RNA Recognition

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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 and in viral infection, and is therefore an attractive target in drug discovery.[1] The clinical utility of antibiotic drugs that target the bacterial ribosome has demonstrated that RNA can indeed be a relevant target.[2] Among other druggable RNA targets, functional sites within the genomic RNA of HIV-1, such as the transactivation-responsive element (TAR), the Rev-responsive element (RRE), or the dimerization-initiation site, have been selected as possible targets for new antiviral strategies.[3]

Like all retroviruses, HIV-1 uses a cellular tRNA molecule, tRNA^{Lys3}, to prime reverse transcription.[4] In virions, the 18 3′-terminal nucleotides of tRNA^{Lys3} are base paired to the genomic RNA. This complex in turn recruits the reverse transcriptase specifically.[5] The aim of this study was to find molecules that bind to tRNA^{Lys3} and serve as leads for inhibitors of the formation of the HIV-1 reverse-transcription initiation complex. The destabilization of this process by oligodeoxyribonucleotides has been reported to result in the efficient inhibition of reverse transcription, thus providing the proof of concept of a possible new antiviral strategy.[6]

Despite recent breakthroughs in the understanding of the interaction between aminoglycosides and ribosomal RNAs,[7] the de novo design of compounds that bind specifically to structured RNAs remains a challenge. A promising approach for the synthesis of shape-specific 2-deoxystreptamine (2-DOS) dimers that can bind selectively to RNA loops has been reported recently.[8] However, the rational design of selective binders of tRNA^{Lys3} is difficult because of the lack of known ligands[9] and the similarity of its shape to that of other tRNA molecules. A combinatorial approach led only to low-affinity structure-specific peptide ligands.[10] However, the availability of crystallographic[11] and NMR spectroscopic data[12,13] and of an efficient expression system made it possible to undertake a fragment-based approach.[14] We used NMR spectroscopic screening[15] to identify ligands on the basis of spectral changes induced by their binding to the target.[16] A primary screen by 1D NMR spectroscopy in the window in which signals that result from the imino hydrogen atoms of the RNA appear ($\delta = 10$–15 ppm), a region devoid of ligand signals, was performed with a focused collection of 50 organic compounds prepared in-house or obtained commercially. As a result, compounds **1** and **2** were found to bind to tRNA^{Lys3} (Figure 1).

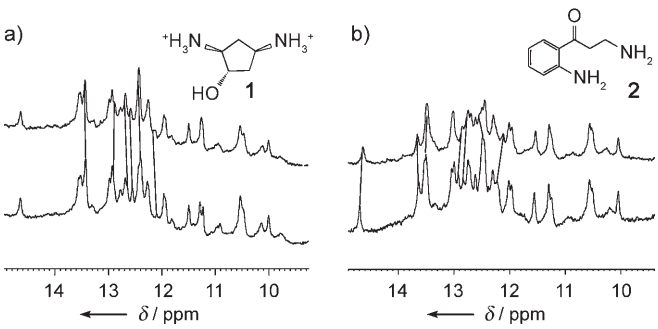

Figure 1. Binding of a) compound **1** (2.1 mM) and b) kynuramine (**2**; 0.6 mM) to tRNA^{Lys3} as monitored by NMR chemical-shift perturbation of the RNA imino protons (top spectra). The reference ^{1}H NMR spectrum of tRNA^{Lys3} (0.3 mM) is shown underneath in each case.

The NMR spectroscopic footprints of **1** and **2** on the target were then determined from 2D HMQC spectra by using ^{15}N-labeled tRNA.[17] Kynuramine (**2**) interacts with the D stem of the tRNA (Figure 2), and **1** binds to at least two specific sites located in the T and D stems.[18] From NMR spectroscopic titrations, the apparent dissociation constants of **1** and **2** for tRNA^{Lys3} were estimated to be 2 and 5 mM, respectively.

A qualitative specificity study was conducted with these two compounds by analyzing their NMR spectroscopic footprints with two other tRNA molecules: tRNA$_f^{Met}$ and tRNA$_m^{Met}$ of *E. coli*. Compound **1** binds to both tRNA molecules, which have very similar T-arm sequences. Interestingly, **2** only binds to the D arm of tRNA$_m^{Met}$. Thus, compound **2**, despite its moderate affinity, appears to interact in a sequence-specific manner with tRNA$_m^{Met}$ and tRNA^{Lys3}, which have identical D stems (Figure 3). The structural elements required for the binding of kynuramine (**2**) were then investigated with the synthesis and binding evaluation of structural analogues **3**–**16** (Scheme 1). Qualitative comparison of chemical-shift perturbations indicated that binding was improved when the aniline moiety was exchanged for another heterocycle, except in the case of compounds **4**, **12**, and **16**. The reduction of the carbonyl group to give the

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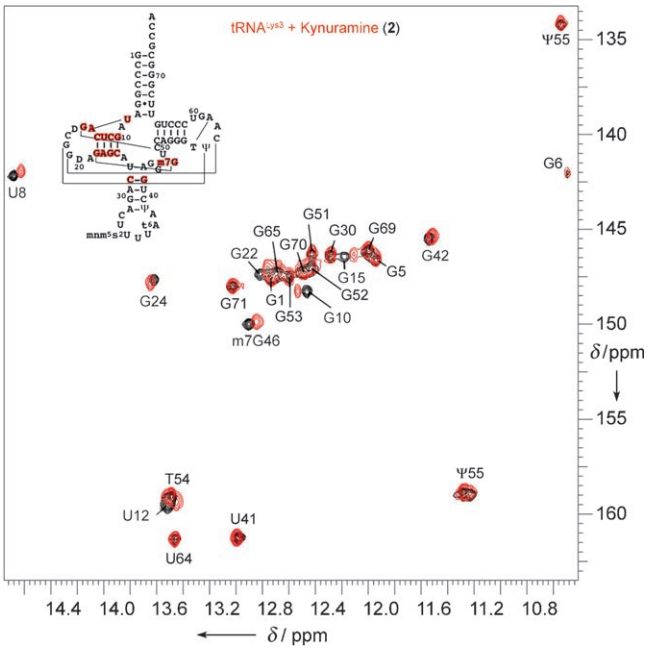


Figure 2. Superposition of the HMQC spectra of tRNA^{Lys3} alone (0.8 mM; black) and tRNA^{Lys3} mixed with compound **2** (1.6 mM; red).

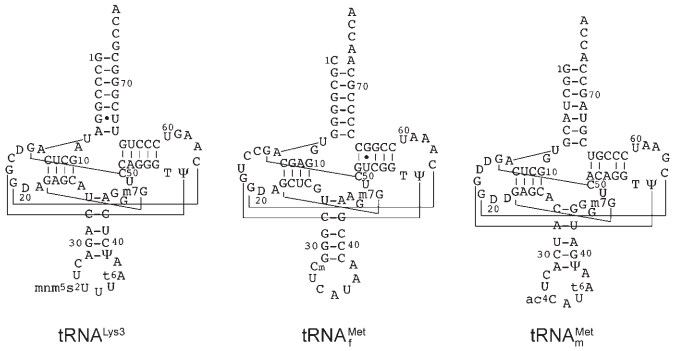
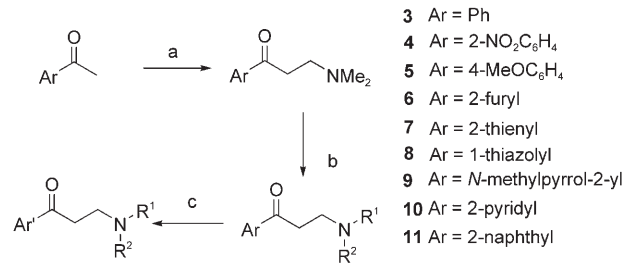


Figure 3. Sequence and secondary structure of the various tRNA molecules used in this study.



3 Ar = Ph
4 Ar = 2-$NO_2C_6H_4$
5 Ar = 4-$MeOC_6H_4$
6 Ar = 2-furyl
7 Ar = 2-thienyl
8 Ar = 1-thiazolyl
9 Ar = *N*-methylpyrrol-2-yl
10 Ar = 2-pyridyl
11 Ar = 2-naphthyl

15 Ar' = 2-$NH_2C_6H_4$, R^1,R^2 = $(CH_2CH_2)_2O$

16 Ar' = 2-$NH_2C_6H_4$, R^1 = R^2 = Me

12 Ar = 2-$NO_2C_6H_4$, R^1,R^2 = $(CH_2CH_2)_2O$
13 Ar = Ph, R^1 = Me, R^2 = $CH_2CH_2NMe_2$
14 Ar = 2-furyl, R^1 = Me, R^2 = $CH_2CH_2NMe_2$

Scheme 1. Reagents and conditions: a) $(CH_2O)_n$, $Me_2NH{\cdot}HCl$, 35% aqueous HCl, EtOH, △, then K_2CO_3; b) CH_3I, CH_2Cl_2, RT, then R^1R^2NH, CH_3Cl, 60°C; c) H_2, Pd/C, MeOH. Compounds **3**–**11** are the products of step a; compounds **3**, **4**, and **6** were used for step b; compound **12** used for step c; and compound **4** is the precursor of compound **16**.

corresponding alcohol led to the loss of the interaction. The binding of **3** was not improved by the introduction of an extra nitrogen atom into the side chain (compound **13**).

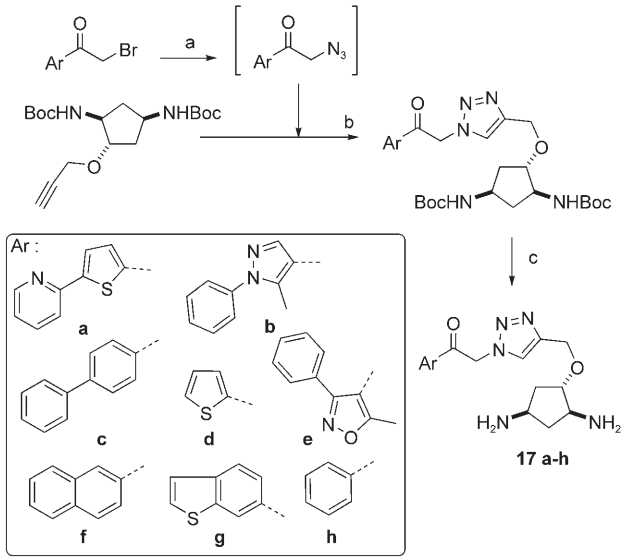
The fluorescent properties of compound **11** (λ_{ex} = 341 nm, λ_{em} = 478 nm) were used to estimate the dissociation constant for its interaction with tRNA^{Lys3} (K_d = (191 ± 86) μM), and the binding of **11** to the D arm of tRNA^{Lys3} was confirmed by a 2D TROSY experiment.

The next step was to attempt to link covalently the two ligands to improve binding. Compound **1** is a 2-DOS surrogate and hence an "RNA-friendly" compound which probably binds to several sites of the tRNA. As evidenced by the discrete NMR chemical shifts, this multiple binding is nevertheless specific. It results from the constrained amine geometry, as described for 2-DOS with 16S ribosomal RNA.[19] This specific multiple binding can be considered an advantage for our strategy, as it should improve the chances of obtaining a productive linkage.

A library of connected fragments **17a**–**h** was prepared by a one-pot procedure in 6–66% overall yield after catch-and-release purification on sulfonic acid resin (Scheme 2). Binding of these compounds to tRNA^{Lys3} was then monitored in a TROSY experiment, which showed improved affinity and specificity for the D-arm region of tRNA^{Lys3} under stoichiometric conditions (Figure 4 for **17f**).

Fluorescence binding assays with tRNA^{Lys3}, tRNA$_m^{Met}$, and tRNA$_f^{Met}$ led to the determination of a K_d value of 1.8 μM for compound **17f** with tRNA^{Lys3} at physiological ionic strength. Interestingly, compound **17f** exhibited significant sequence selectivity for the D arms of tRNA^{Lys3} and tRNA$_m^{Met}$ over that of tRNA$_f^{Met}$ (Table 1 and Figure 3). This specificity correlates with a decreased ionic-strength dependence of the interaction with tRNA^{Lys3}. The strong dependence of the affinity for tRNA$_f^{Met}$ on ionic strength reflects poorly selective binding dominated by electrostatic effects.[20]

In conclusion, a selective ligand of tRNA^{Lys3} with a micromolar dissociation constant has been synthesized for the



Scheme 2. Reagents and conditions: a) NaN_3, H_2O/acetone; b) $CuSO_4$, sodium ascorbate; c) HCl, MeOH, then amberlyst resin, then NH_3, MeOH. Boc = *tert*-butoxycarbonyl.

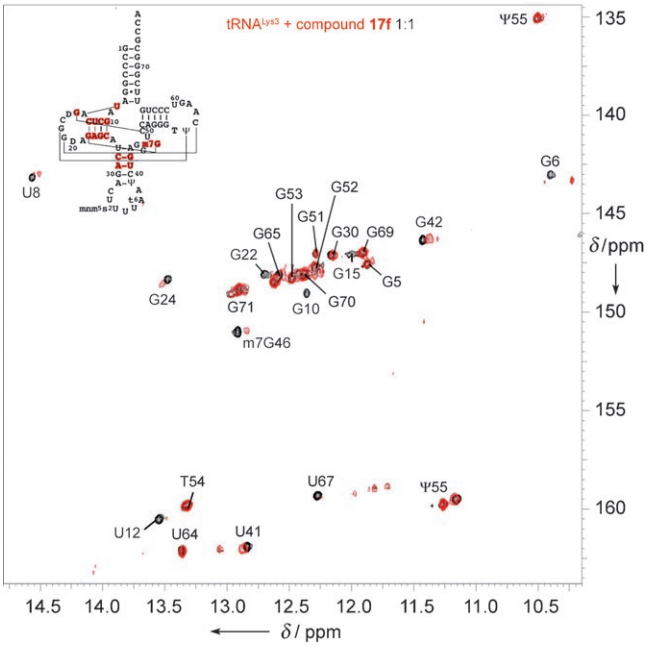


Figure 4. Superposition of the TROSY spectra of tRNA^{Lys3} alone (0.3 mM; black) and tRNA^{Lys3} mixed with compound **17 f** (0.3 mM; red).

Table 1: Dissociation constants K_d [μM] of compound **17 f** for three different tRNA molecules at various ionic strengths.

	tRNA^{Lys3}	tRNA$_f^{Met}$	tRNA$_m^{Met}$
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	–	–

first time. This study outlines the power of a fragment-based strategy in the field of RNA-ligand discovery, providing potential lead compounds for the development of antiviral drugs. The use of compound **17 f** as an inhibitor of reverse transcription in cell-free assays will be investigated. Besides this important application, this study provides new information on the design of small RNA-interacting molecules. This step is crucial for achieving a challenging goal: cellular regulation at the RNA level by small-molecule effectors.

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[1] G. J. R. Zaman, P. J. A. Michiels, C. A. A. van Boeckel, *Drug Discovery Today* **2003**, *8*, 297–206.
[2] a) E. Westhof, *Biochimie* **2006**, *88*, 931–933; b) J. Poehlsgaard, S. Douthwaite, *Nat. Rev. Microbiol.* **2005**, *3*, 870–881.
[3] N. W. Luedtke, Y. Tor in *Small Molecule DNA and RNA Binders: From Synthesis to Nucleic Acid Complexes*, *Vol. 1* (Eds.: M. Demeunynck, C. Bailly, W. D. Wilson), Wiley-VCH, Weinheim, **2003**.
[4] R. Marquet, C. Isel, B. Ehresmann, *Biochimie* **1995**, *77*, 113–124.
[5] Z. Zhang, S. M. Kang, C. D. Morrow, *AIDS Res. Hum. Retroviruses* **1998**, *14*, 979–988.
[6] X. Wei, M. Götte, M. A. Wainberg, *Nucleic Acids Res.* **2000**, *28*, 3065–3074.
[7] B. François, R. J. M. Russel, J. B. Murray, F. Aboul-ela, B. Masquida, Q. Vincens, E. Westhof, *Nucleic Acids Res.* **2005**, *33*, 5677–5690, and references therein.
[8] a) X. Liu, J. R. Thomas, P. J. Hergenrother, *J. Am. Chem. Soc.* **2004**, *126*, 9196–9197; b) J. R. Thomas, X. Liu, P. J. Hergenrother, *J. Am. Chem. Soc.* **2005**, *127*, 12434–12435; c) J. R. Thomas, X. Liu, P. J. Hergenrother, *Biochemistry* **2006**, *45*, 10928–10938.
[9] A few studies have been carried out on the interaction between aminoglycoside derivatives and tRNA^{Phe}: a) S. R. Kirk, Y. Tor, *Bioorg. Med. Chem.* **1999**, *7*, 1979–1991; b) R. Szilaghi, S. Shahzad-Ul-Hussan, T. Weimar, *ChemBioChem* **2005**, *6*, 1270–1276; c) N. E. Mikkelsen, K. Johansson, A. Virtanen, L. A. Kirsebom, *Nat. Struc. Biol.* **2001**, *8*, 510–514.
[10] C. Tisné, F. Guillière, F. Dardel, *Biochimie* **2005**, *87*, 885–888.
[11] P. Benas, G. Bec, G. Keith, R. Marquet, C. Ehresmann, B. Ehresmann, P. Dumas, *RNA* **2000**, *6*, 1347–1355.
[12] C. Tisné, B. P. Roques, F. Dardel, *J. Mol. Biol.* **2001**, *305–314*, 443–454.
[13] C. Tisné, B. P. Roques, F. Dardel, *J. Biol. Chem.* **2004**, *279*, 3588–3595.
[14] a) D. C. Ress, M. Congreve, C. W. Murray, R. Carr, *Nat. Rev. Drug Discovery* **2004**, *3*, 660–672; b) D. A. Erlanson, R. S. McDowell, T. O'Brien, *J. Med. Chem.* **2004**, *47*, 3463–3482.
[15] For the NMR-spectroscopy-guided discovery of RNA binders, see: a) L. Yu, T. K. Oost, J. M. Schkeryantz, J. Yang, D. Janowick, S. W. Fesik, *J. Am. Chem. Soc.* **2003**, *125*, 4444–4450; b) M. Mayer, T. L. James, *J. Am. Chem. Soc.* **2004**, *126*, 4453–4460.
[16] The use of this "target-observation" strategy (as opposed to ligand-observation schemes, such as saturation transfer) is essential to filter out promiscuous binders, which interact through nonspecific electrostatic interactions with the highly charged nucleic acid. Promiscuous binders cause a general broadening of the NMR spectrum of the target, whereas specific binders induce only peak shifts, which allow the identification of the binding site. For a general review, see: H. O. Villar, J. Yan, M. R. Hansen, *Curr. Opin. Chem. Biol.* **2004**, *8*, 387–391.
[17] For the NMR spectroscopic assignment of imino groups, see: C. Tisné, B. P. Roques, F. Dardel, *J. Mol. Biol.* **2001**, *306*, 443–454.
[18] Not surprisingly, the aminoglycosides neomycin and kanamycin were also detected as tRNA^{Lys3} ligands. However, they are too complex for a fragment-based approach. In contrast, compound **1** is an excellent 2-deoxystreptamine surrogate in the design of "RNA-friendly" compounds: L. Micouin, F. P. Dardel, C. Tisne-Vicrobeck, F. Maurice, M. Bonin, A. Perez Luna, C. J. Bournaud, G. Bégis, WO2006024784, **2006**.
[19] a) S. Yoshizawa, D. Fourmy, R. G. Eason, J. D. Puglisi, *Biochemistry* **2002**, *41*, 6263–6270; see also reference [7].
[20] Both enantiomerically enriched (e.r. = 93/7) isomers of compound **17 f** were prepared. The dissociation constant $K_d = (1.2 \pm 0.6)$ μM was found for *(R)*-**17 f** with tRNA^{Lys3} at an ionic strength of 150 mM KCl, and $K_d = (1.1 \pm 0.7)$ μM was found for *(S)*-**17 f** under the same conditions. Thus, the interaction with tRNA^{Lys3} is not stereospecific, and racemic ligands can be used in this approach. For the enantioselective preparation of **1**, see: a) A. Pérez Luna, M.-A. Ceschi, M. Bonin, L. Micouin, H.-P. Husson, S. Gougeon, G. Estenne Bouthou, B. Marabout, M. Sevrin, P. George, *J. Org. Chem.* **2002**, *67*, 3522–3524; b) A. Pérez Luna, M. Bonin, L. Micouin, H.-P. Husson, *J. Am. Chem. Soc.* **2002**, *124*, 12098–12099.

