## Verification of a Designed Intramolecular Hydrogen Bond in a Drug Scaffold by Nuclear Magnetic Resonance Spectroscopy

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**Abstract:** 2D <sup>1</sup>H-<sup>15</sup>N HMBC NMR acquired at natural abundance and DMSO titration monitored by 1D <sup>1</sup>H NMR verified the existence of an intramolecular hydrogen bond that was designed to mimic the pyrimidinone ring of a class of kinase inhibitors. A scalar coupling across the hydrogen bond was detected in organic and aqueous solvent, suggesting a simple and general approach for testing the propensity of intramolecular hydrogen bonds to stabilize pseudo-rings in drug scaffolds.

Rationally redesigning existing drugs is an attractive route to uncover new leads for drug development. Recently, the bioactive conformations of several kinase inhibitors were mimicked by replacing scaffold rings with pseudo-rings formed via intramolecular hydrogen bonds (H bond) (Figure 1). Examples include the anthranilimide 1 derived from the anilinopthalazine kinase inhibitor PTK787,1 urea-based Cdk4 inhibitors such as 2,<sup>2</sup> anthranilimides 3 targeting Tie-2 tyrosine kinase,<sup>3</sup> and a trisubstitued purine p38 inhibitor  $4^4$ . Although the design of an intramolecular H bond is very intriguing, it can be debated whether such a conformation-stabilizing H bond in fact exists, especially in aqueous solution.<sup>5</sup> Here, we illustrate a simple and generally applicable approach using natural abundance <sup>1</sup>H-<sup>15</sup>N HMBC<sup>a</sup> NMR experiments and 1D NMR solvent titrations to verify the existence of designed pseudoring-forming H bonds in drug scaffolds.

The scaffold studied **5** was derived from pyrido[2,3-*d*]pyrimidin-7-one kinase inhibitors (Figure 2), which were originally developed as Src inhibitors and are now recognized to possess broad tyrosine kinase inhibitory activity.<sup>6</sup> The redesign of the pyrido[2,3-*d*]pyrimidin-7-one kinase inhibitors involved mimicking the pyrimidinone ring with a pseudo-ring formed between the nitrogen of a deazapurine scaffold and an urea amino group (Figure 2). The same design approach has recently led to the discovery of 1-alkyl-3-phenyl-1-(6-phenylaminopyrimidin-4-yl)ureas as a new class of tyrosine kinase inhibitors,<sup>7,8</sup> and five urea-based inhibitors are currently undergoing clinical trials.<sup>3</sup>

To test for the intramolecular H bond as proposed in the design, we performed NMR titrations of a water-soluble mimetic of the core scaffold **6** (Figure 2) in chloroform with DMSO (Figure 3).<sup>9</sup> H bonding generally results in deshielding, and an increasingly downfield-shifted proton resonance indicates increased H-bond strength.<sup>10</sup> The urea hydrogen HN11 (9.3 ppm)



Figure 1. Pseudo-rings formed by intramolecular hydrogen bonds.



Figure 2. Structures of pyrido[2,3-D]pyrimidin-7-one kinase inhibitors PD-180970 and PD-166326 and designed mimetics of the core scaffold.



**Figure 3.** DMSO titration of **6** in deuterated chloroform at 300 K. (A) 1D spectra and (B) chemical shifts versus DMSO concentration. Maximal changes at 11.1% (v/v) DMSO for HN11, 0.13 ppm; HN14, 0.47 ppm; H2, 0.00 ppm; H7, 0.10 ppm; H8, -0.07 ppm; and -0.10 ppm for NH<sub>3</sub><sup>+</sup> protons.

is significantly downfield of other HN protons. Furthermore, HN11 is, in contrast to the aniline HN14 (7.6 ppm) and the amino protons, protected from exchange with residual water in the sample. As the concentration of DMSO increases, the resonance line of HN14 shifts downfield as a result of increasing H-bonding interactions with DMSO. In contrast, only small changes are seen for HN11, thus indicating that DMSO cannot compete with the proposed intramolecular H bond. As expected, the chemical shifts of all other hydrogens remain virtually unchanged (Figure 3).

<sup>15</sup>N chemical shifts are exquisitely sensitive to the presence of hydrogen bonds.<sup>11–13</sup> Consequently, we performed  ${}^{1}H{-}{}^{15}N$  HMBC experiments<sup>14,15</sup> at natural abundance to obtain nitrogen chemical shift assignments (Figure 4, Tables S1 and S2). All correlation peaks are consistent with the structure in Figure 2. In agreement with previous observations,<sup>11,12</sup> the putative H-bond acceptor N3 has a nitrogen chemical shift upfield of the chemically similar N1 nitrogen, while the donor nitrogen is shifted downfield.<sup>13</sup> Most interestingly, N3 shows a correlation peak with HN11 (Figure 4A, box). This cross-peak is best

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<sup>&</sup>lt;sup>*a*</sup> Abbreviations: HMBC, heteronuclear multiple bond correlation; NMR, nuclear magnetic resonance; QM, quantum mechanic.



Figure 4. <sup>1</sup>H-<sup>15</sup>N HMBC spectra of 6 (146 and 96 mM respectively) in (A) d<sub>6</sub>-DMSO and (B) in 8.8 mM sodium phosphate buffer (pH 5.05, 12% D<sub>2</sub>O, 88% H<sub>2</sub>O) recorded at natural abundance (90 ms evolution time, 300 K, 600 MHz Bruker Avance instrument with <sup>1</sup>H/ <sup>15</sup>N/<sup>13</sup>C-TXI cryoprobe). Proton resonances are labeled according to Figure 2. Boxes highlight correlations across the intramolecular H bond. Presat spectra are shown above the 2D plots, with stars indicating the approximate position of HN14 and NH3<sup>+</sup> amino protons in the absence of solvent suppression. Spectrum A was recorded over 45 h (576 scans, 200 t<sub>1</sub> experiments), while B was recorded with 1024 scans with the release HMBC pulse program inv4gplrndqf (magnitude detection). For B, a low-power presaturation pulse was added for water suppression. The cross-peaks at 253 ppm are the correlations of the amino nitrogen folded from  $\sim$ 30.8 ppm. The spectra were centered at 180 ppm for nitrogen, with a sweep width of 222.4 ppm and a sweep width of 14 ppm in the proton dimension. (C) Slice through spectrum A. The signalto-noise of the through-H-bond cross-peak is 71:1, while the peak of three-bond correlation H2 to N3 has a signal-to-noise of 351:1. Adequate signal-to-noise can be obtained in 24 h or with more dilute samples, especially if phase-sensitive detection is used.<sup>15</sup>

explained by a scalar correlation through the designed intramolecular H bond because the alternative pathway through five bonds is unlikely as no other long-range correlations beyond three bonds are observed.

Through-H-bond scalar couplings have previously been observed in a variety of systems,<sup>11,12</sup> including Watson–Crick base pairs in RNA<sup>16</sup> and DNA,<sup>17</sup> reverse Hoogsteen RNA base pairs,<sup>18</sup> and a H bond between two histidine side chains in apomyoglobin,<sup>19</sup> between flavin mononucleotide and flavodox-in,<sup>20</sup> in the secondary structure of proteins,<sup>21</sup> involving phosphate groups,<sup>22</sup> and in metalloproteins.<sup>23,24</sup> Similarly, scalar couplings across intramolecular H bonds have been detected in small-molecule model systems.<sup>25–30</sup> These studies generally require isotope labeling of the macromolecule or model compound, and for small molecules, the NMR measurements often need to be performed at low temperatures,<sup>13</sup> in the solid state,<sup>31</sup> or at multiple magnetic fields.<sup>30</sup>

Scalar couplings across H bonds have been interpreted as evidence of their partial covalent nature.<sup>32–37</sup> The observation of a through-H-bond scalar coupling between the proton HN11 and the ring nitrogen N3 of **6** is therefore strong evidence for a stable pseudo-ring structure, as shown in Figure 2. This structure persists in water as indicated by the through-H-bond cross-peak in the <sup>1</sup>H<sup>-15</sup>N HMBC spectrum recorded in sodium phosphate buffer (Figure 4B, box). The stability of the interac-



Figure 5. Model of the core scaffold of 6. Only the atoms shown were included in the ab initio QM calculations.

tion is further illustrated by the fact that HN11 is not effected by presaturation pulses, indicating slow exchange with the bulk solvent. In contrast, HN14 and amino protons are not protected from solvent exchange as indicated by the absence of any crosspeaks in the <sup>1</sup>H-<sup>15</sup>N HMBC (Figure 4B) and line broadening in chloroform (Figure 3A). To evaluate the stability and geometry of the intramolecular H bond, we performed ab initio quantum mechanical calculations with Jaguar 7.0 (FirstDiscovery Suite, Schrodinger, Inc.) using the density functional theory at the B3LYP/6-31G\*\* level and the Poisson-Boltzmann continuum-dielectric method for the treatment of aqueous solvation. These calculations suggest that the intramolecular H bond stabilizes the pseudo-ring conformation over the open form by 5.25 kcal/mol. The N-N distance between N3 and N11 suggested by the calculations is 2.79 Å, with a N3-H11 distance of 1.97 Å and a bond angle of 135.5° (Figure 5). These geometric parameters are within the range of observed H-bond geometries.5

Taken together, the DMSO titrations and <sup>1</sup>H-<sup>15</sup>N HMBC NMR experiments verify an intramolecular H bond designed to form a pseudo-ring mimetic of a kinase inhibitor scaffold. Since the H bond persists in water using a minimal model of the core scaffold, it is likely that the designed conformation is assumed by larger analogues in cellular or in vitro assays. The core scaffold 6 does not show any biological activity because of the lack of side chains, but several related urea inhibitors have recently been disclosed as potent inhibitors for a number of tyrosine kinases.<sup>7,8,38,39</sup> For one of these compounds, a cocrystal structure verifies that the urea adopts a pseudo-bicyclic conformation stabilized by an internal hydrogen bond.<sup>39</sup> Most importantly, we have demonstrated that simple NMR experiments performed without isotope labeling can test the propensity of small molecule scaffolds to form stable intramolecular hydrogen bonds that may lock drug leads into their bioactive conformation.

**Supporting Information Available:** Chemical shift assignments and synthesis of **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

- (1) Furet, P.; Bold, G.; Hofmann, F.; Manley, P.; Meyer, T.; Altmann, K.-H. Identification of a new chemical class of potent angiogenesis inhibitors based on conformational considerations and database searching. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2967–2971.
- (2) Honma, T.; Hayashi, K.; Aoyama, T.; Hashimoto, N.; Machida, T.; Fukasawa, K.; Iwama, T.; Ikeura, C.; Ikuta, M.; Suzuki-Takahashi, I.; Iwasawa, Y.; Hayama, T.; Nishimura, S.; Morishima, H. Structurebased generation of a new class of potent Cdk4 inhibitors: new de novo design strategy and library design. *J. Med. Chem.* **2001**, *44*, 4615–4627.
- (3) Dumas, J.; Smith, R. A.; Lowinger, T. B. Recent developments in the discovery of protein kinase inhibitors from the urea class. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 600–616.
- (4) Wan, Z.; Boehm, J. C.; Bower, M. J.; Kassis, S.; Lee, J. C.; Zhao, B.; Adams, J. L. N-Phenyl-N-purin-6-yl ureas: the design and synthesis of p38alpha MAP kinase inhibitors. *Bioorg. Med. Chem. Lett.* 2003, 13, 1191–1194.

- (5) Steiner, T. The hydrogen bond in the solid state. Angew. Chem., Int. Ed. 2002, 41, 48–76.
- (6) Kraker, A. J.; Hartl, B. G.; Amar, A. M.; Barvian, M. R.; Showalter, H. D.; Moore, C. W. Biochemical and cellular effects of *c*-Src kinaseselective pyrido[2, 3-d]pyrimidine tyrosine kinase inhibitors. *Biochem. Pharmacol.* **2000**, *60*, 885–98.
- (7) Ding, Q.; Gray, N. S.; Li, B.; Liu, Y.; Sim, T.; Uno, T.; Zhang, G.; Pissot Soldermann, C.; Breitenstein, W.; Bold, G.; Caravatti, G.; Furet, P.; Guagnano, V.; Lang, M.; Manley, P. W.; Schoepfer, J.; Spanka, C. Pyrimidine urea derivatives as kinase inhibitors. WO2006000420, 2006; CAN 144:108347.
- (8) Furet, P.; Caravatti, G.; Guagnano, V.; Lang, M.; Meyer, T.; Schoepfer, J. al. Entry into a new class of protein kinase inhibitors by pseudo ring design. Manuscript in preparation.
- (9) Yang, D.; Qu, J.; Li, B.; Ng, F.-F.; Wang, X.-C.; Cheung, K.-K.; Wang, D.-P.; Wu, Y.-D. Novel turns and helices in peptides of chiral alpha-aminoxy acids. J. Am. Chem. Soc. 1999, 121, 589–590.
- (10) Wagner, G.; Pardi, A.; Wuethrich, K. Hydrogen bond length and <sup>1</sup>H NMR chemical shifts in proteins. J. Am. Chem. Soc. 1983, 105, 5948–5949.
- (11) Grzesiek, S.; Cordier, F.; Dingley, A. J. Scalar couplings across hydrogen bonds. *Methods Enzymol.* **2001**, *338*, 111–133.
- (12) Dingley, A. J.; Cordier, F.; Grzesiek, S. An introduction to hydrogen bond scalar couplings. *Concepts Magn. Res.* 2001, 13, 103–127.
- (13) Smirnov, S. N.; Golubev, N. S.; Denisov, G. S.; Benedict, H.; Schah-Mohammedi, P.; Limbach, H.-H. Hydrogen/deuterium isotope effects on the NMR chemical shifts and geometries of intermolecular lowbarrier hydrogen-bonded complexes. J. Am. Chem. Soc. 1996, 118, 4094-4101.
- (14) Bax, A.; Summers, M. F. Proton and carbon-13 assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 1986, 108, 2093–2094.
- (15) Hadden, C. E.; Martin, G. E.; Krishnamurthy, V. V. Improved performance accordion heteronuclear multiple-bond correlation spectroscopy-IMPEACH-MBC. J. Magn. Res. 1999, 140, 274–80.
- (16) Dingley, A. J.; Grzesiek, S. Direct observation of hydrogen bonds in nucleic acid base pairs by internucleotide <sup>2</sup>J<sub>NN</sub> couplings J. Am. Chem. Soc. 1998, 120, 8293–8297.
- (17) Pervushin, K.; Ono, A.; Fernandez, C.; Szyperski, T.; Kainosho, M.; Wuethrich, K. NMR scalar couplings across Watson–Crick base pair hydrogen bonds in DNA observed by transverse relaxation-optimized spectroscopy. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14147–14151.
- (18) Hennig, M.; Williamson, J. R. Detection of N-H···N hydrogen bonding in RNA via scalar couplings in the absence of observable imino proton resonances. *Nucleic Acid Res.* 2000, 28, 1585–1593.
- (19) Hennig, M.; Geierstanger, B. H. Direct detection of a histidinehistidine side chain hydrogen bond important for folding of apomyoglobin. J. Am. Chem. Soc. 1999, 121, 5123-5126.
- (20) Loehr, F.; Yalloway, G. N.; Mayhew, S. G.; Rueterjans, H. Cofactorapoprotein hydrogen bonding in oxidized and fully reduced flavodixin monitored by trans-hydrogen-bond scalar couplings. *ChemBioChem* 2004, 5, 1523–1534.
- (21) Cordier, F.; Grzesiek, S. Direct observation of hydrogen bonds in proteins by interresidue <sup>3h</sup>J<sub>NC'</sub> scalar couplings. J. Am. Chem. Soc. 1999, 121, 1601–1602.
- (22) Loehr, F.; Mayhew, S. G.; Rueterjans, H. Detection of scalar couplings across NH–OP and OH–OP hydrogen bonds in a flavoprotein. J. Am. Chem. Soc. 2000, 122, 9289–9295.
- (23) Blake, P. R.; Park, J. B.; Adams, M. W. W.; Summers, M. F. Novel observation of NH-S(Cys) hydrogen-bond-mediated scalar coupling in cadmium-113 substituted rubredoxin from *Pyrococcus furiosus*. *J. Am. Chem. Soc.* **1992**, *114*, 4931–4933.
- (24) Blake, P. R.; Lee, B.; Summers, M. F.; Adams, M. W. W.; Park, J. B.; Zhou, Z. H.; Bax, A. Quantitative measurement of small through-hydrogen-bond and 'through-space' <sup>1</sup>H<sup>-113</sup>Cd and <sup>1</sup>H<sup>-199</sup>Hg J couplings in metal-substituted rubredoxin from *Pyrococcus furiosus*. J. Biomol. NMR **1992**, 2, 527–533.
- (25) Soentjens, S. H. M.; van Genderen, M. H. P.; Sijbesma, R. P. Intermolecular <sup>2h</sup>J<sub>NN</sub> coupling in multiply hydrogen-bonded ureidopyrimidinone dimers in solution. *J. Org. Chem.* **2003**, *68*, 9070– 9075.

- (26) Loening, N. M.; Anderson, C. E.; Iskenderian, W. S.; Anderson, C. D.; Rychnovsky, S. D.; Barfield, M.; O'Leary, D. J. Qualitative and quantitative measurements of hydrogen bond mediated scalar couplings in acyclic 1,3-diols. *Org. Lett.* **2006**, *8*, 5321–5323.
- (27) Fierman, M.; Nelson, A.; Khan, S. I.; Barfield, M.; O'Leary, D. J. Scalar coupling across the hydrogen bond in 1,3- and 1,4-diols. *Org. Lett.* 2000, 2, 2077–2080.
- (28) Claramunt, R. M.; Sanz, D.; Alarcon, S. H.; Torralba, M. P.; Elguero, J.; Foces-Foces, C.; Pietrzak, M.; Langer, U.; Limbach, H. H. 6-Aminofulvene-1-aldimine: a model molecule for the study of intramolecular hydrogen bonds. *Angew. Chem., Int. Ed.* **2001**, *40*, 420–423.
- (29) Pietrzak, M.; Limbach, H. H.; Perez-Torralba, M.; Sanz, D.; Claramunt, R. M.; Elguero, J. Scalar coupling constants across the intramolecular NHN hydrogen bond of symmetrically and nonsymmetrically substituted 6-aminofulvene-1-aldimines. *Magn. Reson. Chem.* 2001, *39*, S100–S108; special issue.
- (30) Pietrzak, M.; Wehling, J.; Limbach, H. H.; Golubev, N. S.; Lopez, C.; Claramunt, R. M.; Elguero, J. C-13 detected scalar nitrogennitrogen couplings across the intramolecular symmetric NHN hydrogen bond of proton sponge. *J. Am. Chem. Soc.* 2001, *123*, 4338– 4339.
- (31) Benedict, H.; Limbach, H. H.; Wehlan, M.; Fehlhammer, W. P.; Golubev, N. S.; Janoschek, R. Solid state N-15 NMR and theoretical studies of primary and secondary geometric H/D isotope effects on low-barrier NHN-hydrogen bonds. J. Am. Chem. Soc. 1998, 120, 2939–2950.
- (32) Wilkens, S. J.; Westler, W. M.; Weinhold, F.; Markley, J. L. Transhydrogen-bond <sup>h2</sup>J<sub>NN</sub> and <sup>h1</sup>J<sub>NH</sub> couplings in DNA A–T base pair: natural bond orbital analysis. *J. Am. Chem. Soc.* **2002**, *124*, 1190– 1191.
- (33) Wilkens, S. J.; Westler, W. M.; Markley, J. L.; Weinhold, F. Natural *J*-coupling analysis: interpretation of scalar *J*-couplings in terms of natural bond orbitals. *J. Am. Chem. Soc.* 2001, *123*, 12026–12036.
- (34) Benedict, H.; Shenderovich, I. G.; Malkina, O. L.; Malkin, V. G.; Denisov, G. S.; Golubev, N.; Limbach, H.-H. Nuclear scalar spinspin couplings and geometries of hydrogen bonds. *J. Am. Chem. Soc.* 2000, *122*, 1979–1988.
- (35) Lloyd-Jones, G. C.; Harvey, J. N.; Hodgson, P.; Murray, M.; Woodward, R. L. Scalar coupling between the N-15 centres in methylated 1,8-diaminonaphthalenes and 1,6-diazacyclodecane: to what extent is (2H)J(NN) a reliable indicator of N–N distance? *Chem.-Eur. J.* 2003, 9, 4523–4535.
- (36) Dingley, A. J.; Masse, J. E.; Peterson, R. D.; Barfield, M.; Feigon, J.; Grzesiek, S. Internucleotide scalar couplings across hydrogen bonds in Watson-Crick and Hoogsteen base pairs of a DNA triplex. *J. Am. Chem. Soc.* **1999**, *121*, 6019–6027.
- (37) Barfield, M.; Dingley, A. J.; Feigon, J.; Grzesiek, S. A DFT study of the interresidue dependencies of scalar *J*-coupling and magnetic shielding in the hydrogen-bonding regions of DNA triplex. *J. Am. Chem. Soc.* 2001, *123*, 4014–4022.
- (38) Maier, J. A.; Brugel, T. A.; Sabat, M.; Golebiowski, A.; Laufersweiler, M. J.; VanRens, J. C.; Hopkins, C. R.; De, B.; Hsieh, L. C.; Brown, K. K.; Easwaran, V.; Janusz, M. J. Development of *N*-4,6-pyrimidine-*N*-alkyl-*N*'-phenyl ureas as orally active inhibitors of lymphocyte specific tyrosine kinase. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3646– 3650.
- (39) Brugel, T. A.; Maier, J. A.; Clark, M. P.; Sabat, M.; Golebiowski, A.; Bookland, R. G.; Laufersweiler, M. J.; Laughlin, S. K.; VanRens, J. C.; De, B.; Hsieh, L. C.; Mekel, M. J.; Janusz, M. J. Development of *N*-2,4-pyrimidine-*N*-phenyl-*N*'-phenyl ureas as inhibitors of tumor necrosis factor alpha (TNF-alpha) synthesis. Part 1. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3510–3.

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