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N- versus O-alkylation: Utilizing NMR methods to establish reliable primary structure determinations for drug discovery

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ABSTRACT

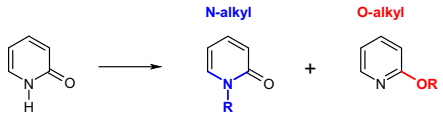
A classic synthetic issue that remains unresolved is the reaction that involves the control of N- versus O-alkylation of ambident anions. This common chemical transformation is important for medicinal chemists, who require predictable and reliable protocols for the rapid synthesis of inhibitors. The uncertainty of whether the product(s) are N- and/or O-alkylated is common and can be costly if undetermined. Herein, we report an NMR-based strategy that focuses on distinguishing inhibitors and intermediates that are N- or O-alkylated. The NMR strategy involves three independent and complementary methods. However, any combination of two of the methods can be reliable if the third were compromised due to resonance overlap or other issues. The timely nature of these methods (HSQC/HMQC, HMBC. ROESY, and ^{13}C shift predictions) allows for contemporaneous determination of regioselective alkylation as needed during the optimization of synthetic routes.

The synthetic organic chemistry literature is now well stocked with reports of new methodologies that can have great utility for the synthetic chemist. Amongst these are procedures for creating C–C, C–O and C–N bonds,[1] with variations of hybridization and chirality, accomplished with synthetic efficiency and atom economy.[2] These advances in methodology have enabled chemists to access a large variety of molecular architectures, and have even allowed for the synthesis of complex molecules such as azadirachtin,[3] taxol[4] and palau'amine,[5] to name but a few.

It is therefore surprising that, despite recent advances, some classic synthetic issues remain unresolved for common and simple chemical transformations often used by chemists. Specifically, this is the case for regioselective control of N- versus O-alkylation of ambident anions,[6] even though reports of such reactions have appeared as early as 50 years ago.[7] By way of example, this issue is illustrated in Scheme 1, where the regioselective alkylation of 2-pyridones (and close analogues) are widely known to produce *N*- and/or *O*-alkyl products.[8]

Efforts to better understand this classical regioselectivity issue were recently published by Breugst and Mayr,[9] where they reported a thorough study on the reactivities of ambident pyridine anions. These authors elegantly demonstrated, amongst other findings, that selective N-alkylation with silver salts is not due to increased electrophilicity of the electrophile, but rather because of simple blockage of the nitrogen position by coordination with silver, therefore ruling out a widely held rationalization. The authors also performed kinetic studies which enabled them to quantify the impact of various parameters, such as the nature of both solvent and electrophile, and the type of ambident anion used (differences between 2- and 4-pyridones, for instance) on the reaction rates. Also very recently, an N-selective alkylation reaction using magnesium reagents as the coordinator-director moiety was reported.[10] Overall, these reports highlight the fact that N- versus O-alkylation regioselectivity varies from one scaffold to another and that within the same scaffold, different synthetic outcomes can be obtained depending on solvent, nature of electrophiles and other conditions such as temperature. This, in part, explains why synthetic chemists have had such difficulty in establishing predictable and robust protocols for N- versus O-alkylation reactions.

This lack of synthetic control currently impacts drug discovery, as medicinal chemists require predictable and reliable protocols for achieving N- versus O-alkylation of substrates, enabling the rational design of single inhibitors, as well as for the synthesis of high-throughput parallel chemistry libraries. Furthermore, the pharmaceutical industry frequently has an interest in employing



Scheme 1. Regioselectivity issues for alkylation of 2-pyridones.

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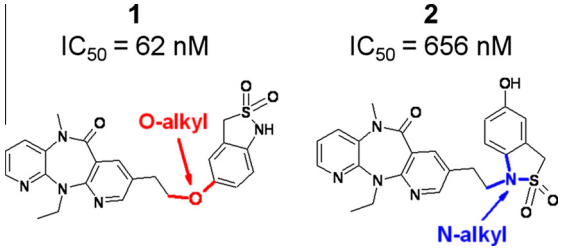
ubiquitous heteroaromatic scaffolds in its drug discovery efforts, such as the functionalized pyridine/pyridones[11] and quinolines/quinolones,[12] as they are known to display attractive drug-like properties. Unfortunately, these scaffolds are often not readily accessible in a chemoselective fashion, and significant time can be required to develop regioselective alkylation procedures. Given this, it is apparent that medicinal and parallel chemistry efforts stand to benefit greatly from a timely analytical strategy for accurately distinguishing N- and O-alkylated products (intermediates and compounds) for uncontrolled alkylation reactions. Step-wise determination of the identity of the products would serve as a valuable tool alongside the optimization of appropriate synthetic conditions and strategies.

Herein, we report an NMR-based strategy that focuses on distinguishing inhibitors and intermediates that are N- versus O-alkylated. The NMR strategy involves three independent and complementary methods, although any combination of two of these can be used to determine the regioselective alkylation of many synthetic compounds in a project. The timely nature of these methods allows for contemporaneous analyses along with the design of synthetic schemes.

Medicinal chemistry issue of N- versus O-analogues: The issue of determining whether a compound is N- or O-alkylated can be demonstrated using inhibitors **1** and **2** (Scheme 2). These compounds arose from a drug discovery program aimed at identifying HIV non-nucleoside reverse-transcriptase inhibitors (NNRTI).[13,14]

The coupling of the tricyclic left-side with the bicyclic right-side routinely involved an ambident anion for the syntheses of large libraries of inhibitors. In some instances, compounds clearly did not fit into established structure–activity relationships (SAR). However, subsequent primary structure determinations by NMR demonstrated the unpredictable nature of the regioselective alkylation. It was found that the *O*-alkyl compound **1** was very potent and possessed an inhibition constant of 62 nM, whereas the *N*-alkyl analogue **2** possessed an inhibition constant of 656 nM.[13] NMR structural data, in confirming the structures, allowed for a more confident understanding of SAR trends. Structural assumptions regarding the products of a reaction or parallel chemistry library (for example, N- vs O-alkylation) could be misleading, and as such close attention should be paid to this issue of structural integrity. Thus, the confirmation of primary structures is critical. In fact, the verification of the correct structures for compounds in older collections has also been insightful for us.

It is clear that one cannot rely on inhibitor activity differences (i.e., unanticipated SAR trends) as a reliable alert for compounds that have inaccurate primary structures. This is because a range of differences in activity are possible, depending on the precise binding roles played by the *N*- or *O*-alkyl appendage/scaffold. For example, this can be visualized in Figure 1, using the X-ray structure of **BILR 355** (inhibition constant of 17 nM) bound to the HIV reverse transcriptase. Figure 1 demonstrates that the right-side of **BILR 355** (highlighted by an arrow) presents its bicyclic portion in a partially solvent-exposed binding mode. This is precisely the



Scheme 2. NNRTI inhibitors **1** and **2**.[13]

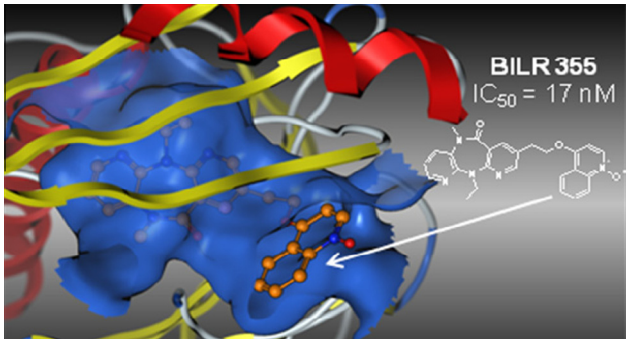


Figure 1. X-ray structure of HIV reverse transcriptase with the NNRTI inhibitor **BILR 355**. The PDB coordinates were deposited with the Protein Data Bank and are available via the PDB code 4KV8.

region that differs between the O- versus N-attachments of compounds **1** and **2** (Scheme 2). In this case, a difference of 10.6-fold in activity is observed between these compounds. However, other ligand–protein complexes involving N- versus O-analogues could be expected to differ in activities that range from little to many fold, depending on whether the alkylation appendage/scaffold is fully solvent exposed, or buried within a pocket. Likewise, one can expect a range of distinctions from physicochemical and in vivo properties.

It is suggested that medicinal chemists should keep in mind this regioselective issue and seek NMR analytical support during the optimization of synthetic protocols. It is our experience that regio-inaccurate structures exist in compound collections, and surprisingly for purchased starting materials from vendors.

Typical analytical methods are insufficient: As we experienced in the above example, it is clear that one cannot rely on simple analytical characterization techniques to distinguish between N- versus O-analogues. Considering mass spectroscopy, both analogues have the same mass, so this method cannot distinguish between the regioselectivity of alkylationanalogues. Also, a typical analysis of ^{1}H NMR spectra (one-dimensional) alone is insufficient for making this distinction. On the other hand, the Supplementary data demonstrates that FTIR can be used to distinguish O- versus N-alkylation for simple compounds such as pyridine **9** versus pyridone **10**, and quinoline **13** versus quinolone **14** (vide infra, Table 1). This method takes advantage of the fact that carbonyl stretching frequencies are potentially easy to recognize as they can fall in a relatively isolated region for small molecules (i.e., ~1640 cm^{-1}).[15] However, this is not always the case for larger compounds. This method becomes unreliable for more drug-like compounds that contain more than one type of carbonyl group. The key IR peaks are often obscured through overlap, thus confounding confident interpretation of regioselectivity of alkylation. The FTIR spectra for compounds **1** and **2** are available in the Supplementary data. Given all of the above, it becomes apparent that there is an urgent and frequent need for a method that can easily distinguish between N- versus O-analogues in a highly reliable and rapid manner.

NMR methods: Over the years, our analytical NMR support group has established a protocol that has allowed for accurate and efficient structure determinations of reaction intermediates and drug-like inhibitors that result from *non-regioselective* alkylation reactions of ambident anions. Three independent and complementary NMR methods have been employed to maximize successful structure determinations, although, in our experience, consistent data from two methods could be considered as satisfactory, if data from the third were compromised (such as peak overlap, vide infra).

NMR Method 1: This through-bond method is considered the most definitive for primary structure determinations, as illustrated