving the DOTA-Gd-coupled Gd-strand and aptamer strand in 50 mM Tris (pH 8.35), 300 mM NaCl and 5 mM $MgCl_2$ buffer to a concentration of 30 µM. The solution was then heated to 90°C and cooled to ambient temperature over the course of an hour. Streptavidin was then added into the solution to a concentration of 30 µM. Adenosine

was then added into the contrast agent solution for $T_1$ measurements and MRI image acquisition.

**$T_1$ measurement:** The $T_1$ was determined using a Bruker mq60 low resolution NMR spectromoter operating at 1.41 T (60 MHz) and 37 ˚C using the following parameters: 4 scans per point, 15 second recycle delay, mono-exponential curve fitting, phase cycling with a first pulse separation of 150 ms and a final pulse separation of 15000 ms using 10 data points for fitting, and a delay sampling window and sampling window of 0.03 seconds.

**Image acquisition:** The MRI image was acquired on 1.5 GE Signa Horizon Echo Speed with 9.0 software (1.5 T, GE). Samples with volumes of 500μL were prepared as described above. The MRI images were acquired at 23 ˚C. Coronal coil was used for the $T_1$-weighted image acquisition. The field of view was 7 and the slice thickness was 4 mm. The repetition time and echo time were 400 ms and 14 ms, respectively.

**Raw data of the magnetic resonance image (Fig. 3C):** The raw data is a matrix (97×33), which can be plotted by software such as MatLab or Origin. We used MatLab to process the data.

Columns 1 to 8

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | 66 | 37 | 148 | 105 | 13 | 257 | 40 | 66 |
| 2 | 132 | 43 | 202 | 82 | 107 | 184 | 38 | 138 |
| 3 | 50 | 173 | 153 | 18 | 40 | 140 | 143 | 53 |
| 4 | 199 | 164 | 184 | 271 | 79 | 116 | 101 | 89 |
| 5 | 53 | 130 | 83 | 156 | 112 | 74 | 83 | 63 |
| 6 | 75 | 74 | 67 | 29 | 132 | 212 | 54 | 78 |
| 7 | 83 | 29 | 45 | 238 | 127 | 136 | 78 | 68 |
| 8 | 41 | 164 | 179 | 164 | 126 | 116 | 70 | 13 |
| 9 | 105 | 139 | 91 | 103 | 39 | 45 | 73 | 80 |
| 10 | 78 | 12 | 161 | 76 | 52 | 88 | 113 | 274 |
| 11 | 54 | 69 | 34 | 122 | 102 | 130 | 41 | 63 |
| 12 | 70 | 42 | 76 | 50 | 92 | 60 | 211 | 62 |
| 13 | 176 | 43 | 28 | 128 | 69 | 121 | 55 | 156 |
| 14 | 228 | 67 | 124 | 81 | 95 | 24 | 74 | 155 |
| 15 | 140 | 34 | 46 | 164 | 128 | 223 | 154 | 59 |
| 16 | 76 | 73 | 77 | 164 | 221 | 191 | 89 | 119 |
| 17 | 93 | 88 | 183 | 128 | 62 | 4 | 22 | 147 |
| 18 | 89 | 207 | 112 | 42 | 60 | 151 | 161 | 67 |
| 19 | 141 | 85 | 161 | 123 | 47 | 114 | 163 | 112 |
| 20 | 116 | 58 | 167 | 111 | 103 | 220 | 90 | 236 |
| 21 | 66 | 128 | 27 | 113 | 93 | 104 | 8 | 151 |
| 22 | 168 | 129 | 137 | 89 | 119 | 97 | 52 | 80 |
| 23 | 89 | 150 | 124 | 82 | 147 | 61 | 54 | 199 |
| 24 | 84 | 81 | 112 | 122 | 140 | 135 | 22 | 160 |
| 25 | 134 | 114 | 46 | 154 | 177 | 182 | 102 | 38 |
| 26 | 159 | 129 | 131 | 324 | 168 | 217 | 137 | 53 |
| 27 | 87 | 86 | 63 | 86 | 47 | 189 | 78 | 125 |
| 28 | 287 | 91 | 75 | 47 | 95 | 117 | 16 | 236 |
| 29 | 143 | 193 | 202 | 42 | 201 | 117 | 65 | 209 |
| 30 | 113 | 120 | 51 | 80 | 94 | 176 | 48 | 269 |
| 31 | 95 | 105 | 131 | 149 | 78 | 71 | 121 | 191 |
| 32 | 81 | 178 | 47 | 175 | 167 | 104 | 176 | 158 |
| 33 | 101 | 77 | 108 | 230 | 77 | 188 | 154 | 104 |

Columns 9 through 16

| | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|
| 1 | 37 | 84 | 140 | 95 | 40 | 131 | 81 | 163 |
| 2 | 58 | 99 | 110 | 35 | 186 | 129 | 56 | 64 |
| 3 | 16 | 55 | 102 | 193 | 117 | 91 | 57 | 62 |
| 4 | 127 | 104 | 191 | 166 | 23 | 126 | 130 | 115 |
| 5 | 104 | 24 | 100 | 230 | 130 | 124 | 128 | 105 |
| 6 | 181 | 62 | 152 | 214 | 202 | 134 | 11 | 187 |
| 7 | 201 | 151 | 151 | 165 | 87 | 66 | 172 | 148 |
| 8 | 130 | 147 | 113 | 180 | 151 | 162 | 434 | 291 |
| 9 | 40 | 138 | 100 | 173 | 87 | 190 | 473 | 507 |
| 10 | 101 | 28 | 31 | 196 | 294 | 397 | 425 | 618 |
| 11 | 100 | 186 | 186 | 282 | 471 | 627 | 599 | 769 |
| 12 | 177 | 159 | 296 | 362 | 351 | 549 | 614 | 618 |
| 13 | 211 | 188 | 321 | 450 | 538 | 580 | 450 | 675 |
| 14 | 34 | 159 | 336 | 625 | 694 | 648 | 431 | 583 |
| 15 | 23 | 358 | 331 | 555 | 647 | 728 | 651 | 633 |
| 16 | 28 | 391 | 318 | 482 | 611 | 674 | 705 | 732 |
| 17 | 151 | 83 | 251 | 401 | 599 | 502 | 578 | 545 |
| 18 | 62 | 188 | 341 | 366 | 885 | 639 | 628 | 588 |
| 19 | 186 | 244 | 410 | 543 | 837 | 631 | 593 | 626 |
| 20 | 86 | 85 | 228 | 492 | 588 | 583 | 665 | 713 |
| 21 | 81 | 143 | 101 | 178 | 507 | 612 | 653 | 694 |
| 22 | 85 | 16 | 112 | 11 | 546 | 686 | 627 | 540 |
| 23 | 79 | 115 | 116 | 196 | 238 | 512 | 678 | 732 |
| 24 | 58 | 12 | 70 | 130 | 81 | 200 | 412 | 592 |
| 25 | 52 | 57 | 32 | 54 | 82 | 148 | 220 | 153 |
| 26 | 84 | 44 | 125 | 71 | 75 | 46 | 78 | 41 |
| 27 | 81 | 200 | 18 | 115 | 91 | 58 | 202 | 66 |
| 28 | 53 | 187 | 40 | 60 | 46 | 89 | 234 | 110 |
| 29 | 251 | 49 | 176 | 68 | 84 | 87 | 50 | 108 |
| 30 | 50 | 44 | 66 | 292 | 114 | 51 | 10 | 34 |
| 31 | 94 | 183 | 50 | 207 | 59 | 127 | 152 | 24 |
| 32 | 138 | 161 | 33 | 112 | 80 | 65 | 79 | 56 |
| 33 | 50 | 146 | 93 | 61 | 186 | 125 | 36 | 215 |

Columns 17 to 24

| | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|---|---|
| 1 | 100 | 37 | 66 | 31 | 125 | 56 | 74 | 111 |
| 2 | 132 | 131 | 76 | 119 | 102 | 132 | 47 | 114 |
| 3 | 109 | 51 | 93 | 43 | 21 | 62 | 68 | 66 |
| 4 | 191 | 101 | 91 | 84 | 57 | 25 | 100 | 157 |
| 5 | 107 | 102 | 159 | 166 | 209 | 131 | 116 | 47 |
| 6 | 121 | 82 | 36 | 147 | 66 | 204 | 158 | 72 |
| 7 | 202 | 204 | 286 | 288 | 87 | 29 | 94 | 109 |
| 8 | 265 | 368 | 364 | 329 | 338 | 385 | 155 | 80 |
| 9 | 535 | 631 | 501 | 427 | 583 | 551 | 316 | 152 |
| 10 | 670 | 669 | 733 | 666 | 675 | 479 | 434 | 368 |
| 11 | 711 | 642 | 692 | 702 | 683 | 483 | 702 | 650 |
| 12 | 577 | 702 | 525 | 660 | 739 | 472 | 659 | 639 |
| 13 | 794 | 773 | 626 | 786 | 718 | 567 | 495 | 628 |
| 14 | 638 | 623 | 618 | 555 | 644 | 881 | 698 | 773 |
| 15 | 738 | 747 | 684 | 531 | 594 | 572 | 511 | 673 |
| 16 | 869 | 551 | 498 | 733 | 677 | 562 | 674 | 695 |
| 17 | 578 | 414 | 511 | 826 | 662 | 666 | 624 | 650 |
| 18 | 514 | 695 | 679 | 645 | 478 | 658 | 497 | 596 |
| 19 | 542 | 648 | 601 | 615 | 654 | 886 | 728 | 548 |
| 20 | 544 | 576 | 624 | 621 | 613 | 672 | 683 | 648 |
| 21 | 535 | 653 | 600 | 565 | 693 | 607 | 651 | 693 |
| 22 | 633 | 755 | 663 | 732 | 812 | 704 | 688 | 776 |
| 23 | 799 | 625 | 731 | 778 | 605 | 669 | 663 | 684 |
| 24 | 688 | 710 | 735 | 563 | 588 | 826 | 510 | 276 |
| 25 | 125 | 422 | 594 | 532 | 424 | 390 | 101 | 168 |
| 26 | 25 | 115 | 178 | 132 | 126 | 176 | 59 | 29 |
| 27 | 47 | 140 | 54 | 62 | 113 | 85 | 179 | 97 |
| 28 | 236 | 69 | 163 | 190 | 71 | 201 | 73 | 26 |
| 29 | 73 | 90 | 59 | 74 | 112 | 175 | 115 | 118 |
| 30 | 33 | 150 | 18 | 113 | 132 | 218 | 125 | 63 |
| 31 | 90 | 83 | 181 | 109 | 163 | 144 | 47 | 159 |
| 32 | 183 | 141 | 133 | 87 | 136 | 109 | 73 | 51 |
| 33 | 77 | 30 | 77 | 111 | 92 | 97 | 77 | 112 |

Columns 25 to 32

| | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 |
|---|---|---|---|---|---|---|---|---|
| 1 | 83 | 98 | 52 | 73 | 15 | 188 | 101 | 114 |
| 2 | 119 | 40 | 141 | 167 | 32 | 104 | 81 | 184 |
| 3 | 193 | 57 | 108 | 36 | 55 | 71 | 95 | 149 |
| 4 | 103 | 281 | 114 | 208 | 78 | 176 | 113 | 170 |
| 5 | 137 | 88 | 62 | 152 | 123 | 117 | 143 | 117 |
| 6 | 108 | 184 | 128 | 42 | 103 | 117 | 287 | 91 |
| 7 | 34 | 127 | 68 | 103 | 75 | 189 | 87 | 370 |
| 8 | 44 | 121 | 75 | 66 | 54 | 217 | 159 | 299 |
| 9 | 45 | 94 | 58 | 147 | 137 | 182 | 134 | 111 |
| 10 | 121 | 116 | 105 | 44 | 77 | 135 | 84 | 164 |
| 11 | 515 | 57 | 123 | 122 | 211 | 61 | 89 | 40 |
| 12 | 592 | 261 | 80 | 80 | 293 | 97 | 168 | 24 |
| 13 | 647 | 589 | 101 | 124 | 297 | 104 | 66 | 134 |
| 14 | 726 | 659 | 183 | 91 | 214 | 220 | 116 | 191 |
| 15 | 513 | 460 | 292 | 266 | 73 | 114 | 141 | 199 |
| 16 | 451 | 631 | 521 | 141 | 88 | 151 | 89 | 86 |
| 17 | 704 | 832 | 449 | 69 | 124 | 4 | 93 | 75 |
| 18 | 865 | 821 | 461 | 125 | 111 | 191 | 76 | 92 |
| 19 | 667 | 704 | 554 | 59 | 43 | 223 | 140 | 128 |
| 20 | 768 | 579 | 98 | 156 | 104 | 24 | 228 | 47 |
| 21 | 767 | 575 | 147 | 32 | 142 | 121 | 176 | 170 |
| 22 | 527 | 140 | 74 | 76 | 205 | 60 | 70 | 111 |
| 23 | 232 | 124 | 70 | 112 | 120 | 130 | 54 | 169 |
| 24 | 101 | 111 | 15 | 93 | 142 | 88 | 78 | 165 |
| 25 | 28 | 120 | 165 | 130 | 176 | 45 | 105 | 137 |
| 26 | 64 | 60 | 22 | 56 | 46 | 116 | 41 | 97 |
| 27 | 24 | 83 | 86 | 102 | 70 | 136 | 83 | 62 |
| 28 | 236 | 177 | 70 | 74 | 88 | 212 | 75 | 42 |
| 29 | 20 | 207 | 98 | 104 | 34 | 74 | 53 | 119 |
| 30 | 130 | 148 | 167 | 159 | 218 | 116 | 199 | 238 |
| 31 | 71 | 39 | 69 | 116 | 54 | 140 | 50 | 143 |
| 32 | 66 | 79 | 125 | 127 | 85 | 184 | 132 | 187 |
| 33 | 102 | 16 | 58 | 61 | 135 | 257 | 66 | 66 |

Columns 33 to 40

| | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |
|---|---|---|---|---|---|---|---|---|
| 1 | 119 | 161 | 193 | 147 | 62 | 86 | 88 | 42 |
| 2 | 48 | 103 | 339 | 86 | 64 | 147 | 150 | 43 |
| 3 | 30 | 244 | 205 | 5 | 162 | 132 | 32 | 102 |
| 4 | 112 | 224 | 86 | 101 | 139 | 53 | 97 | 119 |
| 5 | 39 | 141 | 17 | 72 | 66 | 123 | 139 | 129 |
| 6 | 44 | 291 | 145 | 178 | 2 | 89 | 81 | 94 |
| 7 | 59 | 41 | 29 | 83 | 74 | 103 | 178 | 91 |
| 8 | 215 | 0 | 38 | 0 | 348 | 144 | 66 | 63 |
| 9 | 245 | 235 | 244 | 180 | 237 | 131 | 191 | 129 |
| 10 | 143 | 134 | 68 | 108 | 56 | 74 | 147 | 128 |
| 11 | 68 | 177 | 34 | 187 | 77 | 47 | 137 | 33 |
| 12 | 18 | 41 | 54 | 70 | 38 | 102 | 15 | 148 |
| 13 | 46 | 49 | 134 | 66 | 103 | 179 | 106 | 167 |
| 14 | 118 | 136 | 111 | 130 | 74 | 154 | 111 | 290 |
| 15 | 42 | 147 | 123 | 176 | 100 | 122 | 170 | 103 |
| 16 | 82 | 112 | 57 | 98 | 90 | 96 | 124 | 209 |
| 17 | 216 | 79 | 72 | 242 | 20 | 104 | 123 | 223 |
| 18 | 94 | 27 | 47 | 88 | 36 | 81 | 124 | 131 |
| 19 | 98 | 145 | 56 | 89 | 187 | 115 | 219 | 145 |
| 20 | 248 | 99 | 255 | 118 | 130 | 43 | 180 | 133 |
| 21 | 110 | 219 | 108 | 42 | 67 | 41 | 90 | 39 |
| 22 | 96 | 70 | 78 | 79 | 93 | 192 | 53 | 112 |
| 23 | 123 | 151 | 92 | 52 | 91 | 210 | 92 | 253 |
| 24 | 126 | 203 | 131 | 159 | 64 | 32 | 233 | 140 |
| 25 | 56 | 76 | 107 | 38 | 120 | 65 | 41 | 134 |
| 26 | 78 | 53 | 106 | 14 | 96 | 124 | 109 | 77 |
| 27 | 114 | 93 | 134 | 187 | 186 | 77 | 76 | 102 |
| 28 | 166 | 101 | 107 | 53 | 82 | 81 | 26 | 57 |
| 29 | 135 | 171 | 108 | 82 | 209 | 128 | 62 | 40 |
| 30 | 47 | 267 | 136 | 21 | 138 | 53 | 165 | 129 |
| 31 | 93 | 88 | 137 | 170 | 144 | 246 | 76 | 202 |
| 32 | 94 | 248 | 97 | 94 | 69 | 52 | 36 | 118 |
| 33 | 46 | 52 | 112 | 82 | 7 | 93 | 107 | 94 |

Columns 41 to 48

| | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 |
|---|---|---|---|---|---|---|---|---|
| 1 | 195 | 77 | 79 | 132 | 114 | 75 | 57 | 35 |
| 2 | 125 | 12 | 26 | 38 | 147 | 134 | 161 | 117 |
| 3 | 31 | 101 | 77 | 272 | 249 | 116 | 220 | 83 |
| 4 | 90 | 92 | 128 | 78 | 70 | 176 | 138 | 38 |
| 5 | 122 | 64 | 98 | 194 | 91 | 92 | 159 | 94 |
| 6 | 137 | 160 | 198 | 80 | 81 | 62 | 223 | 116 |
| 7 | 140 | 125 | 92 | 85 | 77 | 73 | 318 | 243 |
| 8 | 176 | 139 | 106 | 141 | 94 | 207 | 391 | 231 |
| 9 | 120 | 105 | 186 | 203 | 210 | 327 | 542 | 370 |
| 10 | 243 | 170 | 212 | 367 | 442 | 392 | 582 | 592 |
| 11 | 213 | 215 | 230 | 279 | 387 | 397 | 552 | 693 |
| 12 | 120 | 202 | 355 | 454 | 551 | 520 | 585 | 512 |
| 13 | 260 | 316 | 351 | 440 | 585 | 579 | 703 | 522 |
| 14 | 257 | 424 | 552 | 428 | 463 | 490 | 595 | 645 |
| 15 | 203 | 398 | 665 | 466 | 450 | 462 | 456 | 491 |
| 16 | 230 | 376 | 555 | 292 | 470 | 465 | 515 | 522 |
| 17 | 343 | 429 | 577 | 420 | 653 | 664 | 539 | 570 |
| 18 | 156 | 305 | 674 | 555 | 503 | 680 | 538 | 523 |
| 19 | 43 | 253 | 650 | 628 | 500 | 598 | 582 | 574 |
| 20 | 152 | 247 | 395 | 442 | 602 | 485 | 395 | 512 |
| 21 | 154 | 43 | 266 | 511 | 695 | 562 | 418 | 494 |
| 22 | 73 | 163 | 109 | 417 | 688 | 658 | 540 | 569 |
| 23 | 236 | 249 | 40 | 144 | 309 | 566 | 583 | 602 |
| 24 | 165 | 154 | 117 | 132 | 78 | 65 | 307 | 690 |
| 25 | 97 | 174 | 134 | 22 | 88 | 96 | 82 | 166 |
| 26 | 63 | 116 | 62 | 130 | 106 | 35 | 36 | 118 |
| 27 | 89 | 116 | 20 | 78 | 164 | 85 | 114 | 49 |
| 28 | 59 | 215 | 81 | 99 | 65 | 69 | 214 | 50 |
| 29 | 31 | 104 | 48 | 118 | 191 | 76 | 34 | 67 |
| 30 | 75 | 119 | 120 | 96 | 212 | 141 | 61 | 85 |
| 31 | 109 | 66 | 120 | 10 | 114 | 46 | 120 | 62 |
| 32 | 68 | 254 | 167 | 77 | 121 | 102 | 160 | 109 |
| 33 | 32 | 89 | 111 | 134 | 19 | 120 | 119 | 43 |

Columns 49 to 56

| | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 |
|---|---|---|---|---|---|---|---|---|
| 1 | 254 | 71 | 70 | 97 | 141 | 67 | 211 | 159 |
| 2 | 83 | 68 | 140 | 161 | 258 | 76 | 181 | 48 |
| 3 | 106 | 91 | 105 | 143 | 83 | 161 | 126 | 30 |
| 4 | 99 | 116 | 90 | 110 | 65 | 75 | 102 | 103 |
| 5 | 83 | 118 | 111 | 113 | 121 | 78 | 136 | 150 |
| 6 | 103 | 223 | 93 | 34 | 127 | 116 | 116 | 97 |
| 7 | 233 | 420 | 246 | 93 | 82 | 48 | 107 | 35 |
| 8 | 334 | 387 | 285 | 400 | 358 | 128 | 188 | 70 |
| 9 | 543 | 375 | 321 | 465 | 261 | 143 | 59 | 62 |
| 10 | 475 | 344 | 421 | 640 | 602 | 486 | 327 | 194 |
| 11 | 385 | 463 | 557 | 607 | 684 | 573 | 588 | 532 |
| 12 | 505 | 720 | 663 | 672 | 674 | 586 | 528 | 461 |
| 13 | 456 | 567 | 470 | 613 | 552 | 458 | 551 | 603 |
| 14 | 561 | 764 | 734 | 578 | 486 | 575 | 664 | 618 |
| 15 | 343 | 411 | 389 | 504 | 586 | 589 | 563 | 570 |
| 16 | 522 | 482 | 336 | 647 | 640 | 388 | 560 | 561 |
| 17 | 623 | 613 | 550 | 523 | 610 | 415 | 575 | 684 |
| 18 | 565 | 592 | 623 | 413 | 508 | 469 | 506 | 571 |
| 19 | 523 | 538 | 577 | 675 | 669 | 421 | 631 | 509 |
| 20 | 493 | 496 | 655 | 644 | 632 | 506 | 549 | 716 |
| 21 | 600 | 696 | 855 | 559 | 464 | 679 | 487 | 546 |
| 22 | 680 | 586 | 653 | 653 | 476 | 604 | 658 | 425 |
| 23 | 656 | 584 | 577 | 648 | 503 | 563 | 460 | 80 |
| 24 | 739 | 604 | 703 | 569 | 367 | 281 | 195 | 117 |
| 25 | 245 | 160 | 353 | 301 | 140 | 31 | 91 | 100 |
| 26 | 72 | 108 | 141 | 83 | 170 | 139 | 72 | 34 |
| 27 | 281 | 160 | 10 | 62 | 56 | 90 | 32 | 48 |
| 28 | 94 | 70 | 34 | 204 | 85 | 107 | 105 | 101 |
| 29 | 128 | 68 | 112 | 16 | 51 | 186 | 41 | 107 |
| 30 | 112 | 117 | 119 | 128 | 31 | 118 | 49 | 161 |
| 31 | 110 | 130 | 108 | 83 | 75 | 149 | 114 | 61 |
| 32 | 45 | 146 | 138 | 112 | 49 | 34 | 20 | 17 |
| 33 | 60 | 134 | 77 | 46 | 208 | 90 | 55 | 6 |

Columns 57 to 64

| | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 |
|---|---|---|---|---|---|---|---|---|
| 1 | 120 | 99 | 74 | 68 | 26 | 46 | 251 | 117 |
| 2 | 159 | 110 | 128 | 62 | 78 | 21 | 108 | 179 |
| 3 | 155 | 101 | 121 | 105 | 68 | 108 | 175 | 33 |
| 4 | 106 | 114 | 79 | 235 | 48 | 100 | 107 | 86 |
| 5 | 153 | 135 | 119 | 96 | 44 | 72 | 63 | 130 |
| 6 | 175 | 134 | 100 | 171 | 154 | 98 | 25 | 68 |
| 7 | 54 | 211 | 103 | 111 | 60 | 83 | 26 | 51 |
| 8 | 74 | 212 | 91 | 95 | 80 | 34 | 86 | 138 |
| 9 | 59 | 46 | 41 | 108 | 26 | 191 | 59 | 110 |
| 10 | 95 | 45 | 122 | 236 | 85 | 172 | 149 | 65 |
| 11 | 240 | 91 | 181 | 148 | 151 | 150 | 106 | 143 |
| 12 | 233 | 76 | 137 | 244 | 95 | 77 | 23 | 192 |
| 13 | 402 | 75 | 77 | 131 | 166 | 104 | 26 | 178 |
| 14 | 612 | 267 | 39 | 138 | 116 | 52 | 50 | 140 |
| 15 | 673 | 232 | 68 | 48 | 107 | 298 | 43 | 59 |
| 16 | 705 | 411 | 111 | 121 | 108 | 152 | 55 | 90 |
| 17 | 753 | 443 | 76 | 13 | 124 | 95 | 134 | 56 |
| 18 | 718 | 321 | 110 | 156 | 67 | 143 | 94 | 48 |
| 19 | 534 | 238 | 57 | 60 | 48 | 139 | 36 | 19 |
| 20 | 650 | 244 | 154 | 117 | 99 | 181 | 129 | 57 |
| 21 | 462 | 142 | 81 | 53 | 22 | 62 | 129 | 95 |
| 22 | 152 | 191 | 83 | 124 | 191 | 64 | 110 | 141 |
| 23 | 201 | 50 | 142 | 90 | 94 | 118 | 77 | 66 |
| 24 | 88 | 162 | 67 | 119 | 152 | 96 | 190 | 109 |
| 25 | 52 | 127 | 148 | 45 | 89 | 137 | 76 | 30 |
| 26 | 141 | 44 | 98 | 114 | 248 | 101 | 95 | 101 |
| 27 | 82 | 52 | 312 | 153 | 132 | 73 | 140 | 47 |
| 28 | 10 | 34 | 95 | 132 | 97 | 82 | 125 | 42 |
| 29 | 169 | 182 | 234 | 100 | 13 | 269 | 115 | 170 |
| 30 | 138 | 61 | 151 | 56 | 79 | 143 | 93 | 102 |
| 31 | 54 | 40 | 138 | 109 | 79 | 127 | 4 | 40 |
| 32 | 90 | 38 | 64 | 28 | 44 | 84 | 67 | 115 |
| 33 | 70 | 54 | 159 | 185 | 28 | 32 | 85 | 157 |

Columns 65 to 72

| | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 |
|---|---|---|---|---|---|---|---|---|
| 1 | 71 | 180 | 36 | 80 | 164 | 41 | 97 | 78 |
| 2 | 85 | 87 | 153 | 76 | 29 | 83 | 62 | 114 |
| 3 | 190 | 22 | 150 | 119 | 74 | 75 | 42 | 166 |
| 4 | 82 | 124 | 157 | 152 | 130 | 53 | 119 | 135 |
| 5 | 117 | 74 | 46 | 263 | 164 | 199 | 238 | 47 |
| 6 | 108 | 97 | 56 | 50 | 173 | 50 | 143 | 93 |
| 7 | 169 | 123 | 27 | 0 | 43 | 132 | 187 | 94 |
| 8 | 159 | 63 | 58 | 158 | 37 | 66 | 66 | 46 |
| 9 | 175 | 138 | 59 | 145 | 68 | 171 | 45 | 121 |
| 10 | 115 | 151 | 133 | 35 | 68 | 111 | 19 | 106 |
| 11 | 195 | 198 | 89 | 72 | 141 | 236 | 112 | 186 |
| 12 | 92 | 86 | 54 | 68 | 176 | 118 | 167 | 125 |
| 13 | 201 | 154 | 77 | 24 | 111 | 234 | 103 | 202 |
| 14 | 85 | 48 | 106 | 140 | 101 | 288 | 29 | 32 |
| 15 | 68 | 167 | 68 | 71 | 76 | 58 | 58 | 244 |
| 16 | 245 | 48 | 166 | 66 | 122 | 43 | 129 | 69 |
| 17 | 160 | 76 | 82 | 71 | 61 | 212 | 76 | 172 |
| 18 | 98 | 249 | 82 | 116 | 76 | 26 | 124 | 222 |
| 19 | 6 | 90 | 131 | 6 | 89 | 26 | 94 | 83 |
| 20 | 127 | 92 | 52 | 151 | 28 | 69 | 122 | 30 |
| 21 | 75 | 106 | 215 | 138 | 160 | 63 | 109 | 110 |
| 22 | 65 | 79 | 142 | 180 | 61 | 166 | 72 | 172 |
| 23 | 73 | 92 | 102 | 96 | 57 | 150 | 122 | 115 |
| 24 | 53 | 53 | 179 | 63 | 67 | 51 | 61 | 95 |
| 25 | 54 | 47 | 76 | 161 | 96 | 70 | 150 | 66 |
| 26 | 123 | 51 | 129 | 118 | 122 | 72 | 202 | 115 |
| 27 | 168 | 61 | 92 | 164 | 43 | 146 | 153 | 18 |
| 28 | 117 | 181 | 78 | 89 | 56 | 129 | 90 | 20 |
| 29 | 58 | 141 | 82 | 30 | 96 | 82 | 77 | 59 |
| 30 | 117 | 195 | 254 | 155 | 129 | 31 | 109 | 153 |
| 31 | 91 | 152 | 141 | 204 | 116 | 101 | 9 | 68 |
| 32 | 128 | 114 | 139 | 89 | 48 | 121 | 37 | 27 |
| 33 | 30 | 99 | 74 | 165 | 43 | 103 | 169 | 79 |

Columns 73 to 80

| | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 |
|---|---|---|---|---|---|---|---|---|
| 1 | 53 | 106 | 14 | 96 | 124 | 109 | 77 | 63 |
| 2 | 93 | 134 | 187 | 186 | 77 | 76 | 102 | 89 |
| 3 | 101 | 107 | 53 | 82 | 81 | 26 | 57 | 59 |
| 4 | 171 | 108 | 82 | 209 | 128 | 62 | 40 | 31 |
| 5 | 267 | 136 | 21 | 138 | 53 | 165 | 129 | 75 |
| 6 | 88 | 137 | 170 | 144 | 246 | 76 | 202 | 109 |
| 7 | 248 | 97 | 94 | 69 | 52 | 36 | 118 | 68 |
| 8 | 52 | 112 | 82 | 7 | 93 | 107 | 94 | 32 |
| 9 | 84 | 66 | 172 | 199 | 76 | 68 | 150 | 257 |
| 10 | 20 | 88 | 97 | 79 | 102 | 191 | 369 | 265 |
| 11 | 32 | 45 | 29 | 138 | 314 | 488 | 540 | 578 |
| 12 | 157 | 82 | 233 | 599 | 647 | 607 | 460 | 418 |
| 13 | 149 | 350 | 589 | 665 | 627 | 554 | 657 | 553 |
| 14 | 40 | 531 | 626 | 411 | 615 | 496 | 522 | 638 |
| 15 | 438 | 596 | 475 | 407 | 601 | 455 | 401 | 582 |
| 16 | 578 | 537 | 412 | 591 | 565 | 391 | 312 | 474 |
| 17 | 482 | 550 | 568 | 536 | 546 | 522 | 395 | 521 |
| 18 | 479 | 501 | 545 | 447 | 484 | 502 | 487 | 538 |
| 19 | 451 | 415 | 472 | 468 | 420 | 577 | 551 | 470 |
| 20 | 608 | 659 | 569 | 578 | 394 | 589 | 617 | 521 |
| 21 | 485 | 590 | 471 | 602 | 619 | 402 | 461 | 575 |
| 22 | 113 | 442 | 500 | 572 | 690 | 509 | 620 | 546 |
| 23 | 75 | 237 | 528 | 535 | 573 | 410 | 536 | 519 |
| 24 | 19 | 91 | 400 | 410 | 498 | 575 | 632 | 612 |
| 25 | 25 | 84 | 43 | 301 | 419 | 390 | 564 | 457 |
| 26 | 119 | 139 | 201 | 214 | 366 | 133 | 336 | 488 |
| 27 | 92 | 40 | 60 | 9 | 115 | 161 | 174 | 267 |
| 28 | 42 | 30 | 114 | 80 | 172 | 113 | 22 | 106 |
| 29 | 106 | 45 | 17 | 74 | 123 | 210 | 141 | 149 |
| 30 | 109 | 73 | 84 | 100 | 38 | 61 | 45 | 161 |
| 31 | 136 | 36 | 105 | 63 | 86 | 169 | 90 | 73 |
| 32 | 71 | 101 | 205 | 72 | 92 | 39 | 71 | 155 |
| 33 | 44 | 112 | 45 | 46 | 67 | 67 | 94 | 165 |

Columns 81 to 88

| | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 |
|---|---|---|---|---|---|---|---|---|
| 1 | 116 | 62 | 130 | 106 | 35 | 36 | 118 | 72 |
| 2 | 116 | 20 | 78 | 164 | 85 | 114 | 49 | 281 |
| 3 | 215 | 81 | 99 | 65 | 69 | 214 | 50 | 94 |
| 4 | 104 | 48 | 118 | 191 | 76 | 34 | 67 | 128 |
| 5 | 119 | 120 | 96 | 212 | 141 | 61 | 85 | 112 |
| 6 | 66 | 120 | 10 | 114 | 46 | 120 | 62 | 110 |
| 7 | 254 | 167 | 77 | 121 | 102 | 160 | 109 | 45 |
| 8 | 89 | 111 | 134 | 19 | 120 | 119 | 43 | 60 |
| 9 | 145 | 220 | 58 | 144 | 66 | 75 | 78 | 84 |
| 10 | 501 | 372 | 214 | 299 | 136 | 98 | 68 | 190 |
| 11 | 697 | 598 | 523 | 458 | 420 | 290 | 51 | 126 |
| 12 | 604 | 559 | 477 | 428 | 577 | 641 | 312 | 15 |
| 13 | 652 | 524 | 467 | 450 | 636 | 706 | 423 | 361 |
| 14 | 662 | 394 | 391 | 457 | 605 | 560 | 601 | 627 |
| 15 | 555 | 463 | 458 | 351 | 596 | 551 | 524 | 472 |
| 16 | 503 | 406 | 540 | 584 | 709 | 547 | 523 | 664 |
| 17 | 649 | 390 | 392 | 713 | 454 | 441 | 524 | 512 |
| 18 | 510 | 348 | 312 | 610 | 294 | 361 | 516 | 358 |
| 19 | 530 | 495 | 415 | 623 | 442 | 429 | 524 | 339 |
| 20 | 626 | 657 | 768 | 812 | 484 | 618 | 638 | 373 |
| 21 | 580 | 431 | 596 | 681 | 550 | 584 | 532 | 374 |
| 22 | 526 | 409 | 468 | 588 | 648 | 478 | 474 | 493 |
| 23 | 451 | 486 | 537 | 505 | 577 | 603 | 432 | 451 |
| 24 | 624 | 637 | 590 | 591 | 483 | 481 | 238 | 250 |
| 25 | 514 | 623 | 528 | 535 | 406 | 423 | 290 | 319 |
| 26 | 430 | 545 | 509 | 299 | 169 | 203 | 188 | 222 |
| 27 | 340 | 236 | 253 | 288 | 208 | 32 | 107 | 79 |
| 28 | 221 | 55 | 128 | 28 | 164 | 103 | 60 | 150 |
| 29 | 94 | 12 | 121 | 107 | 197 | 76 | 152 | 91 |
| 30 | 123 | 39 | 155 | 92 | 86 | 76 | 83 | 24 |
| 31 | 58 | 109 | 52 | 80 | 133 | 108 | 67 | 55 |
| 32 | 124 | 83 | 31 | 71 | 145 | 88 | 250 | 36 |
| 33 | 88 | 14 | 109 | 148 | 195 | 51 | 76 | 109 |

Columns 89 to 97

| | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 108 | 141 | 83 | 170 | 139 | 72 | 34 | 141 | 44 |
| 2 | 160 | 10 | 62 | 56 | 90 | 32 | 48 | 82 | 52 |
| 3 | 70 | 34 | 204 | 85 | 107 | 105 | 101 | 10 | 34 |
| 4 | 68 | 112 | 16 | 51 | 186 | 41 | 107 | 169 | 182 |
| 5 | 117 | 119 | 128 | 31 | 118 | 49 | 161 | 138 | 61 |
| 6 | 130 | 108 | 83 | 75 | 149 | 114 | 61 | 54 | 40 |
| 7 | 146 | 138 | 112 | 49 | 34 | 20 | 17 | 90 | 38 |
| 8 | 134 | 77 | 46 | 208 | 90 | 55 | 6 | 70 | 54 |
| 9 | 82 | 70 | 69 | 78 | 106 | 100 | 94 | 37 | 74 |
| 10 | 171 | 91 | 175 | 80 | 114 | 134 | 92 | 134 | 69 |
| 11 | 48 | 66 | 104 | 109 | 198 | 151 | 110 | 43 | 169 |
| 12 | 137 | 66 | 82 | 13 | 95 | 125 | 108 | 131 | 39 |
| 13 | 140 | 147 | 88 | 105 | 161 | 57 | 173 | 56 | 102 |
| 14 | 191 | 89 | 78 | 99 | 157 | 68 | 43 | 155 | 51 |
| 15 | 261 | 172 | 162 | 149 | 70 | 214 | 5 | 248 | 116 |
| 16 | 453 | 295 | 109 | 92 | 77 | 60 | 255 | 159 | 93 |
| 17 | 375 | 347 | 126 | 82 | 50 | 104 | 105 | 105 | 71 |
| 18 | 262 | 168 | 134 | 66 | 92 | 71 | 167 | 94 | 138 |
| 19 | 286 | 191 | 116 | 170 | 137 | 35 | 178 | 141 | 96 |
| 20 | 420 | 230 | 112 | 116 | 60 | 66 | 57 | 222 | 32 |
| 21 | 381 | 155 | 108 | 193 | 88 | 66 | 109 | 191 | 106 |
| 22 | 357 | 62 | 96 | 71 | 74 | 18 | 131 | 60 | 96 |
| 23 | 423 | 113 | 83 | 182 | 107 | 163 | 111 | 190 | 62 |
| 24 | 97 | 231 | 160 | 99 | 20 | 118 | 140 | 124 | 70 |
| 25 | 87 | 138 | 147 | 213 | 71 | 101 | 169 | 126 | 65 |
| 26 | 82 | 140 | 72 | 166 | 118 | 26 | 137 | 132 | 192 |
| 27 | 129 | 185 | 120 | 76 | 50 | 128 | 90 | 78 | 100 |
| 28 | 201 | 88 | 195 | 251 | 101 | 59 | 155 | 234 | 22 |
| 29 | 87 | 53 | 9 | 90 | 93 | 224 | 268 | 187 | 13 |
| 30 | 42 | 173 | 147 | 139 | 196 | 210 | 123 | 20 | 108 |
| 31 | 34 | 58 | 39 | 132 | 158 | 93 | 158 | 81 | 210 |
| 32 | 96 | 65 | 99 | 79 | 117 | 64 | 139 | 43 | 116 |
| 33 | 90 | 248 | 54 | 50 | 65 | 215 | 58 | 149 | 69 |