Nucleobase coupling by Mitsunobu reaction towards nucleoside analogs

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This paper is dedicated to Prof. Dr. Horst Kunz on the occasion of his 80th anniversary

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Abstract

The coupling of a nucleobase is a key step in the synthesis of most nucleoside analogs, e.g. carbocyclic nucleosides, isonucleosides and acyclic nucleosides. The synthetic strategies for nucleosides based on N-glycosylation are not applied when the nucleobase is not linked to the anomeric center. Thus, other methods have been employed, mainly those based on the alkylation of nucleobases. The Mitsunobu reaction, in which a hydroxy group is replaced by a nucleophile, has also been extensively applied, generating a diversity of molecules, including pharmaceuticals and their precursors. In this review the usefulness of this reaction for the coupling of nucleobases to non-anomeric positions of sugars, carbasugars and other homocyclic and linear structures is highlighted and discussed, covering purines and pyrimidines as pronucleophiles.

Keywords: Mitsunobu reaction, carbocyclic nucleosides, isonucleosides, acyclic nucleosides, synthesis
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# 1. Introduction

## 1.1. Nucleoside analogs

Nucleosides and nucleotides have been extensively used as prodrugs in antiviral and cancer chemotherapies. Nucleoside analogs comprise often a nucleobase coupled to the anomic position of a modified carbohydrate moiety and act as inhibitors of nucleic acid synthesis. After entering the cells through specific transporters, they are phosphorylated, leading to the accumulation of nucleotide analogs in cancer or virus-infected cells. These mono-, di- and triphosphorylated nucleotide analogs act by inhibiting intracellular enzymes, or by incorporating the synthesized DNA and RNA, inducing DNA chain elongation termination, apoptosis or mutations in viral progeny.\(^1\)\(^,\)\(^2\) Nucleoside analogs, namely those with carbasugars or acyclic moieties linked to the nucleobase, or with the nucleobase coupled to a non-anomeric position of the carbohydrate (isonucleosides) have been extensively studied. Recently, a comprehensive review was published covering the chemical synthesis of azasugar, thiosugar and selenosugar nucleosides and their anticancer activity.\(^3\) Both carbocyclic and acyclic nucleosides find application as antiviral drugs, e.g. the carbocyclic nucleoside entecavir and acyclovir, an acyclic nucleoside (Scheme 1). There are also reports of antiviral isonucleosides, e.g. the antitherpetic BMS 181,164, developed by Bristol-Myers Squibb (BMS)\(^4\)\(^,\)\(^5\) but, as far as we know, they do not include the clinically available drugs for the management of viruses.
Scheme 1. Structure of deoxyguanosine mimicking antiviral drugs entecavir (carbocyclic nucleoside), acyclovir (acyclic nucleoside) and the isonucleoside BMS 181,164, developed by Bristol Myers and Squibb (BMS).

The absence of a N-glycosyl bond, which can potentially increase the stability in a biological environment,\textsuperscript{6} poses a problem: the coupling of the nucleobase to the sugar mimetics or to a non-anomeric position cannot be accomplished by reactions carried out for nucleoside synthesis that take advantage of the specific reactivity of the anomeric center. Therefore, key synthetic strategies must be employed to obtain the nucleoside analog. The most common approaches can be classified into two groups: nucleobase coupling and nucleobase building-up. In the first strategy the desired nucleobase (or a precursor) is coupled directly to the sugar (or sugar mimetics), while in the latter, the nucleobase is constructed from a linear (for pyrimidines) or aromatic (for purines) structure, already linked to the sugar. The coupling of the nucleobase can be achieved by a variety of methods.\textsuperscript{7,8} One of them makes use of the Mitsunobu reaction (MR), as illustrated and discussed in this review for the synthesis of nucleoside analogs.

1.2. The Mitsunobu reaction
First reported in 1967,\textsuperscript{9} this reaction has a diversity of synthetic applications as described in comprehensive reviews published in 2009 \textsuperscript{10} and 2015.\textsuperscript{11} MR conducts to the replacement of the substrate free hydroxy group by a nucleophile, with inversion of configuration when the substrate is chiral, mediated by dialkyl azodicarboxylate and trialkyl- or triarylphosphane. The reaction requires the activation of dialkyl azodicarboxylate with the phosphane leading to the formation of betaine 1, which reacts with the pronucleophile (NuH) generating the ionic species 2. Reaction with the alcohol gives a phosphonium salt and ethyl 2-(propionyloxy)hydrazinecarboxylate (3). The attack of the nucleophile follows a S\textsubscript{N}2 mechanism to afford the final product and trialkyl- or triarylphosphane oxide,\textsuperscript{10-12} as shown in Scheme 2, for a reaction mediated by diethyl azodicarboxylate (DEAD) and triphenylphosphane. Interestingly, a S\textsubscript{N}1 mechanism can take place, but is very rare and was never reported to occur in nucleobase coupling.\textsuperscript{10} The pronucleophile usually comprises a -OH, -SH or -NHR group, although other groups with a pKa lower than 11 may also react.\textsuperscript{11} The common azodicarboxylates in MR are DEAD and diisopropyl azodicarboxylate (DIAD), di-tert-butyl azodicarboxylate (DBAD) is also used, while triphenylphosphane is, indeed, the most frequently phosphane applied.

Catalytic MRs should also be highlighted, as they overcome MR issues such as poor atom economy, and the formation of stoichiometric phosphane oxide and/or hydrazine by-products. Aiming at reducing to a catalytic amount the azo MR reagent, Toy \textit{et al.}\textsuperscript{13,14} used iodobenzene diacetate to reoxidize the hydrazine formed, while Hirose \textit{et al.}\textsuperscript{15,16} described the first MR in which hydrazine is reoxidized in the presence of a catalytic amount of iron phthalocyanine and atmospheric oxygen. The first fully catalytic MR was reported by Buonomo & Aldrich\textsuperscript{17} who used catalytic 1-phenylphospholane, employing phenylsilane to recycle the catalyst, integrating it with Taniguchi’s azocarboxylate catalytic system. One year later, Hirose \textit{et al.}\textsuperscript{18} reinvestigated this reaction and in 2016 they reported examples for which the catalytic system in phosphane
reagent is incompatible with that in the azo reagent. Recently, a fully catalytic redox-free MR has been reported\(^1\) using (2-hydroxybenzyl)(methyl)(phenyl)phosphane oxide as catalyst. The oxidation state of phosphorus remains +5 all over the reaction, as the mechanism involves the formation of a phosphonium ring with the oxygen of the phenol hydroxy group linked to phosphorus, which is opened by the alcohol. This reaction has been successful for C-O, C-N and C-S bond formation, leads to inversion of configuration, does not involve stoichiometric oxidant nor reductant, and has water as the single by-product.\(^{19}\) To the best of our knowledge, catalytic MR reactions have not yet found application for the synthesis of nucleoside analogs.

Scheme 2. Mechanism for the Mitsunobu reaction.\(^{12}\)

In nucleoside analog synthesis, the nucleobase acts as a pronucleophile and replaces the hydroxy group of the substrate. Therefore, this synthetic strategy is more efficient in a single unprotected hydroxy group, although steric hindrance may affect the reactivity of some hydroxy groups, resulting in regioselectivity.\(^{20}\) The nucleobase also has an impact on the outcome of the reaction as further discussed in this review.

MR has seen extensive use for nucleobase alkylation, mostly in the context of drug development, including scale up attempts, namely for the synthesis of the antiviral drug entecavir, in gram\(^{21,22}\) and even kilogram\(^{23}\) scales. The application of MR in large scale has so far been limited by the complex nature of the resulting reaction mixtures, which makes them difficult to purify. However, the methodologies based on catalytic Mitsunobu reactions seem to solve this issue.\(^{19,24}\)

2. Nucleobase Coupling

The effectiveness of the nucleobase coupling by MR depends on the alcohol precursor, the nucleobase and the reaction conditions, namely the solvent, the temperature and the order in which the reagents are added.\(^{25-28}\) The usual yield of a nucleobase coupling reaction through MR is in the 50% to 80% range, for carbasugar, other carbohydrates and acyclic substrates, with higher yields being generally obtained with primary alcohols. The low reactivity of the alcohol, a lack of regioselectivity, side reactions and the difficulty in purification of the complex reaction mixture may also compromise reaction yields.\(^{29,30}\) Nonetheless, MR is a suitable and unique alternative to some nucleoside analogs that could not be synthesized by the usual
methodologies and has proven appropriate for the coupling of purines and pyrimidines as described in sections 3 and 4 of this review.

2.1. Reaction conditions in MR and alcohol structure
In carbocyclic nucleoside and isonucleoside synthesis the limiting reagent is the alcohol, while for acyclic nucleosides it is common to use an excess of alcohol in relation to the nucleobase.\textsuperscript{26,28,31-34} This excess may have to be handled in different ways, as demonstrated by Lu et al.\textsuperscript{31} Aiming to overcome low yields caused by alcohol degradation, two successive additions of the non-limiting reagents (alcohol, DIAD and PPh\textsubscript{3}), separated by 6 hours, were made, resulting in a considerable yield increase. Similar procedures have also been successfully used by other authors.\textsuperscript{26,34}

The influence of alcohol structure on reaction yield is clearly demonstrated by Šála et al.\textsuperscript{35} during coupling of 6-chloropurine with bicyclic alcohols 4, 5, 6 and 7 (Scheme 3) via MR protocol. While the yield was higher with a primary alcohol, resulting from less steric hindrance, those for the secondary alcohol substrates were considerably different, with the highest yield obtained for alcohol 5. Lu et al.,\textsuperscript{31} Dai et al.\textsuperscript{32} and Fletcher et al.\textsuperscript{33} used tert-butanol as a model tertiary alcohol but no reaction occurred, while the other alcohols screened gave yields in the 80-90% range, with the nucleobase as the limiting reagent. This was expected as MR reacts mainly via S\textsubscript{N}2 mechanism.

![Scheme 3. MR coupling of 6-chloropurine to different bicyclic alcohols as reported by Šála et al.\textsuperscript{35}](image)

However, there are alcohols where MR coupling did not succeed, even when several reaction conditions were explored. Few such examples are reported in the literature by Chen et al.\textsuperscript{36} during assembly of fluorinated acyclic nucleoside phosphonates containing cytosine and adenine, by Brémond et al.\textsuperscript{37} and Kasula et al.\textsuperscript{38} in an attempted synthesis of aristeromycin analogs embodying 6-chloropurine and 7-deazapurine moieties, respectively. Rosen et al.\textsuperscript{39} also failed the coupling of cytosine and 2-amino-6-chloropurine to mono-fluorinated cyclopropane sugar mimetics. The alternative pathway was based on the conversion of the free
hydroxy group into e.g. mesylate or tosylate, followed by the conventional $S_{N}2$ reaction to couple the nucleobase, in two reaction steps.

The solubility of the nucleobase can also have a noticeable effect on the outcome of the reaction. While a variety of solvents can be used for MR, the most frequently used for nucleobase coupling is THF. The low solubility of nucleobases such as adenine and $N^4$-benzoyl cytosine in this solvent have hampered their use in MR. One good example of the importance of nucleobase solubility is given by Bazile et al. who found out that the yield of MR coupling of 2-fluoroadenine, which has low solubility in THF, was low (only 27%), while that for the coupling of its Boc-protected derivative was 88%.

2.2. Regioselectivity, side reactions and product purification

The reaction conditions affect the regioselectivity during coupling to purines ($N^9/N^7$) and to pyrimidines ($N^1/O-2$); however, this is mainly an issue for pyrimidines, which rarely lead to a single product, while MR with purines leads mostly to $N^9$ ligation; moreover, the $N^3$ position of uracil and thymine pronucleophiles is protected in most cases to prevent the formation of $N^3$ and O-4 linked nucleoside analogs.

The formation of side products is also frequent, resulting in low reaction yields. In carbasugars embodying an $\alpha,\beta$-unsaturated alcohol, e.g. in compound 8 (Scheme 4), the $\beta$-addition of the nucleobase, migration of the double bond and displacement of triphenylphosphane oxide can also take place. Scheme 4 illustrates the results obtained by Kumamoto et al., who was able to prepare nucleoside 10 in only 26% with the concomitant formation of the analog diastereoisomers 9a,b, obtained in 41% yield. This effect was mitigated by replacing $-\text{C≡CH}$ by $-\text{CO_2Me}$, resulting in the formation of only the desired product 12 in 58% yield.

Another reported side reaction is dehydration of alcohol precursor. Both Viña et al. and Weising et al. experienced this problem when attempting to synthesize cis carbocyclic nucleosides (Scheme 5); in the latter case, a relationship between solvent used and the extent of elimination was found, since reactions in MeCN resulted in more elimination than those in THF. Dehydration may also occur in linear alcohols, as shown by
Guo et al.;\textsuperscript{27} in this case, the elimination reaction occurred when the L-serine amino group was protected by Boc, while the same was not verified with a trityl protecting group.

Scheme 5. Alcohol dehydration, as reported by Viña et al.\textsuperscript{46} (A), Weising et al.\textsuperscript{29} (B) and Guo et al.\textsuperscript{27} (C).

Product purification is also a challenge.\textsuperscript{29,47-52} MR reaction mixture is usually quite complex, containing remaining excess reagents, the dialkyl hydrazine-1,2-dicarboxylate derivative, triphenylphosphane oxide, the desired product and, in some cases, also side products. Therefore, tedious purification processes result in low product yields.\textsuperscript{50} One solution practiced is the use of crude products in the following step, leading to separable products.\textsuperscript{49,51} Also investigation of other appropriate nucleobases may be a strategy towards nucleoside analog purification.\textsuperscript{48}

2.3. MR as an alternative to nucleobase coupling failure

Common nucleobase coupling strategies are not efficient for all target molecules, and MR can be the appropriate alternative, as shown by Singh et al.\textsuperscript{53} The attempt to perform a nucleophilic substitution (S\textsubscript{N}2), intended to transform the carbasugar 21 into the entecavir analog 22 failed, leading instead to the addition of the nucleobase in the β position and migration of the double bond to afford compound 23 (Scheme 6); however, MR starting from 24 gave compound 22 in 65% yield.
Scheme 6. Nucleobase β-addition with migration of double bond, a competitive reaction to the proposed S_{N}2 substitution, as reported by Singh et al.\textsuperscript{53}

MR was successfully used by Douadi et al.,\textsuperscript{54} Christian et al.,\textsuperscript{55} and Yang et al.\textsuperscript{56} to afford nucleoside analogs 26, 28 and 30, starting from carbasugar substrates embodying a cyclopent-2-en-1-ol (Scheme 7), while palladium-catalyzed allylic substitution, one of the most common procedures for nucleobase coupling, did not produce the desired carbocyclic nucleoside. MR is also an alternative to nucleophilic substitution of unstable tosylate and mesylate substrates.\textsuperscript{52}

Scheme 7. MR coupling to cyclopentene derivates as an alternative to Pd (0) catalyzed acetyl displacement, as described by Douadi et al.\textsuperscript{54} (A), Christian et al.\textsuperscript{55} (B) and Yang et al.\textsuperscript{56} (C).
Failure to couple nucleobases to epoxides for the synthesis of isonucleosides type 34 encouraged Yoshimura et al. to treat the epoxide with thiophenol or (4-methoxyphenyl)methanethiol giving compounds type 32, which were then submitted to MR. Stereoselective attack of the nucleobase to the episulfonium intermediate type 33 gave the nucleoside analog 34 (Scheme 8). Both 6-chloropurine and 3-benzoylthymine were successfully coupled by this methodology in good yields (83%-80% for 6-chloropurine and 52%-59% for 3-benzoylthymine).

Scheme 8. Nucleobase coupling by MR via episulfonium intermediate 33, performed by Yoshimura et al. 57,58

3. Purine Coupling

The direct MR coupling of the two canonical purine nucleobases, adenine and guanine, is not, in most cases, the most applied methodology for nucleoside analog synthesis. Although adenine has been used as a pronucleophile, 43,59-62 the strategy to obtain an adenine nucleoside relies mainly on the coupling of a nucleobase precursor, followed by transformation of the purine to adenine moiety.

No examples of successful coupling of guanine have been reported in the last ten years, as it does not react under the conditions producing moderate yields with other nucleobases, 52,31,36 which may result from guanine’s extremely low solubility, 36 even under reflux conditions. 31

3.1. Adenine and adenine precursors’ coupling

Like guanine, adenine also has a low solubility in THF. 40 Alternatively, 1,4-dioxane can be used as solvent in MR with this nucleobase, allowing for moderately good coupling yields when reacting with primary alcohols of carbasugar analogs. Illustrative examples were reported by Csuk & Thiedee, 59 who obtained the nucleoside analog 36 in 72% yield when coupling adenine to the difluorocyclopropane derivate 35, and by Franzyk & Stermitz 60 with the preparation of the carbocyclic nucleoside 38 in 68.5 % yield (Scheme 9).
Scheme 9. Successful coupling of adenine as reported by Csuk & Thiedee\textsuperscript{59} (A), and Franzyk & Stermitz\textsuperscript{60} (B).

Nevertheless, in most recent reports, alternative pronucleophiles have been used to obtain adenine nucleoside analogs, namely 6-chloropurine and 6-[bis(tert-butoxycarbonyl)amino]purine (Scheme 10).

Scheme 10. Structure of adenine precursors used in MR.

Both nucleobases led to moderate to good MR yields. Michael & Strazewski\textsuperscript{63} succeeded to couple the two pronucleophiles to carbasugar\textsuperscript{39}, obtaining compounds\textsuperscript{40} and\textsuperscript{42} in 82% and 95% yield, respectively (Scheme 11). However, sugar deprotection of\textsuperscript{40} with TFA gave the unexpected hypoxanthine derivative\textsuperscript{41}. Another example of Boc-protected 6-aminopurine coupling was reported by Liu\textit{ et al.}\textsuperscript{64} who coupled this nucleobase derivative to carbasugars\textsuperscript{43},\textsuperscript{44} and\textsuperscript{45} affording the nucleoside analogs in 77%, 87% and 88%, respectively (Scheme 12).
Scheme 11. High yield coupling of adenine precursors to a bicyclic carbasugar, and the formation of hypoxanthine derivative 41, as described by Michael & Strazewski et al. 63

Scheme 12. High yield coupling of Boc-protected 6-aminopurine to three different secondary alcohols, as reported by Liu et al. 64

Both nucleobase derivatives, when linked to the sugar moiety, can be converted to give the corresponding adenine nucleosides. Reaction of 6-chloropurine to adenine requires high temperatures in methanolic NH3, commonly in high pressure vessels, 40 harsher reaction conditions than those for deprotection of 6-[bis(tert-butoxycarbonyl)amino]purine achieved at room temperature. 65 Moreover, 6-chloropurine is sensible to acid media, as reported by Michael & Strazewski, 63 leading to hypoxanthine derivatives, e.g. compound 41 (Scheme
11). Nevertheless, 6-chloropurine is quite used for nucleoside analog synthesis as it can be transformed into a variety of nucleobases, and in some cases gives better yields than the Boc-protected 6-aminopurine, as reported by Guo et al. for its coupling with N-protected L-serine methyl ester.\textsuperscript{27}

3.2. Guanine precursors’ coupling

Guanine nucleoside analogs are frequently prepared by coupling 2-amino-6-chloropurine and derivatives, namely 2-acetamido-6-chloropurine,\textsuperscript{44} 2-(\textit{tert}-butyloxycarbonyl)amino-6-chloropurine\textsuperscript{33} and 2-[\textit{bis}(\textit{tert}-butyloxycarbonyl)]amino]-6-chloropurine, followed by acid\textsuperscript{66} or basic treatment.\textsuperscript{67} Mohamed \textit{et al.}\textsuperscript{65} achieved the coupling of 2-amino-6-chloropurine to a carbocyclic vinylphosphonate by MR to give the corresponding nucleoside analog in 80% yield, which was transformed in the carbocyclic vinylphosphonate derivative of guanine. Also, Miralles-Llumà \textit{et al.}\textsuperscript{66} took advantage of MR to couple this nucleobase to a cyclobutyl unit obtaining \textit{47} in 87% yield. However the same nucleobase failed to couple with the alcohol \textit{48}, but with the 2-\[\textit{bis}(\textit{tert}-\text{butyloxycarbonyl})\textit{amino}]\textit{6-chloropurine}, MR coupling generated the nucleoside analog \textit{50} in 60% yield (Scheme 13). The versatility of 2-amino-6-chloropurine was also shown by Weising \textit{et al.},\textsuperscript{57} who converted the carbocyclic nucleoside \textit{51} into abacavir and carbovir (two antiviral drugs) in a single step (Scheme 14).

\textbf{Scheme 13.} Coupling of guanine precursors to two different carbasugars has been reported by Miralles-Llumà \textit{et al.}\textsuperscript{66}
Scheme 14. High yield, one step conversion of 51 into the antiviral compounds abacavir and carbovir.\textsuperscript{67}

An alternative to chlorinated guanine precursors is a nucleobase embodying a diphenylcarbamate at position 6 with its 2-amino group protected with the 2-methylethylcarbonyl group. This guanine precursor was coupled to the diprotected glycerol \textsuperscript{52} by Krishnamurthy et al.\textsuperscript{68} via MR in yields ranging from 47\% to 58\% (Scheme 15). This acyclic nucleoside, derived from glycerol, was prepared for its further exploration in oligonucleotide chemistry aiming to discover new nucleic acid mimetics with base-pairing properties.

Scheme 15. Synthesis of the acyclic sugar nucleoside analog 53 derived from 1,3-diprotected glycerol \textsuperscript{52} via MR.\textsuperscript{68}

Hollenstein & Leumann\textsuperscript{69} succeeded to couple the same nucleobase to the acyclic diol \textsuperscript{54} through a highly regioselective MR, affording the acyclic nucleoside analog \textsuperscript{55} in 59\% yield (Scheme 16). This structure served as a precursor to access new DNA mimetics with potential binding affinity to complementary oligonucleotides.

Scheme 16. Synthesis of acyclic nucleoside \textsuperscript{55} as reported by Hollenstein & Leumann.\textsuperscript{69}
A similar purine was proposed by Lu et al., with an acetamido group at position 2, which has a good solubility in THF and high reactivity. The authors succeeded to couple this protected nucleobase with primary, secondary and aromatic alcohols by MR in yields ranging from 70% to 80%. This base was also linked through MR to alkyl chains by Wamberg et al., aiming to generate new molecules for noncovalent anchoring to amphiphilic membranes. While these two examples do not describe nucleoside analog synthesis, they illustrate N9-guanine alkylation through MR and may therefore be potentially useful for the synthesis of isonucleoside, carbocyclic nucleosides and acyclic nucleosides.

### 3.3. Regioselectivity and alkylation of purine amino groups

Purine coupling to carbohydrates and carbasugars through MR is regioselective for the N9 position of the nucleobase, with only some rare examples of formation of N7-linked carbocyclic nucleosides as minor products, and one report of N3 coupling as a major product in isonucleoside synthesis, with both adenine and 8-azaadenine as pronucleophiles.

MR regioselectivity conducting to the N3 linkage has also been found in acyclic nucleosides. An example is the reaction of bis[(tert-butylcarbonyloxy)methyl] 4-hydroxy-but-2-enylphosphonate with 6-chloropurine or 2-amino-6-chloropurine, affording, under MR conditions, the N9 acyclic nucleosides in 74% and 50% yield, respectively, while a 21% N9:N7 (7:3) reaction mixture was obtained when the corresponding 4-bromo derivative reacted with the base by a S_n2 reaction. However, some reports show that, even under MR conditions, the regioselectivity for the N9 isomer can be rather low, reaching proportions of N9/N7 (5.6:1), as shown by Manvar & Shah for the coupling of 2-amino-6-chloropurine to the terminal hydroxy group linked to the ethoxymethylphosphonate chain. N9 regioselectivity was also described by Lu et al. in the synthesis of non-sugar carbon nucleosides through MR, although N7-alkylation also occurred when 2,6-dichloropurine was coupled to some of the alcohols, in 5-18% yields. Solvent, reaction temperature, azodicarboxylate reagent (DEAD or DIAD), and amount of non-limiting reagents also influence regioselectivity. Both Lu et al. and Manvar & Shah described that, by adding alcohol(1.05 equiv.), DIAD (1.05 equiv.) and PPh3 (1.05 equiv.) twice, and running the reaction for 6 hours after each addition, almost exclusively N9 alkylation occurred, while adding the same reagents' amount but in one single portion gave lower regioselectivity and lower yields.

While most examples of purine alkylation through MR happen in the nucleobase N9 and N7 positions, the latter as minor reaction products, there is also the possibility of using MR to alkylate other positions of the purine. MR is highly chemoselective conducting to reaction at the N9 position, even with bases bearing a free amino group, namely 2-aminochloropurines. However, alkylation of the 2-amino group of N9-protected nucleobases was carried out by MR when using an excess of primary and secondary alcohols to give compounds type 57, in high yields (86-93%). Alklyation of the (tert-butoxycarbonyl)amino group in position 6 of the adenine derivative 58 by MR, with both primary and secondary alcohols, also gave nucleoside analogs in high yields (81-91%), deprotected by acid treatment (Scheme 17). Noteworthy, these MRs only succeeded because the amino group is Boc-protected, which increases the acidity of this non-aromatic NH group.
Scheme 17. Alkylation of the 2-amino group and the 6-amino group, of guanine and adenine analogs, respectively, as presented by Fletcher et al.\textsuperscript{75,76}

4. Pyrimidine Coupling

The coupling of pyrimidines by MR is mostly restricted to 3-benzoyluracil and 3-benzoylthymine, which behave similarly as pronucleophiles. Cytosine is not commonly used, although some derivatives, in which the amino group is protected with a benzoyl group,\textsuperscript{77} an acetyl group\textsuperscript{27} or diprotected with bis(tert-butoxycarbonyl)\textsuperscript{78} have been coupled with moderate to good yields.

4.1. N\textsuperscript{1}/O-2 Regioselectivity

MR regioselectivity is the main issue when the pronucleophile is a pyrimidine. Pyrimidines may undergo tautomerization in solution, as shown in Scheme 18. Since both NH and OH groups can act as pronucleophiles in MR, the use of this reaction with pyrimidines will usually result in mixtures of N-alkylated and O-alkylated products. Moreover, it is necessary to protect the N\textsuperscript{3} position, usually with a benzoyl group, to avoid linkage to N\textsuperscript{3} and O-4, which can result in the formation of nucleoside analogs embodying two sugar moieties.\textsuperscript{79,80}

Scheme 18. Tautomerization of uracil and thymine derivatives.

The regioselectivity of the reaction depends on multiple factors. Leung et al.,\textsuperscript{81} carried out the optimization of the MR experimental procedure for the coupling of N\textsuperscript{3}-benzoylthymine to cyclopentanol 60 (Scheme 19, Table 1) by testing different solvents, reagent proportions and reaction temperature (0 °C and rt). The results showed that the highest regioselectivity and the highest yield for N\textsuperscript{1}-alkylation occurred by using DMF as solvent, in comparison with THF and dichloromethane (solvent as the only variable). It was also shown that, by increasing the proportion of the nucleobase relatively to the reagents Ph\textsubscript{3}P and DIAD, at room temperature, the N\textsuperscript{1}-alkylated product yield increased from 53% to 64%, although at the cost of producing more of O-2 linked product, which yield increased from 8% to 25%.\textsuperscript{81}
Scheme 19. 3-Benzoylthymine coupling performed by Leung et al.\textsuperscript{81}

Table 1. Reaction optimization performed by Leung et al.\textsuperscript{81}

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Weising et al.\textsuperscript{29} tested MeCN and THF for the coupling of a carbocyclic ribo-nucleoside with analog N\textsuperscript{3}-benzoylthymine, to control regioselectivity and avoid elimination. The results obtained show that in MeCN a product ratio of 1:1 (N\textsuperscript{1}:O-2) was obtained, while in THF the ratio was 1:4 (N\textsuperscript{1}:O-2), favoring O-2 ligation. This conclusion should be envisioned as specific for this reaction, as there are examples of regioselective N\textsuperscript{1} coupling by MR with THF.\textsuperscript{78}

While in many cases the yield of MR with pyrimidines is quite good,\textsuperscript{23,59,75-77} there are no reported cases of nearly quantitative yields of N\textsuperscript{1}-linked products, most probably due to the formation of O-2 derivatives.

5. Coupling of Other Nitrogen Heterocycles

MR can be employed to synthesize carbocyclic nucleosides comprising N-heterocyclic bases with a structure not derived from canonical nucleobases, namely deazapurines. These compounds, including 1-deazapurines,\textsuperscript{85} 3-deazapurines\textsuperscript{49,51,86-88} and 7-deazapurines\textsuperscript{44} have been successfully coupled to alcohols by MR.

Unlike regular purines, the coupling of 3-deazapurine may not be regioselective. Yang et al.\textsuperscript{87} reported an example were alcohol precursors with unsaturated bonds (compounds 63, 64 and 65, Scheme 20) led to N\textsuperscript{9} and N\textsuperscript{7} linked isomers, when 3-deaza-6-chloropurine was used as a pronucleophile. However, this regioselectivity may change when the base embodies bulky substituents that produce stereochemical hindrance to the attack of a particular position, as shown by Jha et al.\textsuperscript{49} for the N\textsuperscript{7} position (Scheme 21). The authors obtained the N\textsuperscript{9} carbocyclic nucleoside by reaction of the carbasugar 66 with very minor formation of the N\textsuperscript{7} isomer (<5-7%).
Scheme 20. Non-regioselective coupling of 6-chloro-3-deazapurine to alcohols, as reported by Yang et al.\textsuperscript{87}

\[ \text{ROH: } 63 \quad \text{TrO} \quad \text{OH} \quad 42\% \quad 53\% \]
\[ 64 \quad \text{AcO} \quad \text{OH} \quad 38\% \quad 57\% \]
\[ 65 \quad \text{OH} \quad 32\% \quad 43\% \]

Scheme 21. N\textsuperscript{9} Regioselective coupling of a 3-deazapurine to the carbasugar 66 resulting from stereochemical hindrance.

6-Azaaracil (68) is a pyrimidine analog that can be coupled by MR with high N\textsuperscript{1} regioselectivity, even without benzylation of N\textsuperscript{3}, unlike pyrimidines. The enhanced acidity of N\textsuperscript{3} resulting from the adjacent N\textsuperscript{6} favored the synthesis of the desired carbocyclic nucleoside 70. Sugar deprotection afforded 71 in 54% yield over the two steps (Scheme 22).\textsuperscript{89}

Scheme 22. Regioselective coupling of unprotected 6-azauracil as reported by Jin et al.\textsuperscript{89}

Pursuing the development of novel and selective inhibitors of phosphoribosyltransferase, an enzyme that is crucial for the survival of the parasite \textit{Plasmodium falciparum}, Klejch et al.\textsuperscript{28} attached a diversity of acyclic
phosphonates with a free hydroxy group to the antiviral drug favipiravir and to a 4-chlorinated allopurinol precursor via MR (Scheme 23). Experimental conditions were extensively investigated, e.g., reagents proportion, time, temperature, solvent, but the coupling of favipiravir occurred mainly in the O-2 position (49-90% isolated yields for different alcohol substrates), or exclusively in this position (Scheme 23), while the coupling of the chlorinated allopurinol precursor produced only the desired N° regioisomer. In the search for furanose and pyranose isonucleosides as potential cholinesterase inhibitors for Alzheimer’s disease treatment, the purine theobromine (compound 77) has also been coupled to different carbohydrate moieties by MR, with regioselective alkylation of N° (Scheme 24).

Scheme 23. MR regioselectivity resulting from reaction of favipiravir and 4-chlorinated allpurinol with the OH group of acyclic phosphonates.

### 6. Microwave-assisted MR Coupling

Microwave-assisted MR (MW-MR) is often an alternative to shorten reaction time and improve reaction yield. One example relies on MR of theobromine 77 with the primary alcohol 76. Under conventional conditions, the reaction was carried out for 48 h and gave a very low yield (16%) of the N°-linked isonucleoside 78. However, the yield increased to 44% after 30 min under microwave irradiation (Scheme 24). With the same strategy, it was also possible to couple adenine to carbohydrate 76 in 33% yield, while under conventional conditions no reaction occurred.
Scheme 24. Theobromine coupling by MR under conventional heating and microwave irradiation.

Carbocyclic nucleosides 80-83, analogs of neplanocin, a natural product with potent antitumor and antiviral activities, were synthesized by Liao et al. The MR yield for base coupling of these 7-deazapurines increased from 20-30% under conventional heating conditions for sixteen hours, to 40-50% after 1 minute under microwave irradiation (Scheme 25).

Scheme 25. Carbocyclic neplanocin analogs by MR under conventional conditions and by MW assisted MR.

Weising et al. tested MR with THF and MeCN for pyrimidine coupling to the carbasugar 15, in both conventional conditions and under microwave irradiation (100 W, 45 ºC) (Scheme 26). Interestingly the yield of 84 was not significantly increased (from 18% to 20%, in MeCN), and the ratio N1:O-2 was 1:1 in both reaction conditions. The same trend was shown for N3-benzoyluracil under MW assisted MR. However, the use of the MW-MR made the purification of the products easier because a smaller excess of reagents was required. Under the same reaction conditions, the coupling of 15 to N-4 acetylcytosine by MW-MR afforded exclusively the O-2 linked nucleoside analog 86 in 70 % yield. MW-MR was also used to couple 6-chloropurine and 2-amino-6-chloropurine, and the N9-nucleoside analogs were obtained in 80% and 70% yield, respectively, when reaction was carried out in THF, as MeCN led to a lower yield (49%) for 6-chloropurine coupling with 15. Similar microwave-assisted conditions (100 W, 50 ºC) were reported for the coupling of 2-amino-6-chloropurine to alcohol 87 in 59% yield after one hour.

These illustrative examples demonstrate that MW-MR may be a step forward for nucleobase coupling by giving considerably shorter reaction times, facilitating nucleoside analog purification, and in some instances also improving reaction yields.
Scheme 26. Microwave-assisted Mitsunobu reactions as reported by Weising et al.\textsuperscript{29,67}

7. Conclusions

Throughout this survey, multiple aspects affecting the synthesis of nucleoside analogs by MR were highlighted and discussed. Indeed, no single factor, by itself, can determine the success of the reaction, but some conclusions can be withdrawn. First, some alcohol precursors are not suitable for nucleobase coupling, due to their low reactivity, as the tertiary or highly hindered secondary alcohols, some of them suffering side reactions, e.g. elimination or $\beta$ addition on $\alpha,\beta$-unsaturated alcohols. Nucleobases with low solubility have a dramatic effect on yield, and reaction optimization is usually successful if a suitable solvent is found, or if the nucleobase is chemically modified. Some of the reaction parameters that can affect yields, such as temperature or order of addition of reagents, cannot be so easily rationalized, as they change depending on the substrate, pronucleophile, solvent and reagents. Thus, the data available in the literature do not allow any prediction or general recommendation to improve yields. This is particularly noteworthy when it comes to MR with the pyrimidines 3-benzoyluracil and 3-benzoylthymine. Even though both solvent and temperature affect the formation of the undesired O-2 coupled products, no exact conditions have been reported favoring $N^1$-alkylation; and base coupling conditions must be optimized case by case, aiming at a higher yield of the product resulting from $N$-ligation. Purine coupling is less problematic, due to MR $N^9$ regioselectivity. $N^7$-Ligation generates only minor products, and MR coupling with purines leads quite often to high yields of the $N^9$ regiosomers, when appropriate alcohol precursors are used. MR was also applied to alkylate amino group substituents in adenine and guanine precursors, when nucleobase reactive functionality is protected.

Microwave-assisted MR is considerably faster than conventional ones, and in some cases lead to higher product yields, encouraging further developments in nucleoside analog synthesis with this green chemical approach.
MR has also been used for the coupling of unnatural nucleobases, such has deaza analogs of purines, 6-azauracil, as well as other aromatic nitrogen heterocycles, broadening the scope of its application. It is not our aim to present a comprehensive review on nucleoside base coupling by MR, but instead to highlight the benefits and the drawbacks found in the synthesis of nucleoside analogs, when the classical reactions for the nucleobase coupling do not succeed or give lower yields and regioselectivity. Nonetheless, by considering the numerous examples of the successful application of MR in the synthesis of carbocyclic nucleosides, acyclic nucleosides and isonucleosides, it can be concluded that MR is a viable experimental tool for the coupling of nucleobases to alcohols, opening the door to novel nucleoside analogs.

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