NMR-Guided Fragment-Based Approach for the Design of AAC(6')-Ib Ligands

Thomas Lombès, Guillaume Bégis, Frédérique Maurice, Serge Turcaud, Thomas Lecourt, Frédéric Dardel, Laurent Micouin

NMR-Guided Fragment-Based Approach for the Design of AAC(6')-Ib Ligands

Thomas Lombs, Guillaume Bøgis, Frødørique Maurice, Serge Turcaud, Thomas Lecourt, Frødøric Dardel, Laurent Micouin, Thomas Lombès, Guillaume Bégis, Frédérique Maurice, et al.

DOI: 10.1002/cbic.200700677

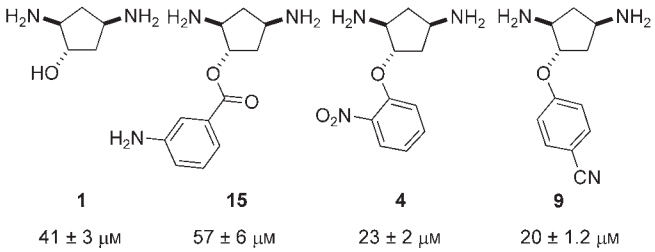
NMR-Guided Fragment-Based Approach for the Design of AAC(6′)-Ib Ligands

Thomas Lombès,[a] Guillaume Bégis,[a] Frédérique Maurice,[b] Serge Turcaud,[a] Thomas Lecourt,[a] Frédéric Dardel,*[b] and Laurent Micouin*[a]

Aminoglycosides are potent, broad-spectrum antibiotics of major clinical importance.[1] Unfortunately, the emergence of resistance is increasingly restricting their use as antibacterials.[2] Among the common mechanisms of resistance, enzymatic modification of aminoglycosides by N-acetylation at the 6′-position is one of the most widespread. Two functional classes of N-acetyltransferases (AAC(6′)) have been described: AAC(6′)-I, which confers resistance to amikacin but not to gentamicin, and AAC(6′)-II, with the reciprocal selectivity. Among these two classes, the aminoglycoside 6′-*N*-acetyltransferase type Ii (AAC(6′)-Ii) has been intensively investigated, with the crystallographic determination of its structure and the design of several bisubstrate inhibitors.[3] Moreover, the first inhibitor of this enzyme active in cells has recently been reported, providing the proof of concept for the pharmaceutical potential of such compounds.[4] The structure of AAC(6′)-Iy has also been reported.[5]

As part of a general program on the fragment-based design of aminoglycoside mimics,[6] we have been interested in the study of another AAC(6′)-I subclass: AAC(6′)-Ib. Unlike in the cases of AAC(6′)-Ii and Iy, which are chromosomally encoded (and hence confined to a single bacterial species), the gene for AAC(6′)-Ib is carried by mobile elements such as the integrons, and is present in over 70% of AAC(6′)-producing Gram-negative clinical isolates.[7] Furthermore, some isoforms of this class have recently evolved in clinical isolates to provide resistance to both amikacin and gentamicin[8] or to some fluoroquinolines.[9] The low sequence identity (< 20%) of ACC(6′)-Ib with the well-characterized ACC(6′)-Ii and ACC(6′)-Iy prompted us to evaluate a fragment-based approach for the design of ligands of this enzyme, rather than a rational design based on the structural data available for ACC(6′)-Ii and ACC(6′)-Iy enzymes. Our aim was to design ligands that were structurally distinct from aminoglycosides, which would be less likely to be deactivated by other resistance enzymes.[10] Here we report an NMR-guided design of micromolar ligands for this enzyme, as well as their conversion into a bisubstrate inhibitor.

In preliminary studies, we used NMR detection techniques to identify compound **1** (Scheme 1) as a primary fragment for the design of ligands.[11] This compound is readily available



Scheme 1. Dissociation constants of selected ligands determined by fluorescence spectroscopy experiments.

from cyclopentadiene on a large scale in a racemic or enantioenriched form.[12] Both saturation transfer difference (STD)[13] and reverse n.O.e. pumping experiments[14] were used to verify its ability to mimic the central desoxystreptamine core of kanamycin binding to the enzyme (Figure 1). Furthermore, the specificity of this binding was confirmed by a substantial decrease in the STD signal of compound **1** when the experiment was conducted in the presence of kanamycin, showing competitive binding of this fragment to the active site of the enzyme (for details see the Supporting Information).

Compound **1** was then coupled through ether or ester linkages with several substituted aromatic moieties capable of mimicking ring 1 of aminosides, leading to racemic neamine analogues (Scheme 2).

All the compounds were then evaluated by reverse n.O.e. pumping experiments for their ability to bind AAC(6′)-Ib.

It was expected that the common aminocyclopentanic moiety would bind in most cases, leading to intermolecular n.O.e. transfers, whereas efficient n.O.e. transfers from the aromatic appendage would only occur with the best ligands (Figure 2). This NMR screening provided a qualitative estimation of binding affinity for all the compounds, which can be classified into three groups: no signals from the aromatic components were observed with compounds **5** and **13**, whereas weak n.O.e transfer onto the aromatic components of compounds **14**, **15**, **8**, **18**, **12** and **11** could be detected, and strong n.O.e. transfers were observed with compounds **7**, **4**, **6**, **16** and **9**. Although this "ligand-observe" set of experiments provided a qualitative affinity score for the enzyme, we could not at this stage exclude the possibility that the binding might be occur-

[a] *T. Lombès, Dr. G. Bégis, S. Turcaud, Dr. T. Lecourt, Dr. L. Micouin*
Chimie Thérapeutique, UMR 8638
Université Paris Descartes, CNRS
4 avenue de l'Observatoire, 75006 Paris (France)
Fax: (+33) 1-43-29-14-03
E-mail: laurent.micouin@univ-paris5.fr

[b] *Dr. F. Maurice, Dr. F. Dardel*
Cristallographie et RMN Biologiques
Université Paris Descartes, CNRS
4 avenue de l'Observatoire, 75006 Paris (France)
Fax: (+33) 1-53-73-99-25
E-mail: frederic.dardel@univ-paris5.fr

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

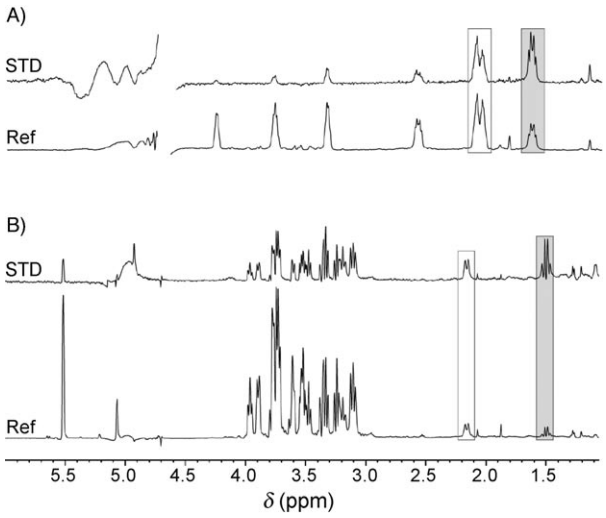


Figure 1. STD analysis of the binding of kanamycin (bottom) and diaminocyclopentanol **1** (top; the water signal has been removed for clarity) to AAC(6′)-1b. The boxed peaks correspond to the cyclic methylenic protons located between the two amino groups. In each case, the shaded box corresponds to the proton *syn* to the amines and shows the strongest STD signal.

ring out of the enzyme active site. Since the size of AAC(6′)-Ib (43 kD, 2×196 amino acids) precluded the direct investigation of foot-printing by "target-observe" 2D NMR techniques (such as HSQC or TROSY experiments), the binding of representative ligands was then investigated by fluorescence spectroscopy. We indeed observed that binding of compounds **1**, **15**, **4** and **9** induced strong quenching of the fluorescence of tryptophan residues (50–70%). As AAC(6′)-I enzymes share conserved tryptophan residues within their active sites, on which the 2-DOS moiety has been observed to stack, this fluorescence quench might be indirect evidence that these compounds are indeed interacting with tryptophans located in the AAC(6′)-Ib active site. Furthermore, it allowed the calculation of their dissociation constants by titration. Thus, K_d values were established to be 41 ± 3 μM for compound **1**, 57 ± 6 μM for compound **15**, 23 ± 2 μM for compound **4**, and 20 ± 1.2 μM for compound **9** (Scheme 1).

This correlates very well with the NMR results, as the additional interactions observed with **4** and **9** result in a twofold improvement of affinity, whereas the small loss observed with **15** is correlated with very weak intermolecular n.O.e. transfer.

The final validation of this NMR-guided ligand optimization was performed by preparing a bisubstrate ligand from compound **9**. For this study, we decided to resynthesise this compound in enantioenriched form, since the coupling of a racemic material with the CoA moiety would deliver diastereomers (Scheme 3). Compound **22** was then selectively reduced, deprotected and coupled with CoA by the one-pot procedure recently reported by Auclair and co-workers (Scheme 3).

The dissociation constant of compound **24** was then evaluated by fluorescence titration as 950 ± 190 nM, showing an improved affinity relative to the starting optimized fragment **9**. Standard AAC(6′)-Ib inhibition essays with kanamycin as a substrate showed that compound **24** is an inhibitor, with a K_i value of about 500 ± 300 nM, whereas neither CoA nor compound **9** showed any significant inhibitory properties. No synergistic activity could be observed when **24** was tested in combination with kanamycin against a *E. coli* strain harbouring the AAC(6′)-Ib encoding gene, as would be expected with a compound bearing an unmodified CoA moiety.[15]

In conclusion, we have shown in this work that "ligand-observe" NMR techniques such as reverse n.O.e. pumping can be used to drive the detection and optimisation of ligands for AAC(6′)-Ib, one of the most clinically important resistance enzymes to aminoglycosides. Interestingly, this fragment-based approach has been conducted without any need for NMR

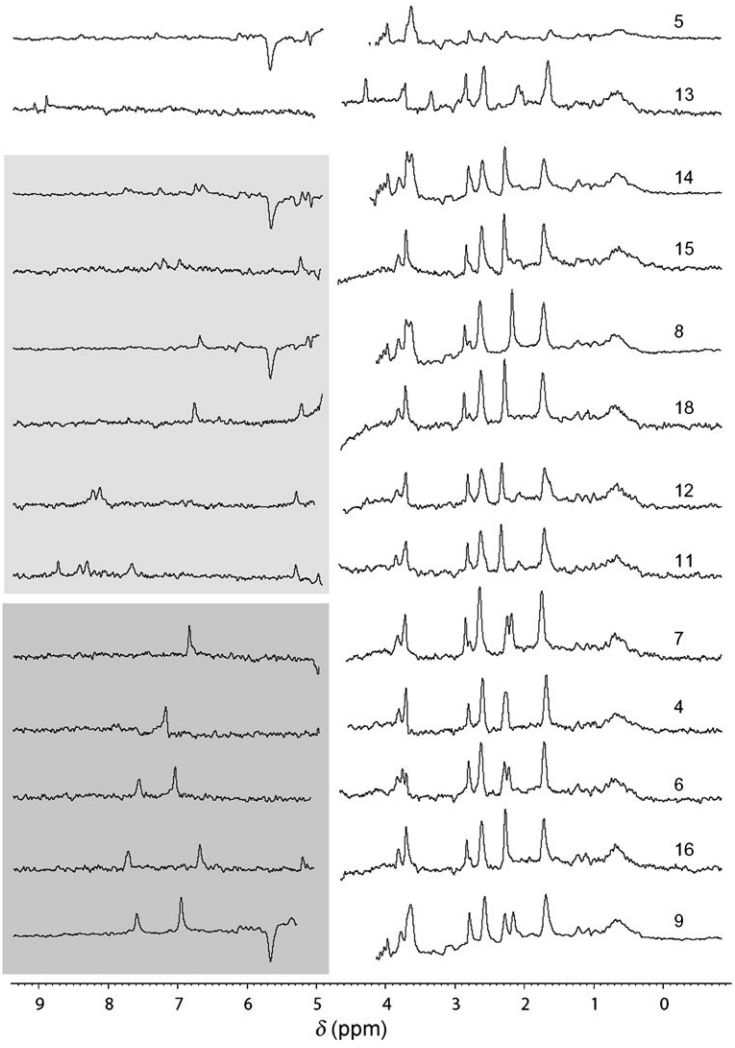
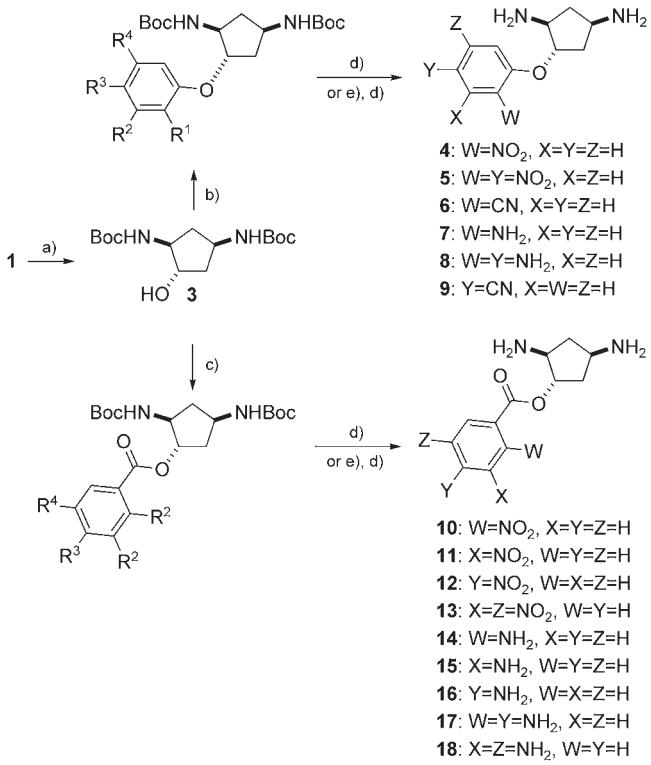
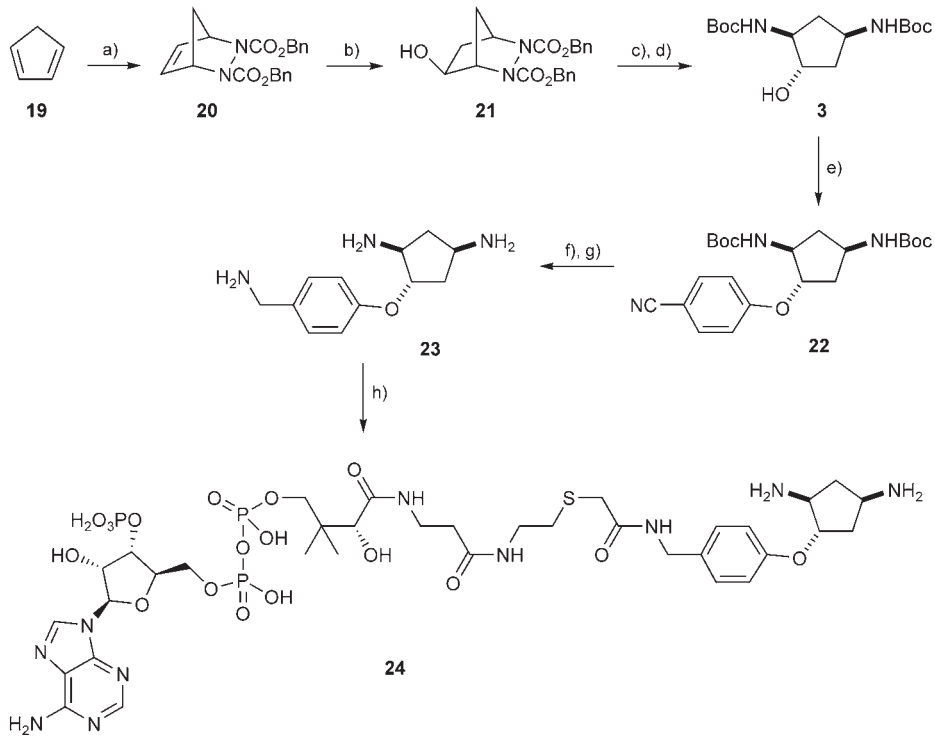


Figure 2. Qualitative binding analysis by reverse-n.O.e pumping experiments of compounds **4**–**18**. Spectra are ordered by increasing affinities. The water signal (4.7 ppm) has been removed for clarity.



Scheme 2. a) Boc_2O, NaOH, dioxane/H_2O, 84%; b) KHMDS, THF, ArF, 66–88%; c) DCC, cat. DMAP, CH_2Cl_2, ArCOOH, 47–66%; d) HCl (gas), AcOEt, quantitative; e) H_2, 10% Pd/C, AcOEt or THF, 43–93%.



Scheme 3. a) DBAD, CH_2Cl_2, quantitative; b) CatBH, 1% $[Rh(COD)Cl]_2$, 2% (*R*,*R*)-BDPP, DME, −50 °C then H_2O_2, 77%, *ee* = 83%; c) H_2, PtO_2, AcOH, quantitive; d) Boc_2O, NaOH 88% from **21**; e) *p*-fluorocyanobenzene, KHMDS, 4 °C to RT, 68%. f) H_2, 10% Pd/C, MeOH, 18 h; g) HCl_g, AcOEt, 10 min., 73% from **22**; h) Auclair's procedure (ref. [4]), preparative HPLC purification, 40%.

structural assignment of the target, with the validation of the binding site for the ligands by fluorescence spectroscopy and the preparation of a bisubstrate inhibitor. Despite the modest inhibition constant of compound **24** in relation to bisubstrate inhibitors described for AAC(6′)-Ii, this result shows that ligands and inhibitors of AAC(6′)-Ib can be constructed from non-aminoglycoside-like fragments, and that NMR can be a powerful tool for qualitatively estimation of their binding affinities to the enzyme. The design of more drug-like, nonbisubstrate inhibitors, as well as cocrystallisation studies, are underway.

Acknowledgements

F.M. was supported by a studentship from the Ministère de la Recherche. Dr. A. Lombès is acknowledged for help in UV experiments. Dr. A. Regazzetti is acknowledged for MS experiments. This work was supported by ANRS, CNRS and the Ministère de la Recherche.

Keywords: aminoglycosides · antibiotics · coenzyme A · fragment-based synthesis · NMR spectroscopy

[1] S. B. Vakulenko, S. Mobashery, *Clin. Microbiol. Rev.* **2003**, *16*, 430–450.
[2] S. Magnet, J. S. Blanchard, *Chem. Rev.* **2005**, *105*, 477–498.
[3] a) L. E. Wybenga-Groot, K.-A. Draker, G. D. Wright, A. M. Berghuis, *Structure* **1999**, *7*, 497–507; b) D. L. Burk, N. Ghuman, L. E. Wybenga-Groot, A. M. Berghuis, *Protein Sci.* **2003**, *12*, 426–437; c) K.-A. Draker, D. B. Northrop, G. D. Wright, *Biochemistry* **2003**, *42*, 6565–6574; d) K.-A. Draker, G. D. Wright, *Biochemistry* **2004**, *43*, 446–454; e) J. W. Williams, D. B. Northrop, *J. Antibiot.* **1979**, *32*, 1147–1154; f) F. Gao, X. Yan, O. M. Baettig, A. M. Berghuis, K. Auclair, *Angew. Chem.* **2005**, *117*, 7019–7022; *Angew. Chem. Int. Ed.* **2005**, *44*, 6859–6862.
[4] F. Gao, X. Yan, T. Shakya, O. M. Baettig, S. Ait-Mohand-Brunet, A. M. Berghuis, G. D. Wright, K. Auclair, *J. Med. Chem.* **2006**, *49*, 5273–5281.
[5] M. W. Vetting, S. Magnet, E. Nieves, S. L. Roderick, J. S. Blanchard, *Chem. Biol.* **2004**, *11*, 565–573.
[6] F. Chung, C. Tisné, T. Lecourt, F. Dardel, L. Micouin, *Angew. Chem.* **2007**, *119*, 4573–4575; *Angew. Chem. Int. Ed.* **2007**, *46*, 4489–4491.
[7] A. C. Fluit, F. J. Schmitz, *Clin. Microbiol. Infect.* **2004**, *10*, 272–288.
[8] I. Casin, B. Hanau-Bercot, I. Podglajen, H. Vahaboglu, E. Collatz, *Antimicrob. Agents Chemother.* **2003**, *47*, 697–703.
[9] A. Robicsek, J. Strahilevitz, G. A. Jacoby, M. Macielag, D. Abbanat, C. H. Park, K. Bush, D. C. Hooper, *Nat. Med.* **2006**, *12*, 83–88.
[10] The potential to escape bacterial resistance by enzymatic inactivation through the design of conformationally restricted aminoglycosides has recently been proposed

in a series of very elegant works: a) J. L. Asensio, A. Hidalgo, A. Bastida, M. Torrado, F. Corzana, J. L. Chiara, E. Garcia-Junceda, J. Canada, J. Jimènez-Barbero, *J. Am. Chem. Soc.* **2005**, *127*, 8278–8279; b) A. Bastida, A. Hidalgo, J. L. Chiara, M. Torrado, F. Corzana, J. M. Pérez-Canadillas, P. Groves, E. Garcia-Junceda, C. Gonzalez, J. Jimènez-Barbero, J. L. Asensio, *J. Am. Chem. Soc.* **2006**, *128*, 100–116; c) F. Corzana, I. Cuesta, A. Bastida, A. Hidalgo, M. Latorre, C. González, A. García-Junceda, J. Jiménez-Barbero, J. L. Asensio, *Chem. Eur. J.* **2005**, *11*, 5102–5113.

[11] F. Maurice, G. Begis, L. Micouin, F. Dardel, *C. R. Chim.* **2006**, *9*, 413–419.

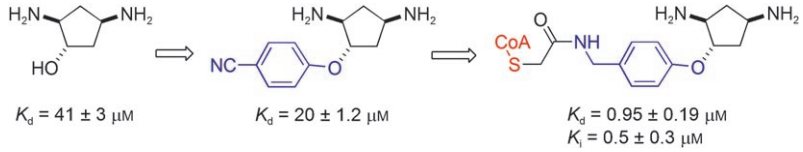
[12] a) A. Pérez Luna, M.-A. Ceschi, M. Bonin, L. Micouin, H.-P. Husson, S. Gougeon, G. Estenne Bouthou, B. Marabout, M. Sevrin, P. George, *J. Org. Chem.* **2002**, *67*, 3522–3524; b) A. Pérez Luna, M. Bonin, L. Micouin, H.-P. Husson, *J. Am. Chem. Soc.* **2002**, *124*, 12098–12099.

[13] M. Mayer, B. Meyer, *J. Am. Chem. Soc.* **2001**, *123*, 6108–6117.

[14] A. Chen, M. J. Shapiro, *J. Am. Chem. Soc.* **2000**, *122*, 414–415.

[15] Compounds bearing a CoA moiety are probably unable to penetrate the bacterial envelope, as observed in ref. [3e] and discussed in ref. [2].

Received: November 9, 2007
Published online on ■■ ■■, 2008



Reverse n.O.e. pumping in the detection and optimisation of ligands: Ligand-observed NMR experiments have been used to drive the detection and optimisation of non-aminoglycosidic ligands for AAC(6′)Ib, one of the most clinically important resistance enzymes to aminoglycosides. This fragment-based approach has been conducted without any need for NMR structural assignment of the target, and has been validated by the preparation of a bisubstrate inhibitor.

T. Lombès, G. Bégis, F. Maurice, S. Turcaud, T. Lecourt, F. Dardel, L. Micouin**

■■ – ■■

NMR-Guided Fragment-Based Approach for the Design of AAC(6′)-Ib Ligands