NMR-guided fragment-based approach for the design of tRNA(Lys3) ligands.

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RNA is a key player in many cellular processes or in viral infection and is thus an attractive target in drug discovery. The clinical utility of antibiotic drugs targeting the bacterial ribosome has demonstrated that RNA can indeed be a relevant target. Among other druggable RNAs, functional sites within the genomic RNA of HIV-1, such as TAR, RRE or the dimerisation initiation site, have been selected as possible targets for new anti-viral strategies.

Like all retroviruses, HIV-1 uses a cellular tRNA, tRNA$_{\text{Lys}}$ for priming reverse transcription. In virions, the 18 3'-terminal nucleotides of tRNA$_{\text{Lys}}$ are base-paired to the genomic RNA. This complex in turn specifically recruits the reverse transcriptase. The aim of this study was to find molecules that bind to tRNA$_{\text{Lys}}$ and serve as leads for inhibitors of the formation of the HIV-1 reverse transcription initiation complex. Indeed, destabilisation of this process by oligodeoxyribonucleotides has been reported to result in an efficient inhibition of reverse transcription, providing the proof of concept of a possible new antiviral strategy.

Despite recent breakthroughs in the understanding of the interaction between aminoglycosides and ribosomal RNAs, the de novo design of new compounds that specifically bind to structured RNAs is still a standing challenge. A recent promising approach for the synthesis of shape-specific 2-deoxystreptamine (2-DOS) dimers that can bind selectively to RNA loops has been reported. Rational design of selective tRNA$_{\text{Lys}}$ binder is however difficult because of the lack of known ligands and of its shape-similarity to other tRNAs. A combinatorial approach only led to low-affinity structure-specific peptidic ligands. However, the availability of crystallographic and NMR data and of an efficient expression system made it possible to undertake a fragment-based approach.

We used NMR screening to identify ligands from spectral changes induced by their binding on the target. A primary screen was performed over a focussed collection of 50 in-house or commercial organic compounds using 1D NMR on the RNA imino proton window (10-15 ppm), which is devoid of ligand signals. As result, compounds 1 and 2 were detected to bind to tRNA$_{\text{Lys}}$ (Figure 1).

Their NMR footprints on the target were then determined on 2D HMQC spectra using 15N-labeled tRNA. Kynuramine 2 interacts with the D-stem (Figure 2), and 1 exhibits binding to at least two specific sites located in the T and D-stems. From NMR titrations, the apparent dissociation constants of 1 and 2 for tRNA$_{\text{Lys}}$ were estimated to be 2mM and 5mM, respectively.

A qualitative specificity study was conducted with these two compounds by analysing their NMR footprints with two other tRNAs: E. coli tRNA$_{\text{Met}}$ and tRNA$_{\text{m}}$. 1 binds to both tRNA which have very similar T-arms sequences. Interestingly, 2 only binds to the D-arm of tRNA$_{\text{m}}$, showing that this compound, despite its moderate affinity, appears to interact in a sequence-specific manner with tRNA$_{\text{m}}$ and tRNA$_{\text{Lys}}$, with identical D-stems (Figure 4). Structural elements required for the binding of kynuramine 2 were then investigated (Scheme 1). Qualitative comparison of chemical shift perturbations indicated that binding
was improved when the aniline moiety was changed for other heterocycles, except for compounds 4, 12 and 16. Reduction of the carbonyl function to the corresponding alcohol led to the loss of interaction. Binding of 3 was not improved by introduction of an extra nitrogen atom on the side chain (compound 13).

A library of connected fragments was prepared using a one-pot procedure leading to compounds 17a-h in 6-66% overall yield after a catch-and-release purification on sulfonic acid resin (Scheme 2). Binding of these compounds to tRNA<sub>Lys</sub><sup>3</sup> was then monitored in a TROSY experiment, showing improved affinity and specificity with the D-arm region (Figure 3 for 17f) under stoichiometric conditions.

![Figure 3](image-url)

Fluorescence binding assays were then conducted on tRNA<sub>Lys</sub><sup>3</sup>, tRNA<sub>Met</sub> and tRNA<sub>f</sub>Met leading to a Kd of 1.8 μM for compound 17f with tRNA<sub>Lys</sub><sup>3</sup>, at physiological ionic strength. Interestingly, compound 17f exhibited a significant sequence selectivity for the D-arm of tRNA<sub>Lys</sub><sup>3</sup> and tRNA<sub>Met</sub> against that of tRNA<sub>f</sub>Met (Table 1 and Figure 4). This specificity is correlated with a reduced ionic-strength dependence of the interaction with tRNA<sub>Lys</sub><sup>3</sup>. The strong ionic-dependence of the affinity to tRNA<sub>Met</sub> reflects poorly selective binding, dominated by electrostatic effects.[19]

![Figure 4](image-url)
In conclusion, a selective ligand of tRNA$_{\text{Lys}}^{33}$, with micromolar dissociation constant has been synthesised for the first time. This study outlines the power of a fragment-based strategy in the field of RNA-ligand discovery, providing potential lead compounds for antiviral drug development. The use of compound 17f as an inhibitor of reverse transcription in cell-free assays will be investigated. Beside this important application, this work provides new information on the design of small RNA interacting molecules. This step is crucial for achieving a challenging goal: the cellular regulation at RNA level by small molecular effectors.

**Table 1.** Dissociation constants of compound 17f for three different tRNAs at various ionic strengths.

| KCl (150 mM) | 1.8 ± 0.9 | 13.2 ± 5.9 | 4.1 ± 0.9 |
| KCl (50 mM)  | 1.1 ± 0.3  | 2.5 ± 0.8  | 1.1 ± 0.2  |
| no KCl       | 0.3 ± 0.1  | -          | -          |

Kd (μM)

In conclusion, a selective ligand of tRNA$_{\text{Lys}}^{33}$, with micromolar dissociation constant has been synthesised for the first time. This study outlines the power of a fragment-based strategy in the field of RNA-ligand discovery, providing potential lead compounds for antiviral drug development. The use of compound 17f as an inhibitor of reverse transcription in cell-free assays will be investigated. Beside this important application, this work provides new information on the design of small RNA interacting molecules. This step is crucial for achieving a challenging goal: the cellular regulation at RNA level by small molecular effectors.

**Keywords:** RNA recognition · tRNA · aminoglycoside mimics · Drug design · Fragment-based synthesis
An NMR-guided fragment-based approach has been used for the synthesis of a micromolar tRNA$^{\text{Lys}}_3$ D-stem binder from two millimolar ligands detected by flow-injection NMR screening.
Supporting information

NMR-guided fragment-based approach for the design of tRNA\textsuperscript{Lys}\textsubscript{3} ligands

Florence Chung, Carine Tisné,\textsuperscript{*} Thomas Lecourt, Frédéric Dardel\textsuperscript{*} and Laurent Micouin\textsuperscript{*}

Chemistry

Compound 1 and its N-Boc derivative were prepared according to reported procedures.\textsuperscript{[1]} Kynuramine 2 was purchased from Sigma.

Typical procedure for the synthesis of Mannich Base:

Paraformaldehyde (1.43 g, 1 equiv.), dimethylamine hydrochloride (11.64 g, 3 equiv.) and acetophenone (13 mL, 2.3 equiv.) were dissolved in ethanol (10 mL). Hydrochloric acid 35% (0.2 mL) was added and the mixture refluxed for 5 hours. The yellowish solution was diluted with cold acetone (50 mL) and chilled for several hours at 0 °C. The crystals were filtrated, washed with acetone (2x20 mL), dissolved in water (20 mL) and then extracted in ethyl acetate (2x35 mL). The aqueous layer was treated with potassium carbonate (pH=10) and extracted in ethyl acetate (5x35 mL). The organic phases were dried over sulphate magnesium and concentrated under reduced pressure to give the Mannich base as an oil (77%).

![Mannich Base](image)

\textsuperscript{1}H NMR \(\delta\) 2.19 (s, 6H), 2.66 (t, 2H, \(J = 7.3\) Hz), 3.05 (t, 2H, \(J = 7.3\) Hz), 7.35 (t, 2H, \(J = 7.4\) Hz), 7.44 (t, 1H, \(J = 7.4\) Hz), 7.86 (d, 2H, \(J = 7.1\) Hz); \textsuperscript{13}C NMR \(\delta\) 36.9, 45.5, 54.4, 128.1, 128.6, 133.0, 137.0, 199.0, MS (ESI): 178 [M+H]\textsuperscript{+}.

![Nitro Compound](image)

\textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\) 2.21 (s, 6H), 2.70 (t, 2H, \(J = 7.2\) Hz), 2.94 (t, 2H, \(J = 7.2\) Hz), 7.42 (dd, 1H, \(J = 7.5, 1.3\) Hz), 7.58 (td, 1H, \(J = 7.5, 1.3\) Hz), 7.70 (td, 1H, \(J = 7.5, 1.0\) Hz), 8.1 (dd, 1H, \(J = 7.5, 1.0\) Hz); \textsuperscript{13}C NMR (CDCl\textsubscript{3}) \(\delta\) 41.3, 45.3, 54.2, 124.4, 127.7, 130.5, 134.4, 138.0, 145.6, 201.8; MS (ESI): 223 [M+H]\textsuperscript{+}. 
H NMR (CDCl₃) δ 2.25 (s, 6H), 2.73 (t, 2H, J = 7.4 Hz), 3.08 (t, 2H, J = 7.4 Hz), 3.13 (s, 3H), 6.89 (d, 2H, J = 8.9 Hz), 7.90 (d, 2H, J = 8.9 Hz); ¹³C NMR (CDCl₃) δ 36.0, 45.0, 54.1, 55.0, 113.2-113.3, 129.6-129.9, 130.1, 163.0, 196.9; MS (ESI): 208 [M+H]⁺.

H NMR (CDCl₃) δ 2.17 (s, 6H), 2.64 (t, 2H, J = 7.3 Hz), 2.91 (t, 2H, J = 7.3 Hz), 6.45 (dd, 1H, J = 3.5, 1.6 Hz), 7.12 (d, 1H, J = 3.5 Hz), 7.51 (d, 1H, J = 1.6 Hz); ¹³C NMR (CDCl₃) δ 36.7, 45.4, 54.2, 112.3, 117.1, 146.4, 152.7 188.2; MS (ESI): 168 [M+H]⁺.

H NMR (CDCl₃) δ 2.10 (s, 6H), 2.61 (t, 2H, J = 7.3 Hz), 2.95 (t, 2H, J = 7.3 Hz), 6.99 (dd, 1H, J = 4.9, 3.8 Hz), 7.49 (dd, 1H, J = 4.9, 0.9 Hz), 7.60 (dd, 1H, J = 3.8, 0.9 Hz); ¹³C NMR (CDCl₃) δ 37.6, 45.4, 54.5, 128.2, 131.9, 133.7, 144.3, 191.9; MS (ESI): 184 [M+H]⁺.

H NMR (CDCl₃) δ 2.25 (s, 6H), 2.77 (t, 2H, J = 7.1 Hz), 3.30 (t, 2H, J = 7.1 Hz), 7.65 (d, 1H, J = 3.1 Hz), 7.97 (d, 1H, J = 3.1 Hz); ¹³C NMR (CDCl₃) δ 36.6, 45.3, 54.0, 126.3, 144.7, 167.0, 192.8; MS (ESI): 185 [M+H]⁺.

H NMR (CDCl₃) δ 2.26 (s, 6H), 2.69 (t, 2H, J = 7.4 Hz), 2.94 (t, 2H, J = 7.4 Hz), 3.9 (s, 3H), 6.11 (dd, 1H, J = 4.1, 2.4 Hz), 6.78 (dd, 1H, J = 2.4, 1.6 Hz), 6.97 (dd, 1H, J = 4.1, 1.6 Hz); ¹³C NMR (CDCl₃) δ 37.2, 45.4, 55.1, 107.9, 119.2, 130.7, 131.1, 189.8; MS (ESI): 181 [M+H]⁺.
O

\[ \text{ONMe}_2 \]
\[ \text{10} \]

\[^1\text{H NMR (CDCl}_3\) \delta 2.29 (s, 6H), 2.78 (t, 2H, } J = 7.2 \text{ Hz), 3.40 (t, 2H, } J = 7.2 \text{ Hz), 7.47 (td, 1H, } J = 7.7, 1.1 \text{ Hz), 7.83 (td, 1H, } J = 7.7, 1.7 \text{ Hz), 8.05 (dd, 1H, } J = 7.7, 1.1 \text{ Hz), 8.68 (dd, 1H, } J = 7.7, 1.7 \text{ Hz); } ^{13}\text{C NMR (CDCl}_3\) \delta 35.8, 45.5, 54.5, 121.9, 127.2, 136.9, 149.0, 153.4, 200.7; MS (ESI): 179 [M\text{+H}]^+.\]

O

\[ \text{ONMe}_2 \]
\[ \text{11} \]

\[^1\text{H NMR (CDCl}_3\) \delta 2.47 (s, 6H), 3.02 (t, 2H, } J = 7.3 \text{ Hz), 3.46 (t, 2H, } J = 7.3 \text{ Hz), 7.59 (m, 2H), 7.89 (m, 2H), 8.01 (m, 2H), 8.52 (s, 1H); } ^{13}\text{C NMR (CDCl}_3\) \delta 37.0, 45.6, 54.5, 123.8, 126.8, 127.8, 128.5, 129.6, 129.7, 132.5, 134.2, 135.6, 199.0; MS (ESI): 228 [M\text{+H}]^+.\]

**Typical procedure for transamination**

Compound 3 (213 mg, 1 equiv.) and methyl iodide (374 µL, 5 equiv.) were dissolved in dichloromethane (4 mL) and stirred at room temperature for 3 hours. The mixture was concentrated, dissolved in chloroform (4 mL) and stirred with \(N,N,N'\)-trimethylethyldiamine (305 µL, 2 equiv.) at 60 °C for 18 hours. The crude product was washed with AcOEt (10 mL), H\(_2\)O (2x 25 mL) and dried over MgSO\(_4\). Purification by silica gel column chromatography (AcOEt/MeOH (98/2), AcOEt/MeOH (95/5), AcOEt/MeOH (90/10)) afforded the compound (24%).

O

\[ \text{Me} \]
\[ \text{NM}_{\text{13}} \]
\[ \text{13} \]

\[^1\text{H NMR (CDCl}_3\) \delta 2.23 (s, 6H), 2.31 (s, 3H), 2.42-2.54 (2t, 4H, } J = 6.8 \text{ Hz), 2.87 (t, 2H, } J = 7.4 \text{ Hz), 3.18 (t, 2H, } J = 7.4 \text{ Hz), 7.47 (t, 2H, } J = 7.4 \text{ Hz), 7.55 (t, 1H, } J = 7.4 \text{ Hz), 7.97 (d, 2H, } J = 7.4 \text{ Hz); } ^{13}\text{C NMR (CDCl}_3\) \delta 36.3, 42.4, 45.6, 52.7, 55.3, 57.1, 127.9, 128.5, 133.0, 136.9, 199.3; MS (ESI): 235 [M\text{+H}]^+.\]
Typical procedure for nitro reduction

Compound 4 hydrochloride (69 mg, 0.27 mmol) was dissolved in methanol (4 mL) and stirred with 10% palladium hydroxide (7 mg) under H₂ atmosphere for 2 hours. The mixture was filtrated through celite and concentrated under vacuum (92%).
Protected diaminocyclopentanol (100 mg, 0.32 mmol) and propargyl bromide (105 μL, 0.96 mmol) were dissolved in 2.5 mL of anhydrous THF under argon atmosphere at 0 °C. After the slow addition of KHMDS (0.5M in toluene, 640 μL, 0.32 mmol) the mixture was stirred at room temperature for 18 hours. H₂O (10 mL) was then added and the crude was extracted by EtOAc (2x 25 mL). The organic phases were washed with brine and dried with MgSO₄. After purification by silica gel column chromatography (EtOAc:Cyclohexane 20:80, 30:70) the product was obtained as an oil (60%).

**Typical procedure for the synthesis of triazoles:**

α-bromo-2’-acetonapthone (225 mg, 2 equiv.) and sodium azide (61 mg, 2.05 equiv.) were stirred at room temperature in a 1/2 water/acetone mixture (4.5 mL) for 1 hour. Alkyne (160 mg, 1 equiv.) in acetone (1.5 mL) was then added, followed by the addition of sodium ascorbate (0.5 equiv.) and copper sulfate (0.5 equiv.). The resultant mixture was then stirred at room temperature until complete consumption of the alkyne (2 days) monitored by TLC (AcOEt:Cyclohexane 1:1). The precipitate was filtrated, rinsed with water and dried under vacuum. The crude product was then stirred for 1 hour in a 2M solution of methanolic hydrochloride (6 mL). After evaporation of the solvent, Amberlyst 15 resin (380 mg, 4 equiv.) and MeOH (6 mL) were added and stirred overnight. The resin was filtrated, rinsed with MeOH (5x5 mL), and stirred for 3 hours in a 2M solution of methanolic ammonia (6 mL). The resin was filtrated and rinsed with MeOH. Evaporation of the organic phase led to compound 17f as an oil (46%).
126.9, 127.6, 128.5, 128.9, 129.5, 130.2, 131.5, 132.5, 136.1, 144.9, 191.4; HRMS (ESI) m/z calcd for C_{20}H_{24}N_{5}O_{2} [M+H]^+: 366.1930. Found: 366.1921.

\[
\begin{align*}
\text{17a} & \quad \text{H NMR (CD}_3\text{OD)} \delta 1.95 (m, 1H), 2.35 (m, 2H), 2.78 (m, 1H), 3.73(m, 1H), 3.91 (m, 1H), 4.47 (m, 1H), 4.90 (2d, 2H, } J = 12.6 \text{ Hz), 6.41 (m, 2H), 8.11 (m, 1H), 8.30 (m, 1H), 8.50-8.40 (m, 2H), 8.53 (m, 1H), 8.72 (m, 1H), 8.91 (m, 1H);} \\
\text{13}C \text{ NMR (CD}_3\text{OD)} \delta 32.7, 34, 48.5, 55.1, 57, 61.5, 80.5, 125.7, 126.4, 132.0, 134.8, 140.6, 142.4, 144.9, 147.3, 184; \text{ MS (ESI): 399 } [M+H]^+.
\end{align*}
\]

\[
\begin{align*}
\text{17b} & \quad \text{H NMR (400 MHz, CD}_3\text{OD, 50°C) } \delta 2.07 (m, 1H), 2.57 (m, 2H), 2.80 (s, 3H), 3.00 (dt, } J = 13.6, 6.9 \text{ Hz), 3.94 (m, 1H), 4.13 (m, 1H), 4.69 (m, 1H), 5.11 (2d, 2H, } J = 12.9 \text{ Hz), 6.32 (s, 2H), 7.74-7.83 (m, 5H), 8.62 (s, 2H);} \\
\text{13}C \text{ NMR (75 MHz, CD}_3\text{OD)} \delta 11.1, 32.6, 33.9, 47.2, 55.0, 57.0, 61.5, 80.3, 117.4, 125.5, 129.3, 134.0, 141.0, 144.6, 185.4; \text{ HRMS (ESI) m/z calcd for } C_{20}H_{26}N_{7}O_{2} [M+H]^+: 396.2148. \text{ Found: 396.2172.}
\end{align*}
\]

\[
\begin{align*}
\text{17c} & \quad \text{H NMR (CD}_3\text{OD) } \delta 1.21 (dt, 1H, } J = 13 \text{ Hz, 8.5 Hz), 1.83 (m, 1H), 2.01 (m, 1H), 2.33 (dt, 1H, } J = 13.0, 6.5 \text{ Hz), 3.20 (m, 1H), 3.43 (m, 1H), 3.80 (m, 1H), 4.66 (2d, 2H, } J = 12.3 \text{ Hz), 7.47 (m, 3H), 7.71 (m, 2H), 7.83 (m, 2H), 8.03 (s, 1H), 8.15 (m, 2H);} \\
\text{13}C \text{ NMR (CD}_3\text{OD) } \delta 38.8, 41.0, 48.5, 57, 62.1, 86.0, 125.5, 127.1, 126.9, 128.3, 128.8, 128.6, 132.8, 139.3, 144.9, 146.8, 191.0; \text{ HRMS (ESI) m/z calcd for } C_{22}H_{26}N_{7}O_{2} [M+H]^+: 392.2087. \text{ Found: 392.2103.}
\end{align*}
\]
\[ ^{1} \text{H NMR (CD}\text{OD)} \delta 1.17 (dt, 1H, J = 13.0, 8.7 Hz), 1.78 (m, 1H), 1.97 (m, 1H), 2.31 (dt, 1H, J = 13.0, 6.5 Hz), 3.15 (m, 1H), 3.38 (m, 1H), 3.76 (m, 1H), 4.65 (2d, 2H, J = 12.2 Hz), 7.29 (dd, 1H, J = 4.9, 3.7 Hz), 7.98 (d, 1H, J = 4.9 Hz), 8.06 (s, 1H), 8.09 (d, 1H, J = 3.7 Hz); \]
\[ ^{13} \text{C NMR (75 MHz, CD}\text{OD)} \delta 39.1, 41.4, 49.1, 57.0, 62.0, 86.2, 125.5, 128.5, 133.7, 135.5, 140.3, 144.9, 184.5; \]
\[ \text{HRMS (ESI) m/z calcd for C}_{14}\text{H}_{20}\text{N}_{5}\text{O}_{2}\text{S} [\text{M+H}]+: 322.1338. \text{Found: 322.1307}. \]

\[ ^{1} \text{H NMR (CD}\text{OD, 50°C)} \delta 2.18 (m, 1H), 2.55 (m, 2H), 3.00 (m, 1H), 3.10 (s, 3H), 3.92 (m, 1H), 4.13 (m, 1H), 4.67 (m, 1H), 5.06 (d+m, 2H, J = 12.7 Hz), 5.75 (s, 2H), 7.98 (m, 3H), 8.41 (m, 2H), 8.64 (s, 1H); \]
\[ ^{13} \text{C NMR (CD} \text{OD)} \delta 12.8, 29.3, 33.9, 49.5, 55.0, 58.3, 61.9, 80.0, 114.3, 126.8, 128.6, 129.1, 143, 130.2, 161.7, 176.7, 185.6; \]
\[ \text{HRMS (ESI) m/z calcd for C}_{20}\text{H}_{25}\text{N}_{6}\text{O}_{3} [\text{M+H}]+: 397.1988. \text{Found: 397.1981}. \]

\[ ^{1} \text{H NMR (CD}\text{OD)} \delta 1.30 (dt, 1H, J = 13.3, 7.9 Hz), 2.03 (m, 1H), 1.91 (m, 1H), 2.31 (dt, 1H, J = 13.3, 6.7 Hz), 3.24 (m, 1H), 3.49 (m, 1H), 3.83 (m, 1H), 4.68 (2d, 2H, J = 11.6 Hz), 7.57 (d, 1H, J = 5.4 Hz), 7.75 (d, 1H, J = 5.4 Hz), 8.04 (m, 3H), 8.63 (s, 1H); \]
\[ ^{13} \text{C NMR (CD}_{2}\text{OD)} \delta 37.9, 39.9, 48.5, 22.6, 56.8, 62.1, 84.0, 122.7, 124.1, 124.4, 125.6, 128.5, 130.6, 139.7, 144.8, 145.4, 191.4; \]
\[ \text{HRMS (ESI) m/z calcd for C}_{18}\text{H}_{22}\text{N}_{5}\text{O}_{2}\text{S} [\text{M+H}]+: 372.1494. \text{Found: 372.1463}. \]
\[17h\]

$^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 1.87 (dt, 1H, $J = 13.1, 8.9$ Hz), 2.27 (m, 2H), 2.70 (dt, 1H, $J = 13.1, 7.7$ Hz), 3.66 (m, 1H), 3.86 (m, 1H), 4.37 (m, 1H), 4.81 (2d, 2H, $J = 12.6$ Hz), 6.25 (s, 2H), 7.62 (m, 2H), 7.75 (m, 1H), 8.11 (m, 2H), 8.26 (s, 1H); $^{13}$C NMR (75 MHz, CD$_3$OD) 32.5, 33.9, 47.1, 55.0, 56.4, 61.9, 80.1, 126.7, 128, 128.8, 134.0, 134.2, 191.1; HRMS (ESI) m/z calcd for C$_{16}$H$_{22}$N$_5$O$_2$ [M+H]$^+$: 316.1774. Found: 316.1764.

Biologicals

Sample preparation

Human tRNALys3 was expressed in vivo in E. coli from a recombinant plasmid and purified as previously described.$^{[2]}$

NMR experiments

Experiments were recorded on a Bruker Avance DRX 600 spectrometer equipped with a 3mm triple-resonance flow-injection probe. The probe was connected to a Gilson 215 liquid handler controlled by the NMR console (Bruker BEST system). For tRNA:ligand mixtures, solvent suppression was achieved by using the ‘jump-and-return’ sequence to avoid the saturation of imino protons.$^{[3]}$ All NMR experiments were conducted at 15°C. Samples for 1D NMR screening contained 0.3 mM of tRNA and 1.2 mM of ligand in 10mM phosphate buffer pH 6.5, in a total volume of 200 μl in 96-well plates. The injected sample volumes were 160 μl. For ligands identified in the primary screen, $^1$H-$^{15}$N HMQC spectra$^{[4]}$ or TROSY spectra$^{[5]}$ were recorded using a sample containing 0.2 mM to 0.4 mM $^{15}$N labelled tRNA for equivalent of ligand concentration (1, 2, 4 and 7) in 10mM phosphate buffer pH 6.5. For the measurement of the dissociation constant (Kd) between kynuramine or 1 and tRNALys3, a sample containing 0.55 mM of tRNALys3 in 10mM phosphate buffer pH 6.5 was titrated by increasing concentrations of ligand in the same buffer: 2, 4, 5, 7, 10, 15 and 20 mM. Kd values were extracted by non-linear least square fitting of the variation of imino proton chemical shifts to a single-site binding hyperbolic function. Confidence limits on the Kd were estimated by Monte-Carlo sampling using the MC-fit program.$^{[6]}$
Figure 1: NMR titration of tRNA\textsubscript{Lys3} (0.55 mM) by kynuramine 2. The NMR signal of G24 imino proton is followed in 1D NMR experiment at 15°C.\textsuperscript{[3]}

Figure 2: Superimposition of HMQC spectra of tRNA\textsubscript{Lys3} alone (0.2 mM) in black and tRNA\textsubscript{Lys3} mixed with compound 1 (1.4 mM) in red, no KCl. A preferential affinity for the T-arm can be observed at a 7/1 ligand/target ratio, but shift of G15 shows a possible secondary binding site on the D-arm.
Figure 3: Superimposition of HMQC spectra of tRNA\textsubscript{Lys}\textsubscript{3} alone (0.8 mM) in black and tRNA\textsubscript{Lys}\textsubscript{3} mixed with compound 2 (1.6 mM) in red, no KCl.
Figure 4: Superimposition of TROSY spectra of tRNA<sup>Lys</sup><sub>3</sub> alone (0.3 mM) in black and tRNA<sup>Lys</sup><sub>3</sub> mixed with compound 17f (1.2 mM) in red, 150 mM KCl.
Figure 5: Superimposition of TROSY spectra of tRNA$_{\text{Lys}}^{3}$ alone (0.3 mM) in black and tRNA$_{\text{Lys}}^{3}$ mixed with compound 17f (0.3 mM) in red, 150 mM KCl.
**Fluorescence titrations**

Fluorescence titrations were conducted at 25.0°C on a JASCO spectrofluorimeter. Excitation and emission wavelengths were 341 nm and 478 nm respectively. The excitation and emission bandwidths were 5 nm.

Fluorescence titrations experiments were performed by adding increasing concentrations of nucleic acid to a fixed amount of ligand (2 μM) in buffers with different ionic strength (10 mM KPO₄ pH 6.5, for three salt concentrations, 0 mM, 50 and 150 mM NaCl). Fluorescence intensities were corrected for dilution and were fitted using equation (1). Confidence limits on the Kd were estimated by Monte-Carlo sampling using the MC-Fit program.[6]

\[
I = I_0 - I_\infty + \frac{I_0 - I_\infty}{2nN_t} \left( K_d + L_t + nN_t \left( K_d + L_t + nN_t \right)^2 - 4L_t N_t \right) \tag{1}
\]

where I₀: Fluorescence intensity without RNA, I: fluorescence intensity at a given concentration of RNA, I₀: fluorescence intensity at the plateau, n: number of RNA binding sites on the ligand, Lᵣ: total concentration of RNA, Nᵣ: total concentration of ligand.

![Diagram](image)

**Figure 6**: Comparison of fluorescence titration of compound 17f with tRNALys3 (in blue) and tRNAfMet (in red) at 150 mM KCl.
Figure 7: Fluorescence titration of compound 17f with tRNA$^{\text{Lys}}_3$, tRNA$^{\text{Met}}_i$ and tRNA$^{\text{Met}}_m$ at various ionic strengths.