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THERMODYNAMICS IN DRUG DESIGN. HIGH AFFINITY AND SELECTIVITY

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ABSTRACT

In drug discovery, active compounds identified by screening or other approaches usually bind to their targets with micromolar or weaker affinities. To become effective drugs, the binding affinities of those compounds need to be improved by three or more orders of magnitude. This task is not trivial if one considers that it needs to be done while satisfying several stringent constraints related to bioavailability, membrane permeability, water solubility, pharmacokinetics, toxicity, etc.. In addition, successful candidates need to exhibit appropriate selectivity and in the case of anti-infectives low susceptibility to mutations associated with drug resistance. These constraints emphasize the need for accurate ways of predicting the various effects of introducing diverse chemical functionalities or scaffold modifications during lead optimization, in particular effects on affinity and selectivity. Recently, it has become evident that the attainment of extremely high affinity, selectivity or adaptability is related to the proportion in which the enthalpy and entropy changes contribute to the binding affinity, and that appropriate control over these variables is critical during the design process. Since modern microcalorimetry provides extremely accurate measurements of the enthalpy and entropy contributions to binding affinity, it provides the basis for the development of rigorous algorithms aimed at: 1) Binding affinity optimization; 2) Improvement of binding selectivity between similar targets; 3) Incorporation of binding adaptability to mutations that cause drug resistance. In this chapter, the role of thermodynamics and enthalpy/entropy profiling in lead optimization will be discussed.

THE BINDING AFFINITY

The identification of drug candidates by screening large libraries of compounds and their subsequent optimization is an important step in the development of new pharmaceutical drugs. Modern high-throughput screening procedures are able to evaluate millions of compounds and identify those that exhibit the highest affinity or inhibitory potency with relative accuracy. Usually, compounds identified by screening have only marginal potency and their binding affinity needs to be improved by orders of magnitude. From a thermodynamic point of view, the binding affinity, K_a, is a function of the Gibbs energy of binding:

$$K_a = e^{-\Delta G / RT} \tag{1}$$

where R is the gas constant and T the absolute temperature. The Gibbs energy of binding is in turn defined by the enthalpy (ΔH) and entropy (ΔS) changes:

$$\Delta G = \Delta H \angle T\Delta S \tag{2}$$

therefore,

$$K_a = e^{-(\Delta H - T\Delta S)/RT}$$

$$K_a = e^{-\Delta H / RT} \times e^{\Delta S / R} \tag{3}$$

Since initial leads typically have affinities in the micromolar range and effective drugs require nanomolar and sometimes even higher affinities, an increase of at least three orders of magnitude in binding affinity is required. This increase is equivalent to an additional 4 kcal/mol in the Gibbs energy of binding. According to Equation 2, the binding affinity can be optimized by making ΔH more negative, ΔS more positive or by a combination of both. However, since enthalpy and entropy changes originate from different interactions, enthalpically or entropically optimized compounds are not equivalent even if they have the same affinity against the intended target.

IMPORTANT CONTRIBUTIONS TO BINDING ENTHALPY AND ENTROPY

Enthalpic and entropic contributions to the Gibbs energy originate from different types of interactions. Enthalpic contributions primarily reflect the strength of the inhibitor interactions with the protein (hydrogen bonds, van der Waals interactions) relative to those with the solvent (in this paper our discussion refers to the intrinsic binding enthalpy and not to enthalpic effects associated with protein conformational changes or linked protonation events, see reference [1] for a complete discussion). Because the unfavourable enthalpy change associated with the desolvation of polar groups is very large (~ 8 - 9 kcal/mol at 25ºC for NH_2, NH, OH, etc. [2], the interactions of polar groups with a protein target need to be strong enough to overcome the unfavourable desolvation enthalpy of those groups. For that reason the enthalpic optimization of a ligand cannot be accomplished by simply increasing the number of polar functionalities in the compound. Unless those functionalities (e.g. hydroxyl or amino groups) establish strong hydrogen bonds with the target, the desolvation penalty will be the predominant term and the overall enthalpy will be unfavourable. The compensation between enthalpy of interaction and enthalpy of desolvation can be appreciated by examining the binding energetics of existing HIV-1 protease inhibitors [3]. Saquinavir for example binds to the protease with an unfavourable enthalpy change of 1.9 kcal/mol at 25ºC [4]. Under the same conditions, TMC-126 binds to the protease with a favourable binding enthalpy of -12 kcal/mol [4]. Saquinavir has a total of 11 hydrogen bond donors and acceptors while TMC-126 has 10. It is evident that the binding enthalpy does not correlate with the number of groups that can participate in hydrogen bonds, emphasizing that the introduction of hydrogen bond donors or acceptors at arbitrary or even weak hydrogen bonding positions can be detrimental to the binding enthalpy and binding affinity. In fact saquinavir binds to the protease with a dissociation constant, K_d, of 0.4 nM whereas TMC-126 binds to the protease with a K_d of 0.004 nM [4].

Two major terms contribute to the binding entropy. The most important one is the change in solvent entropy arising from the complete or partial desolvation of the drug molecule and some regions of the protein upon binding. The second contribution is due to changes in conformational degrees of freedom experienced by the drug molecule and protein upon binding. The change in solvation entropy is tremendously favourable if the surfaces that are buried upon binding are predominantly hydrophobic.

Moreover, the unfavourable enthalpy associated with the desolvation of hydrophobic groups (e.g. CH_3, CH_2, CH) is one order of magnitude smaller than that of polar groups. The combination of a large favourable entropy and a small desolvation enthalpy contributes to yield a highly favourable Gibbs energy for hydrophobic burial which is estimated to be around 25 cal/mol×Å^2 [5-7]. The major drawback of this approach to affinity optimization is that the compound rapidly becomes insoluble in water.

Changes in conformational degrees of freedom occur in both the drug molecule and the protein molecule. In solution, the drug molecule is capable of adopting multiple conformations due to rotations around bonds. Upon binding, interactions with the protein constrain the drug to a single conformation resulting in a loss of conformational entropy. With the protein, a similar loss in conformational degrees of freedom is usually observed as side chains within the binding cavity and some backbone elements (e.g. flexible loops that become immobilized) lose conformational degrees of freedom. Since any loss in conformational entropy is unfavourable, one approach to minimize those adverse effects has been to introduce conformational constraints in the drug molecule. One rotatable bond that becomes immobilized upon binding carries a Gibbs energy penalty close to 0.5 kcal/mol due to the loss of conformational entropy [8]. Everything else being equal, a conformationally constrained molecule has a higher binding affinity because it does not carry that entropy penalty.

It must be noticed that the binding of most compounds is associated with a change in heat capacity (ΔC_p) which makes the enthalpy and entropy changes temperature dependent. Since ΔC_p is usually negative, the enthalpy change will become more negative at higher temperatures and in some cases a change in sign can be observed. For example, the binding enthalpy of saquinavir will change from 1.9 kcal/mol at 25ºC to -2.2 kcal/mol at 37ºC due to a binding ΔC_p of -340 cal/K×mol [9]. The change in the numerical values of the thermodynamic parameters with temperature does not imply that the mode of binding of a particular inhibitor has changed. Structural or molecular correlations of thermodynamic parameters should be made at one particular temperature, usually 25ºC, at which characteristic values for different interactions have been tabulated and are well known [2]. In this paper we have followed that convention and use 25ºC as the reference temperature for analysis.

Additional contributions to the binding enthalpy or entropy like those associated with protein conformational changes coupled to binding [1] need to be considered explicitly if the goal is to account for absolute values of the Gibbs energy, enthalpy or entropy changes. Those terms are less critical in scoring functions since drug molecules targeting the same protein site will generally elicit the same conformational change in the protein and contribute a constant term [1]. The same situation occurs with contributions arising from the reduction in rotational/ translational degrees of freedom which are similar for all ligands. Other interactions like salt bridges are less common in drug design due to the infrequent use of charged molecules as drugs, but need to be considered if present.

THE LOCK AND KEY PARADIGM

The most important drug design paradigm currently in use is derived from the classic key-and-lock hypothesis of enzyme specificity originally advanced by Emil Fischer in 1890 (see [10] for a review). The design paradigm is commonly referred to as the "shape complementarity principle" and essentially entails the synthesis of conformationally constrained drug molecules pre-shaped to the geometry of the target-binding site. A molecule that is pre-shaped to the target and conformationally constrained provides specificity and simultaneously enhances affinity. It provides specificity because the probability of finding different proteins with geometrically identical binding sites is generally low. There are important exceptions such as the kinases, all of which bind ATP and have similar binding sites, or blood coagulation factors, all of which are serine proteases with highly homologous catalytic sites. Drug design against individual members of these protein classes is notoriously difficult due to selectivity issues, emphasizing the need for better ways of approaching and solving the issue of selectivity. In addition to improving selectivity, conformational constraints also improve affinity because they minimize the loss of conformational degrees of freedom of the drug molecule upon binding. Organic and medicinal chemists have been able to successfully implement this strategy and design conformationally constrained molecules against a variety of targets.

Shape complementarity, however, does not guarantee binding. For binding to occur a favourable Gibbs energy is required.

Since the effective binding energetics is the difference between the magnitude of the drug/target interactions and the interactions with the solvent, it is always possible to generate a significant binding affinity by making the interactions of the drug with the solvent unfavourable; i.e. by increasing the hydrophobicity of the drug. This is in fact a common strategy, and as a result a high proportion of affinity-optimized drug candidates is highly hydrophobic and rigid (pre-shaped to the geometry of the binding site). The tendency towards higher hydrophobicity in new drug candidates is well known [11-13].

EXTREMELY HIGH AFFINITY

Compounds optimized according to the strategy described above have characteristic thermodynamic signatures as illustrated for compound 1 in Fig. 1.

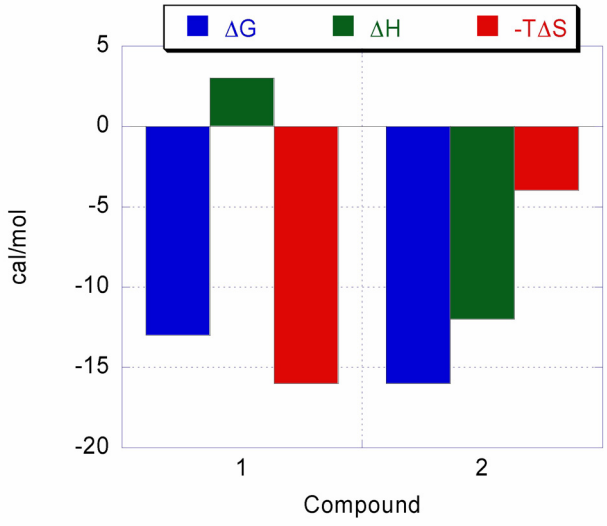

Figure 1. Thermodynamic signatures for two different hypothetical compounds. Compound 1 is entropically optimized, its binding is characterized by an extremely large entropy value that needs to compensate the unfavourable binding enthalpy. Compound 2 is favoured both enthalpically and entropically. As such, it can achieve extremely high affinity without the enthalpy or entropy changes assuming extreme values.

The combination of conformational constraints and hydrophobicity results in compounds that are entropically optimized and characterized by unfavourable or only slightly favourable enthalpy changes. All first generation HIV-1 protease inhibitors (nelfinavir, saquinavir, indinavir and ritonavir) fall under this category [4,9]. The binding of these compounds is entropically driven and at 25ºC their binding enthalpy is either unfavourable (indinavir, saquinavir, nelfinavir) or only slightly favourable [4,9].

There are several unwanted consequences associated with thermodynamic signatures like the one associated with compound 1 in Fig. 1. First, the enthalpic and entropic contributions to the binding affinity point in opposite directions, partially compensating each other. As a result, the binding affinity cannot achieve extremely high values. A significant portion of the favourable entropy change is used to offset the unfavourable enthalpy without a real gain in binding affinity. Since hydrophobicity is the main contributor to the favourable entropy change, these drugs exhibit the characteristic problems associated with poor water solubility. Finally, in the case of anti-viral drugs and other anti-infectives the lack of flexibility associated with the presence of excessive conformational constraints in the drug molecule prevents accommodation to binding site variations due to mutations and consequently a high susceptibility to drug resistance [14,15].

The thermodynamic signature of compound 2 in Fig. 1 is different. In this case, both the enthalpy and entropy changes contribute favourably to the binding affinity. As a result neither contribution has to assume extreme values in order to achieve a much larger binding affinity. In fact, the combination of favourable binding enthalpy and entropy is the key to reach extremely high binding affinities. In particular, the entropy change does not need to be exceptionally large to maintain high affinity allowing the designer the possibility of reducing the hydrophobicity of the compound and improve aqueous solubility. It provides the designer with a wider spectrum of possibilities since enthalpically favourable compounds do not need to be more polar as demonstrated for the case of HIV-1 protease inhibitors. In the case of anti-infectives, a favourable binding enthalpy also permits the introduction of flexible elements that will lower the susceptibility to mutations associated with drug resistance [16].

SELECTIVITY

Not all the forces that contribute to binding affinity contribute in the same proportion to specificity or selectivity. The two most relevant examples are hydrogen bonding and hydrophobicity, major contributors to the binding enthalpy and entropy respectively. The strength of hydrogen bonds and therefore their contribution to binding affinity is highly dependent on the distance and angle between donor and acceptor groups. As such, the engineering of several hydrogen bonds at critical locations will define a stereochemical fingerprint that will contribute significantly to affinity as well as selectivity.

On the other hand, the hydrophobic effect is non-specific and driven by the tendency of the compound to escape water rather than by a specific attraction to the target molecule. In fact, within the classic design paradigm, hydrophobicity provides the binding force while "shape complementarity" through conformational constraints provides specificity and selectivity.

From the above considerations, it is evident that maximal selectivity or specificity will be achieved if shape complementarity is combined with a cluster of strong stereospecific hydrogen bonds between drug and protein. Analysis performed in this laboratory and implemented in in-house design algorithms indicate that maximal selectivity is achieved with a critical number of hydrogen bonds. Either a very small or a large number of hydrogen bond donors or acceptors in the drug molecule can lead to poor selectivity. For example, if a molecule has a significant number of hydrogen bond donors and acceptors but binds to its target with only weak or unfavourable binding enthalpy, it indicates that some of those groups only make weak hydrogen bonds and are unable to compensate their unfavourable desolvation enthalpy. Against a homologous target, the same drug molecule may establish a different combination of similarly weak hydrogen bonds, giving rise to a similar energetic situation and resulting in poor selectivity. If, on the other hand, a drug molecule has few hydrogen bond donors and acceptors but binds with a strong favourable enthalpy, it would be indicative of the formation of strong hydrogen bonds with the target. Since the formation of these strong bonds requires a precise geometric arrangement for proper stereochemistry, the likelihood of finding a similar arrangement in other protein is limited, resulting in higher selectivity. In addition, since none of the groups participate in weak bonds, the possibility of alternative patterns is also minimized.

The above situation is illustrated schematically in Fig. 2. In this figure, drug molecule 1 with six hydrogen-bond capable groups will bind to proteins A and B with similar weak binding enthalpy (in both cases only three bonds are satisfied and three groups do not form bonds but pay desolvation penalty). Drug molecule 2, on the other hand, with only three hydrogen-bond capable groups will bind protein A with strong favourable enthalpy (the three bonds are satisfied) but no protein B (only one bond is satisfied and two groups only pay desolvation penalty). This example illustrates the advantages of fewer, geometrically well defined, strong bonds to improve selectivity. Of course, the cluster of bonds should define a pattern unique to the intended target.

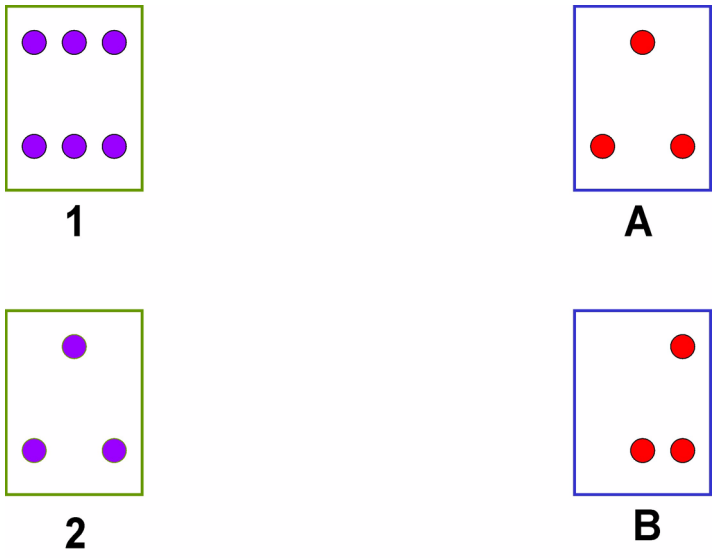

Figure 2. Selectivity is better achieved when a compound has few hydrogen bond donors and acceptors, all of which form strong bonds with the target. In this example, compound 1 has six groups and will bind with weak or unfavourable enthalpy to both protein A and protein B on the right (in both cases three of the groups do not form bonds and contribute unfavourably to the binding enthalpy due to the desolvation penalty). Compound 2 has three groups that perfectly match protein A but not protein B. Compound 1 binds with highly favourable enthalpy to protein A but will display unfavourable enthalpy towards protein B.

BINDING THERMODYNAMICS AND THE RULE OF 5

The Lipinski's "rule of five" [11-13] provides a powerful set of criteria for the solubility and permeability and, consequently, oral bioavailability of drug candidates. It basically stipulates that poor absorption or permeability is more likely when: 1) there are more than 5 hydrogen bond donors (expressed as the sum of NH's and OH's); 2) the molecular weight is over 500; 3) the log P is over 5 (or MlogP is over 4.15); and, 4) there are more than 10 hydrogen bond acceptors (expressed as the sum of N's and O's) [11].

Since some of the terms in the rule of five are related to hydrophobicity or groups that participate in hydrogen bonding interactions, it is important to assess if there is a correlation between thermodynamic parameters for binding and the rule of five. The four panels in Fig. 3 show the dependence of the binding enthalpy of HIV-1 protease inhibitors with the four criteria that define the rule of five. It is clear in the figure that there is no correlation between the number of hydrogen bond donors and the binding enthalpy. In fact, close inspection of the figure reveals that with four donor groups, for example, a compound can be characterized by a large positive (unfavourable) or large negative (favourable) binding enthalpy.

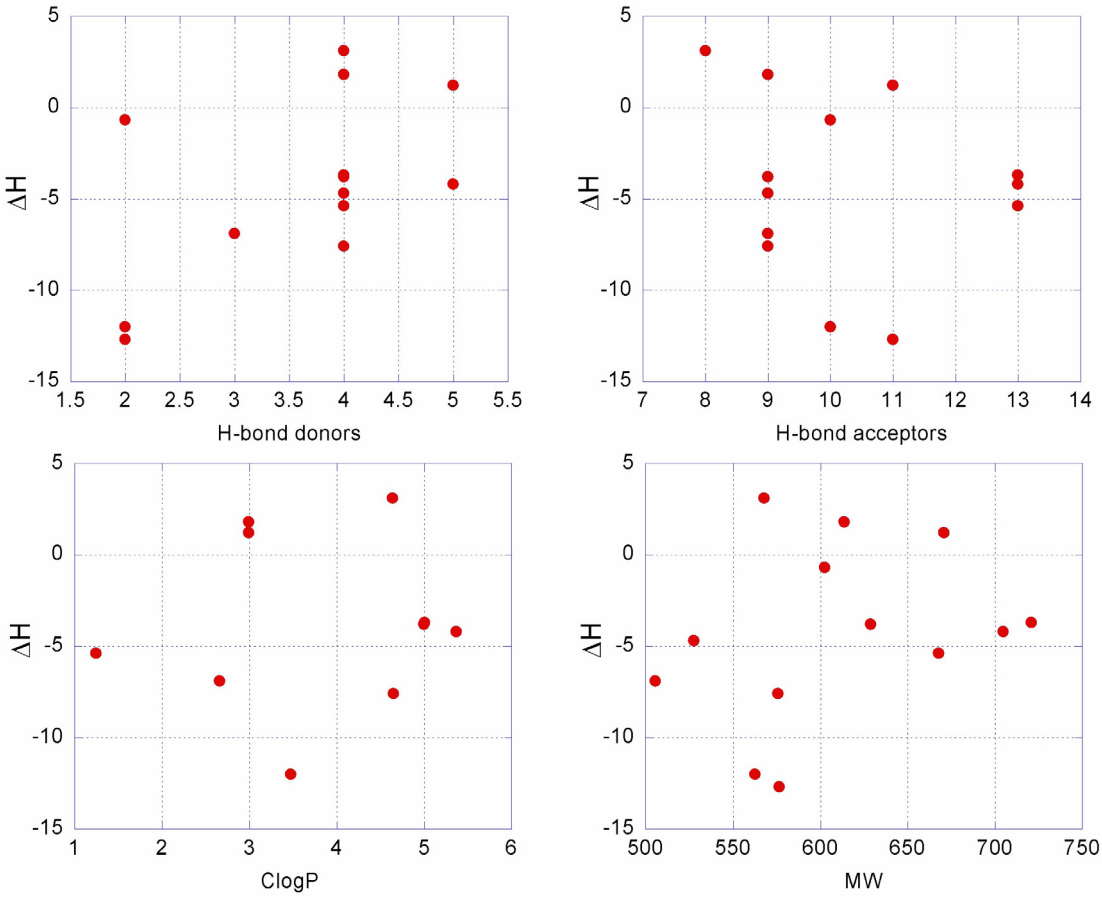

Figure 3. The correlation between the binding enthalpy (cal/mol) of HIV-1 protease inhibitors (included in the graph are indinavir, saquinavir, nelfinavir, ritonavir, amprenavir, lopinavir, atazanavir, KNI-577, KNI-272, KNI-764, TMC-114, TMV-126) with the number of hydrogen bond donors, hydrogen bond acceptors, octanol/water partition coefficient (C logP) and molecular weight, the four variables that define Lipinski's rule of five [11-13].

A similar situation can be observed with the number of hydrogen bond acceptors. As discussed above, the binding enthalpy is the sum of the favourable enthalpy of interaction and the unfavourable enthalpy of desolvation. If the hydrogen bonds are not strong or if a potential group is buried without making a bond, the unfavourable enthalpy of desolvation will be the dominant term. The figure also indicates the lack of correlation of the binding enthalpy with the calculated octanol/water partition coefficient and with the molecular weight of the inhibitors. Together, these observations indicate that a compound can be enthalpically or entropically optimized and that this character is not correlated with the parameters that define the rule of five. This conclusion is particularly important for enthalpically optimized compounds, due to the erroneous belief that these compounds require a large number of hydrogen bond donors and acceptors.

As discussed above, enthalpically optimized compounds afford significant advantages in affinity and selectivity, and in the case of anti-infectives also in adaptability to mutations associated with drug resistance as discussed elsewhere [3].

ISOTHERMAL TITRATION CALORIMETRY

The discussion above emphasizes the advantages of enthalpic optimization and, consequently the need for accurate measurements of binding enthalpies and binding entropies in addition to binding affinity. During the last decade, the development of highly sensitive isothermal titration calorimeters has revolutionized the analysis of binding reactions. Unlike any other technique, isothermal titration calorimetry (ITC) provides a complete thermodynamic characterization of the binding reaction since it directly measures the enthalpy of binding and the association constant, and consequently the entropy change. Furthermore, experiments performed at different temperatures provide the temperature dependence of the enthalpy change, i.e. the heat capacity change associated with the binding reaction. Unlike spectroscopic or other techniques, there is no need to obtain the temperature dependence of the association constant in order to estimate enthalpy changes through the van't Hoff equation. The van't Hoff analysis lacks accuracy due to three facts: 1) the narrow temperature range permissible in biological experiments; 2) the association constant very often appears temperature-independent within the accessible experimental range due to effects related to the existence of a change in heat capacity with binding; and 3) poor precision in the association constant determination that translate into large errors for ΔH and ΔS. Isothermal titration calorimetry does not require a van't Hoff analysis since it measures directly the heat released or absorbed by a binding reaction and therefore the enthalpy change. As a result, enthalpy changes can be routinely measured with accuracies close to 0.1 kcal/mol [17].

CONCLUSIONS

Even though many combinations of ΔH and ΔS values will elicit the same binding affinity (i.e. the same ΔG and therefore the same K_a), the properties and the response of these compounds to changes in the environment or in the protein target are not the same because they originate from different types of interactions.

Enthalpically optimized compounds have important advantages over their entropically optimized counterparts. First, because their affinity does not rely on hydrophobicity much higher affinities can be achieved without completely losing water solubility. Second, because enthalpic interactions arise from stereochemically specific interactions (e.g. hydrogen bonds), it is possible to achieve higher selectivity, provided that the compound contains only a critical number and arrangement of hydrogen bond donors and acceptors.

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