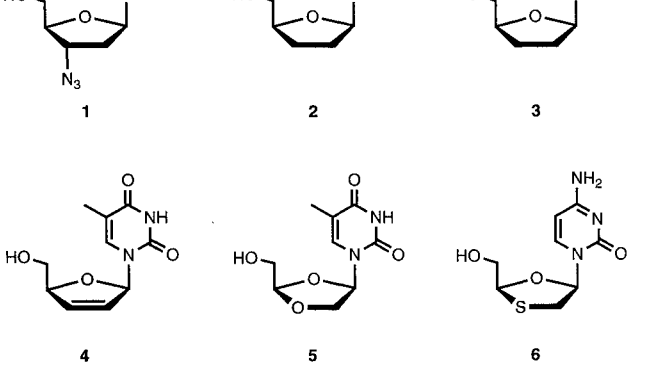
Nitrogen Glycosylation Reactions Involving Pyrimidine and Purine Nucleoside Bases with Furanoside Sugars

Lawrence J. Wilson, Michael W. Hager, Yahya A. El-Kattan, Dennis C. Liotta*
Department of Chemistry, Emory University, Atlanta, GA 30322, USA
Fax +1(404)7274010; E-mail dliotta@dooley.cc.emory.edu
Received 3 January 1995; revised 30 May 1995

Different approaches for the synthesis of nucleoside analogs (potential HIV inhibitors) are described. Starting from a suitably substituted furanose ring, it is demonstrated that a high facial stereocontrol of the glycosylation reaction can be effected. Different reaction conditions including Lewis acid promoted, S_N2 displacement and some enzymatic methodologies for the stereoselective synthesis of these compounds are reviewed.

1. Introduction

The class of compounds known as 2′,3′-dideoxynucleosides have been used as both anticancer and antiviral drugs.[1] Recently, they have received increased attention due to their activity against the human immunodeficiency virus (HIV).[2] In fact, the emergence of 3′-azido-3′-deoxythymidine (**1**, Scheme 1) as an effective anti-HIV agent has demonstrated the biological importance of nucleosides lacking a 3′-hydroxy functionality. These compounds are structural analogs to the naturally occurring 2′-deoxy nucleosides, the building blocks of DNA. As one might expect, the structural similarity of these compounds allows the 2′,3′-dideoxy derivatives to mimic their 2′-deoxy counterparts during DNA synthesis, a biological function critical to cellular reproduction. However, when 2′,3′-dideoxynucleosides are accepted as substrates by a DNA polymerase and are incorporated into a growing strand of DNA, the absence of a 3′-hydroxy group prohibits further strand elongation. It is primarily through the inhibition of DNA synthesis either by the chain termination process discussed above or by competitive inhibition (e.g., Reverse Transcriptase) that 2′,3′-dideoxynucleosides exhibit their anticancer and antiviral activity.



Scheme 1

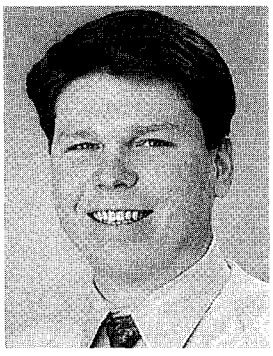
The use of 2′,3′-dideoxynucleoside derivatives (e.g., AZT, **1**; ddC, **2**; ddI, **3**) as anti-HIV agents in the treatment of AIDS has spurred both the search for nucleosides with more favorable therapeutic indexes and the development of general synthetic approaches to them. The commonly used chemical methods for synthesizing these materials can be classified into two broad categories: (i) those which modify intact nucleosides by altering the carbohydrate portion (divergent approach), and (ii) those which incorporate the nucleoside base onto a carbohydrate derivative at a suitable stage in the synthesis (convergent approach). Methods that utilize the latter concept provide greater flexibility for achieving structural modifications. Since the nucleoside β anomers typically exhibit greater biological activity, an important factor in the latter strategy involves the ability to deliver the base to the β-face of the carbohydrate ring during the glycosylation reaction. [Throughout this manuscript, the location of each substituent under consideration is described as α when it lies below the plane of the ring system and β when it lies above it. The wedged lines indicate the

absolute stereochemistry, and the bold lines (hashed or solid) indicate only the relative stereochemistry.]

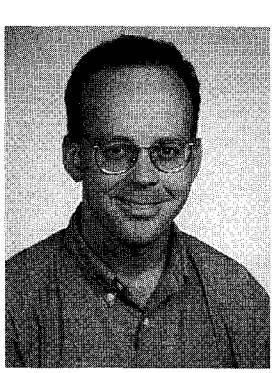
1.1. Divergent Approaches: Modification of Intact Nucleosides

The modification of intact nucleosides was the first method available for synthesizing 2′,3′-dideoxynucleosides. In fact, all three compounds currently approved by the FDA for the treatment of AIDS (AZT, **1**; ddC, **2**; ddI, **3**) were first synthesized using this approach. These routes rely largely, but not exclusively, on strategies which involve the deoxygenation of furanonucleosides. The main advantage of these approaches is that control of the glycosidic stereochemistry is not an issue, i.e., it is either set by nature or can be controlled synthetically via anchimeric assistance of an appropriate 2′-substituent. They

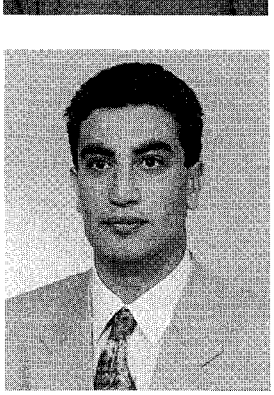
Biographical Sketches



Lawrence J. Wilson received his B.S. degree in chemistry from Furman University in 1985, where he worked under the direction of Donald G. Kubler in the area of sugar mutarotation reaction kinetics. He attended Florida State University from 1985 to 1987, and worked on organometallic chemistry involving rhodium under the direction of Professor Marie E. Kraft. He then moved on to receive his Ph.D. from Emory University in Atlanta, GA in 1992 under the direction of Professor Dennis C. Liotta. His thesis involved the invention and elaboration of stereoselective methods to construct nucleosides, including selective nitrogen glycosylation reactions. In 1992, he joined the laboratory of Professor Paul A Wender at Stanford University in Palo Alto, CA as a post-doctoral associate where he worked in the area of enediynes and DNA cleaving agents. He is presently at the Procter & Gamble Company in Cincinnati, Ohio, where he works in the area of drug discovery in the Cardiac section of the pharmaceuticals division. His current interests are combinatoral chemistry, solid phase synthesis, and molecular diversity.



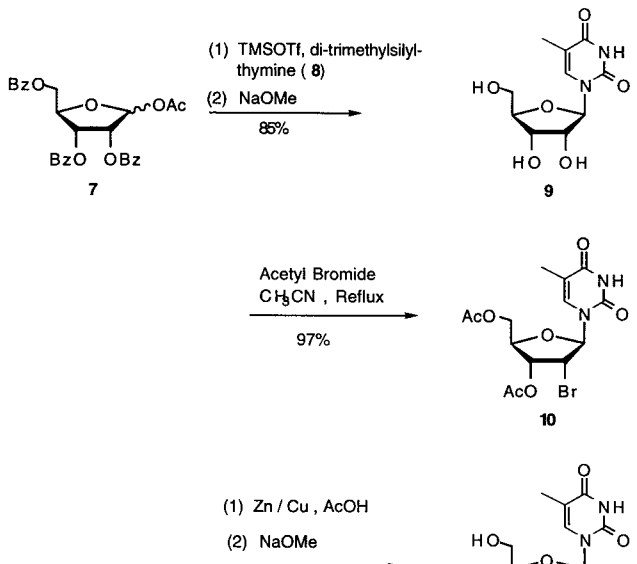
Michael W. Hager received a B.S. degree in 1987 from the University of North Carolina at Chapel Hill and a Ph.D. in organic chemistry in 1993 from Emory University under the direction of Professor Dennis C. Liotta. He is currently a postdoctoral research associate with Professor John Frost at Michigan State University.



Yahya A. El-Kattan received his MS degree in biochemistry from the university of Montpellier II, France. He than obtained a Ph.D. in organic chemistry from the same university under the direction of Professor Jean-Louis Imbach and Dr. Gilles Gosselin. His Ph.D. work involves the synthesis of a new series of cyclic and acyclic nucleosides to be used as antiviral agents. In 1992 he joined the laboratory of Professor Dennis C. Liotta at Emory University, Atlanta, GA as a post-doctoral associate where he worked on the synthesis of different analogs of the new antiviral agent FTC, and in the area of boron neutron capture therapy. He is presently at the university of South Alabama, as a research associate, in the laboratory of Professor June E. Ayling, where he works on the synthesis of different heterocyclic molecules for the regulation of the level of nitric oxide.

Professor Dennis Liotta received his Ph.D. in Organic Chemistry in 1974 from The City University of New York and completed his post-doctoral training at Ohio State University. In 1976 he joined Emory University as an Assistant Professor in the Department of Chemistry, was promoted to Associate Professor in 1982, and to Professor in 1988. Dr. Liotta was appointed Chairman of the Department in 1993. In recent years his research efforts have centered on the development of novel methodology for the preparation of antiviral and anticancer therapeutic agents. He is a Fellow of the Alfred P. Sloan Foundation, the recipient of a Camille and Henry Dreyfus Teacher Scholar Fellowship and an Alexander von Humboldt Senior Scientist. He is currently the Secretary-Treasurer of the Divison of Organic Chemistry of the American Chemical Society, a member of an Advisory Panel of the American Cancer Society, a member of the AIDS and Related Research Study Section of the National Institutes of Health and Director of the Molecular Therapeutics Program at the Emory University Winship Cancer Center.

are limited primarily by the availability of naturally occurring nucleoside starting materials (e.g., thymidine, cytidine, uridine, adenosine, guanosine). However, since these approaches do not involve nitrogen glycosylation, they fall outside of the scope of this discussion.



Scheme 2

Interestingly, not all approaches can be classified as either purely divergent or convergent. An example of a divergent/convergent route is provided in the synthesis of D4T **(4)** by Mansuri and co-workers (Scheme 2).[3] 5-Methyluridine **(9)**, available from 1-*O*-acetyl riboside **7** and silylated thymine **8**, could be converted to its 2′-bromo-3′,5′-*O*-diacetate derivative **10** in one step by heating in the presence of acetyl bromide. Vicinal elimination with Zn/Cu couple, followed by deprotection of the 5′-*O*-acetyl group, produced D4T **(4)** in 40% yield from **7**.

1-(2-Bromo-2-deoxy-3,5-di-*O*-acetylribosyl)thymine (10); Typical Procedure:

Acetyl bromide (13.8 mL, 113 mmol) was added dropwise over 0.5 h to a suspension of 5-methyluridine **(9)** (5 g, 19.38 mmol) in MeCN (250 mL) heated at reflux. On completion of addition, the solution was allowed to cool and then concentrated. The residue was dissolved in CH_2Cl_2 (60 mL) and washed with H_2O (60 mL). The organic phase was concentrated to leave the desired product **10** (7.8 g, 97%).

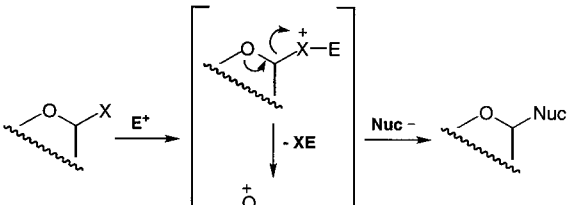
1-(5-*O*-Acetyl-2,3-dideoxy-β-D-*glycero*-pento-2-enofuranosyl)thymine (4); Typical Procedure:

A Zn/Cu couple (3 g, Fairfield chemicals) was heated at reflux in AcOH (20 mL) for 0.5 h. The suspension was filtered and washed with MeOH. The couple was suspended in MeOH (70 mL), compound **10** (1 g, 2.4 mmol) was added, and the mixture was stirred for 0.5 h. The mixture was filtered and concentrated to leave an oil, which was purified by flash column chromatography, MeOH/CH_2Cl_2 (1:9). The desired product was isolated as a white solid (0.64 g, 53%).

In many respects this synthesis combines the best features of convergent and divergent syntheses in that it can be used to prepare a variety of purine and pyrimidine nucleosides while still making use of readily available starting materials. For example, starting with adenosine, ddA was synthesized using this strategy.

1.2. Convergent Approaches: Nitrogen Glycosylation Reactions

Convergent approaches to nucleoside derivatives are more versatile than nonconvergent approaches, since they involve the coupling of a modified carbohydrate moiety with a purine or pyrimidine base at a suitable stage in the synthetic plan through a nitrogen glycosylation reaction. Since these approaches enable one to vary both the base and carbohydrate components of nucleosides, they should, in principle, represent the preferred strategy for preparing substrates for structure-function studies. In practice, however, this presumption only holds true when the method in question provides a reasonable degree of control on the glycosylation reaction stereochemistry.



Scheme 3

Table 1. Activation Methods for Glycosylations

Entry	X	E^+ (Lewis Acid)	Nucleophile
1	OR	TMSOTf, $SnCl_4$	OR, CR, NR_2, SR
2	SPh	NBS	OR, NR_2
3	S(O)Ph	Tf_2O	OR, NR_2
4	SMe	$NOBF_4$	OR
5	OH	$Ph_2Sn{=}S$, Tf_2O	OR
6	SePh	AgOTf	OR
7	*S*-(2-pyridyl)	AgOTf	CR
8	*O*-(4-pentenyl)	NBS, "I^+"	OR, NR_2

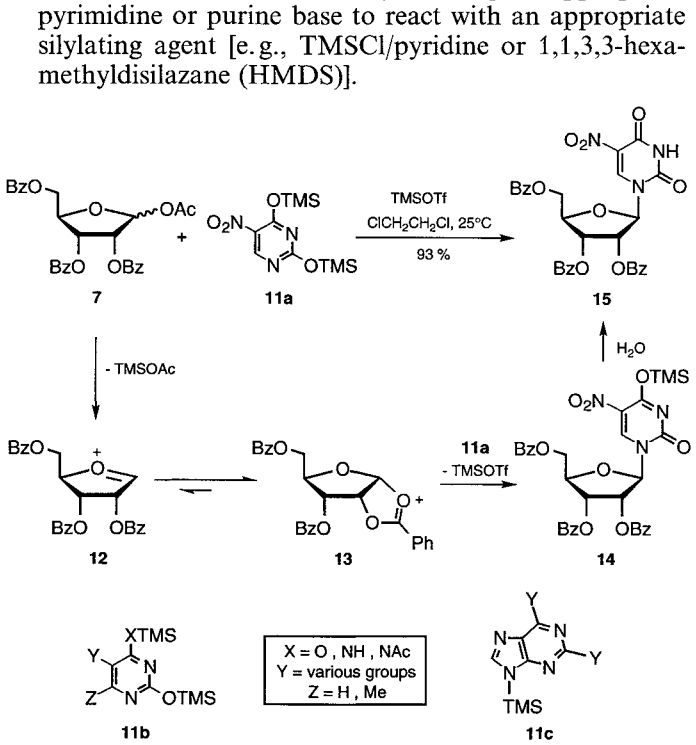
In oxygen and nitrogen glycosylation reactions with carbohydrates, there are many ways to activate the anomeric center towards the incoming nucleophile (Scheme 3, Table 1). The most common approach involves the use of Lewis acids (e.g., E = Ag and Sn salts, TMSOTf)[4-6] in conjunction with 1-*O*-acyl/alkyl furano- or pyranosides (X = OAc or OMe) which undergo oxonium ion formation. A second method utilizes groups at the anomeric center that can be activated by electrophiles other than Lewis acids (e.g., X/E = S(O)Ph/Tf_2O;[7] PhS/NBS;[8] MeS/$NOBF_4$;[9] *O*-(4-pentenyl)/NBS[10]). In these cases,

product formation results from either a direct nucleophilic displacement or proceeds through an oxonium ion. The final method involves the displacement of 1-halo carbohydrates either through Lewis acid assisted oxonium ion formation or under S_N2 type conditions.

2. Lewis Acid Promoted Reactions of Furanosides with Silylated Bases

2.1. Vorbrüggen Method: Use of Trimethylsilyl Trifluoromethanesulfonate and 2-Acyloxy Sugars

In the coupling reaction of nucleoside bases with ribose type carbohydrates, it is the silyl variant of the Hilbert–Johnson procedure,[11] developed by Vorbrüggen, which has made this process applicable and practical.[5] The conditions for this reaction involve use of oxygen and/or nitrogen trimethylsilylated pyrimidine (**11b**, Scheme 4) or purine bases (**11c**) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the Lewis acid. Other Lewis acids are successful in facilitating this reaction, with the exception of tin compounds.[12,13] The silylated nucleoside bases are formed by allowing the appropriate pyrimidine or purine base to react with an appropriate silylating agent [e.g., TMSCl/pyridine or 1,1,3,3-hexamethyldisilazane (HMDS)].



Scheme 4

2′,3′,5′-Tri-*O*-benzoyl-5-nitrouridine; Typical Procedure:[5]
To a solution of bistrimethylsilylated 5-nitrouracil (18.33 mL, 11 mmol, 0.6 N soln in 1,2-DCE) and the acetylriboside (5.04 g, 10 mmol) in 1,2-DCE (75 mL) was added TMSOTf (23 mL, 12 mmol, 0.528 N standard soln in 1,2-DCE) and the reaction mixture stirred for 2 h at 24 °C. After standard workup, the crude product (6.8 g) afforded pure crystalline product on recrystallization from ethanol in three crops; yield: 5.7 g (93 %).

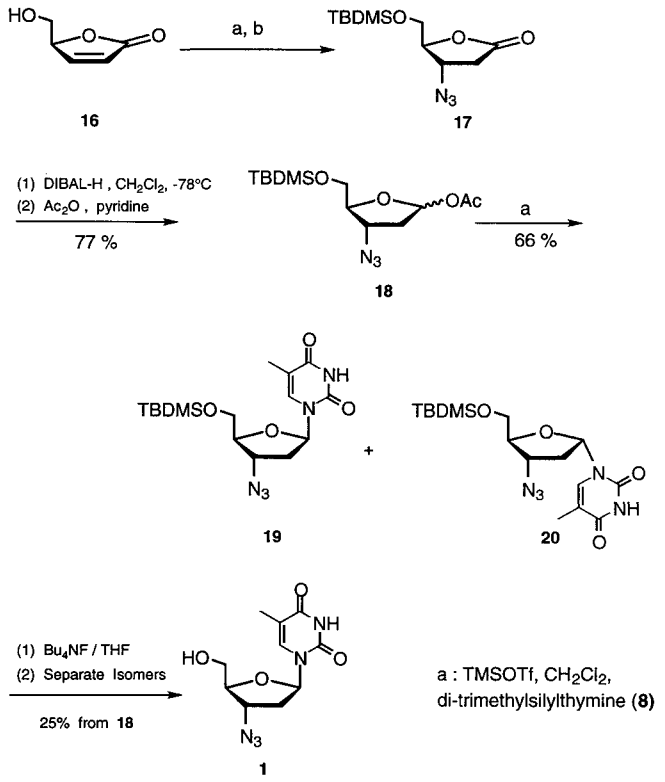
An example of this process is in the coupling of silylated 5-nitrouracil (**11a**) and 1-*O*-acetylriboside **7** (Scheme 4).[5] In this reaction, TMSOTf activates the acetate group, resulting in formation of an oxonium ion **12**. Due to the neighboring benzoyloxy substituent, a bridging oxonium ion **13** is formed, which is favored over the other cation **12** due to resonance stabilization. The reactive intermediate **13** then undergoes attack at C_1 by the nitrogen base in a stereoselective fashion, and results in formation of **14**. The nucleoside product **15** results after quenching the reaction. The conversion of this type of nucleoside to an anti-HIV target through deoxygenation methodology has been presented previously [Scheme 2, D4T (**4**)].

The β selectivity of this reaction is explained through formation of the bridged oxonium ion **13**, which, due to its concave nature, effectively blocks the bottom face.[5,11] Another extension of this idea is that the oxygen atom of the 2-benzoyl carbonyl moiety forms an activated α-leaving group which undergoes an S_N2 type displacement by the silylated base. Neighboring group participation of this type is well documented in carbohydrate chemistry, and is usually Lewis acid independent. This reaction allows the coupling of a large variety of silylated bases (**11b, 11c**), and is the most general procedure of this type. The silylated base/TMSOTf is essential to produce the desired nitrogen substitution products (N_1 and N_9 for pyrimidine and purine bases, respectively).[5,12,13] When tin(IV) chloride is employed, the N_3 isomers (for pyrimidines) and N_7 isomers (for purines) are produced as side products, presumably due to prior complexation between the tin and nitrogen atoms (N_1 and N_9).[12,14] However, all products produced possess the required β configuration due to the neighboring acyloxy substituent (i.e., formation of **13**). The reaction can also be performed by mixing the silylating agent, nucleoside base, Lewis acid, and 1-*O*-acetylribofuranoside together as a "one step/one pot" procedure.

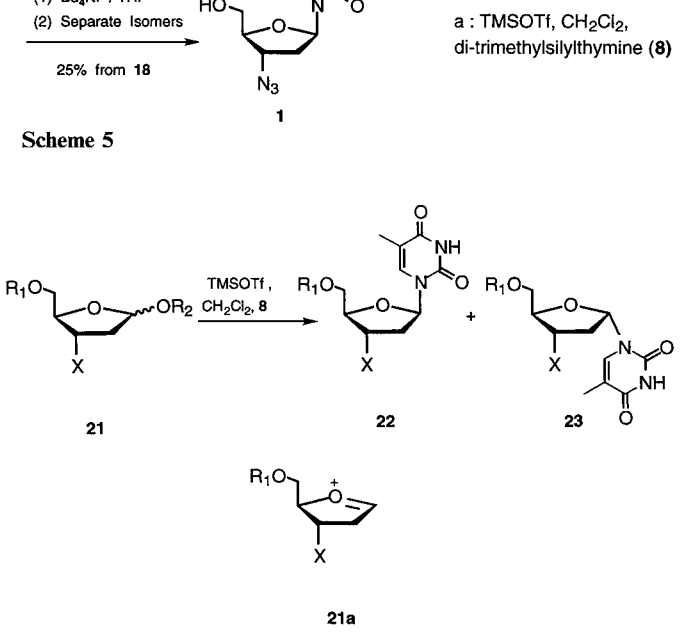
2.2. 2-Deoxyribose Couplings

There are many examples of nitrogen glycosylations involving 2-deoxyribose sugars that contain a variety of substituents at the 3 position due to the interest in anti-HIV activity. Most of these routes involve the methodology developed by Vorbrüggen, and utilize a silylated base and TMSOTf in the glycosylation reaction. However, in most of these examples, little to no facial selectivity is observed in the glycosylation reaction due to the lack of neighboring group participation and the absence of any stereoelectronic effects (i.e., the anomeric effect).

The synthesis of AZT (**1**) by Chu and co-workers illustrates an example of a fully convergent 2′,3′-dideoxynucleoside synthesis (Scheme 5).[15] The route was devised due to the relatively expensive cost of thymidine. The starting material, (*S*)-4-hydroxymethyl-2(*H*)-furanone (**16**) is available from D-mannitol (4 steps). Subsequent protection as the bulky *tert*-butyldimethylsilyl ether, and stereoselective azide incorporation at C_3 gave lactone **17** (no yield reported). Reduction with diisobutylaluminum hydride and acetylation gave the 1-*O*-acetyl-3-azido-2-deoxyriboside **18** in 77 % yield. Vorbrüggen coupling with silylated thymine (**8**) gave rise to an equal mixture of β (**19**) and α (**20**) products. The isomers were then deprotected, and the desired nucleoside (AZT, **1**) could be separated by column chromatography. Although the design of this route is quite novel, the lack of stereoselectivity in the glycosylation reaction limits its synthetic utility.



Scheme 5



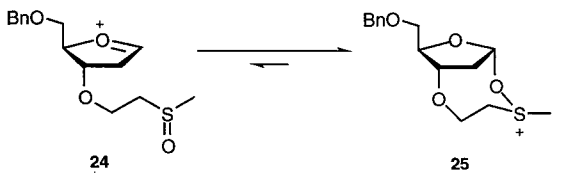
Scheme 6

The Vorbrüggen method is also successful with other furanoside derivatives, as well as pyranosides.[5] However, the same lack of selectivity seen with the 3-azidoriboside **18** in the coupling reaction with silylated thymine **8** is a general trend with other 2-deoxyribose derivatives (i.e., **21** gives **22** and **23**; Scheme 6).[16] There are many examples which support this observation, with a large variation of the substituent in the 3 position (X, **21**), and selected examples are listed in Table 2. For the parent 2′,3′-dideoxy system (X = H, entry 1), no selectivity is observed with silylated thymine or silylated cytosine [not shown; ddC **(10)** precursor].[17,18] Various heteroatom derivatives (N, O, S; entries 2, 5–8, and 11) result in selectivity that range from 1.6:1 (entry 11) to 1:2 (entry 5). A carbon atom at the 3 position (entries 3, 10, and 12) follows the same trend of no selectivity.

Table 2. Selectivities of the Coupling of **21** and **8**

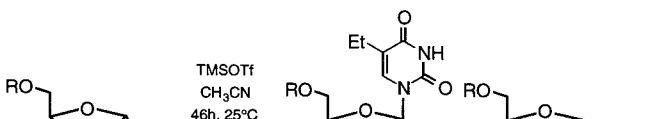
Entry	X	R^1	R^2	Yield (%)	Ratio (**22**/**23**)
1	H	TBDPS	Ac	–	1:1.2
2	N_3	TBDMS	Ac	66	1:1
3	CN	TBDMS	Ac	91	4:5
4	F	4-PhPhCO	Me	89	5:1
5	BnS	Ac	Ac	30–69	1:2
6	PhS	TBDMS	Ac	30	1:1
7	PhSe	Ac	Me	50	1:1.5
8	BnO	Bn	Ac	54	1.5:1
9	MeS(O)EtO	Bn	Ac	89	8:1
10	$BzOCH_2$	4-BrBn	Me	66	1:1[11]
11	PhthlN	Ac	Ac	63	1.6:1
12	Me	TBDPS	Ac	91	1.6:1

These results show that, in general, 2-deoxyribosides couple with silylated bases without selectivity with respect to the anomeric center. This is due to formation of the oxonium ion **21a** (Scheme 6), which would not be expected to have any significant facial bias since the substituents at C_3 and C_4 are not in close proximity to induce any steric effect upon the incoming group at C_1. Another contributing factor is reaction equilibration during product formation (see Scheme 8). There are two exceptions to this trend which show some promise. The first involves the 3-fluoro derivative of **21** (X = F, entry 4), for which a 5:1 selectivity is observed for silylated thymine **8**.[19] Although modest selectivity favoring the β isomer is observed, no explanation for this result is presented. The large electronegativity of the fluorine atom may be playing a significant role (i.e., dipole–dipole repulsion). The second example involves a 3-position neighboring group (entry 9). For the 3-*O*-ethylmethyl sulfoxide compound [X = MeS(O)EtO, **21**], a selectivity of 8:1 is obtained.[20] This result is explained by stabilization of the oxonium ion (**24**, Scheme 7) through assistance of the sulfoxide oxygen favoring an 8-membered bridged intermediate **25**. This would effectively block the α face, similar to the 2-benzoyl group (**13**, Scheme 4), and promote β attack of the silylated base in a similar mechanism. This method is useful only for certain bases, since the coupling with silylated cytosine results in a lower ratio (3:1) favoring the β isomer. No further work on this result has been performed, although other 3-substituted ribosides of this type could in principle direct the glyosylation stereochemistry. Other 3-hydroxy protecting/directing groups have been tested with C and S glycosylations involving 2-deoxyfuranoses giving similar to lower selectivity.



Scheme 7

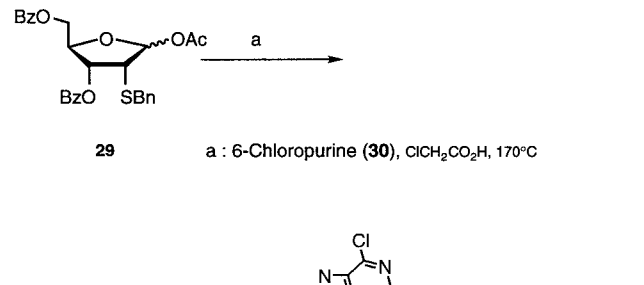
Another potential problem in the coupling of ribose compounds lacking a neighboring group was identified by Vorbrüggen.[5] He discovered that equilibration of the product(s) is possible under the reaction conditions (Scheme 8). When the stereochemically pure α-*O*-trimethylsilyl-5-ethyluridine derivative **26** was exposed to TMSOTf in acetonitrile, the result was formation of both C_1 isomers **27** and **28**. In this reaction it seems that an oxonium ion **21a** (X = O-4-MeBz) is being formed. This result shows the distinct possibility that isomerization occurs during product formation, and indicates that any kinetic result may be difficult to obtain with 2-deoxyribose compounds (i.e., **21**) in the presence of a Lewis acid (e.g., TMSOTf). It also confirms the general observation that, in order to obtain any selectivity in this reaction, a neighbouring group is needed. Although recent studies show that some stereoselectivity [(α/β) 3:1] can be achieved by using an excess of tin(IV) chloride in acetonitrile, this reaction proceeds via an acyclic intermediate.[21a,b]



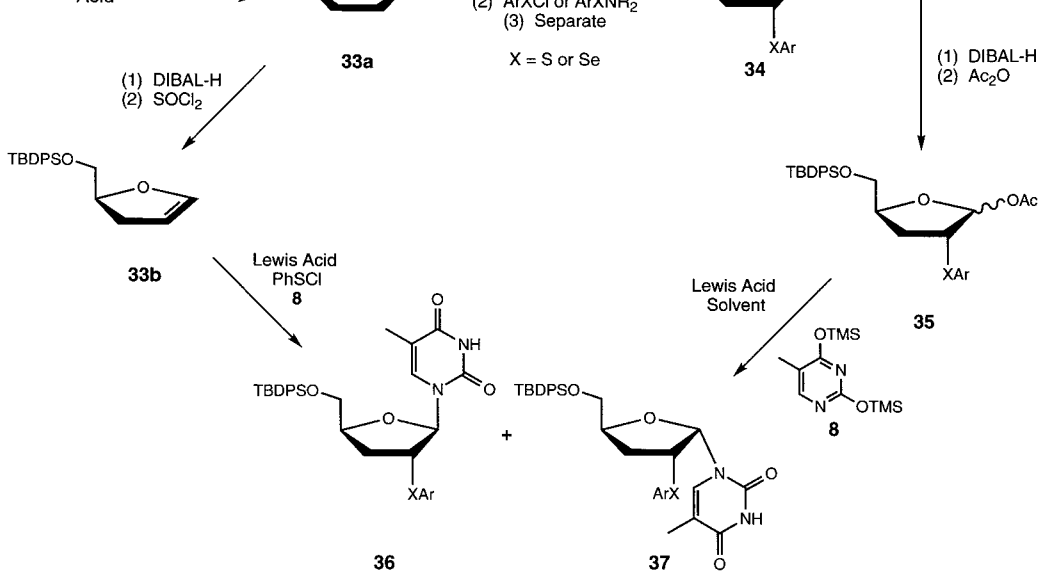
Scheme 8

2.3. 2-α-Hetero/Halo-2-deoxyribose Couplings

The next important area in the convergent synthesis of nucleosides involves the use of 2-substituted ribosides, where the 2 substituent is capable of controlling the selectivity of the glycosylation reaction, similar to the 2-acyloxy substituent (Scheme 4). The first reported case of this type was in the synthesis of 2′-α-thio-2′-deoxypurine nucleosides (Scheme 9).[22] Under fusion conditions with chloroacetic acid, 6-chloropurine **(30)** reacted with 2-benzylthioribose **29** to give a mixture of two isomers [**(31/32)** 2:1] in 38% yield. Other conditions, such as the use of $HgBr_2$, produced less desirable results. Although the stereoselectivity was low in this reaction, later results with tin Lewis acids would prove the utility of the sulfur group.



Scheme 9



Scheme 10

6-Chloro-9-(2-*S*-benzyl-3,5-di-*O*-benzoyl-2-thio-α,β-D-ribofuranosyl)-9*H*-purine; Typical Procedure:
To α,β-1-*O*-acetate **29** (385 mg, 0.760 mmol) was added 6-chloropurine (148 mg, 0.970 mmol, 90% pure from ultraviolet extinctions) and chloroacetic acid (5 mg, 0.05 mmol). The materials were mixed well, forming a gum, and were fused under vacuum by immersion in a bath at 172–175 °C for 90 sec. The cooled residue was dissolved in $CHCl_3$ (5 mL). On chilling (0 °C), the solution deposited 57 mg of unreacted 6-chloropurine (assume 91 mg reacted, 78%). Concentration of the filtrate afforded 460 mg of a dark oil. The NMR spectrum disclosed the presence of unreacted acetate, 5-(benzyloxymethyl)-2-(*S*-benzyl)furan-2-thiol and the desired nucleoside in the ratio 5:54:41. The nucleoside fraction was isolated by preparative TLC of the residue on two plates (2 mm thick), developed with $CHCl_3$–MeOH (98:2), dried, and developed again. The band R_f 0.8–0.9 contained sugar and the furan. The band of R_f 0.5 was eluted with $CHCl_3$–MeOH (9:1) to give the nucleoside; yield: 120 mg (38%). The anomeric ratio β:α was 2:1 by NMR analysis.

The first examples of this type of reaction with notable selectivity involve the use of 2-α-phenylselenenyl and phenylsulfenyl groups (**35**, XAr = PhSe or PhS, Scheme 10). The advantage of these routes is that the derived starting material, *tert*-butyldiphenylsiloxy lactone **33a**, is readily available in either chiral form from D- or L-glutamic acid. Chu and co-workers showed that the 2-α-phenylselenenyl derivative (entry 1, Table 3) gave a 99:1 ratio favoring the β isomer **36** (XAr = PhSe) in the coupling reaction with silylated thymine **8**.[23] This derivative was then converted to D4T **(4)** by oxidative elimination and deprotection. In this case, the starting material **34** (XAr = SePh) was made from lactone **33a** by allowing the silyl ketene acetal of **33a** to react with phenylselenenyl chloride [(trans/cis) 2:1]. Separation, reduction and acetylation gave the requisite 2-α-phenylselenenyl-1-*O*-acetylriboside **35**. Recently, this methodology was extended to the coupling of silylated uracil, cytosine, 6-chloropurine, and 6-chloro-4-fluoropurine with slightly lower ratios. In these cases, the phenylselenenyl group was removed by oxidative elimination to give D4T **(4)**, or reduced under radical conditions (BEt_3, Bu_3SnH) to ddC **(2)** and ddl **(3)**.

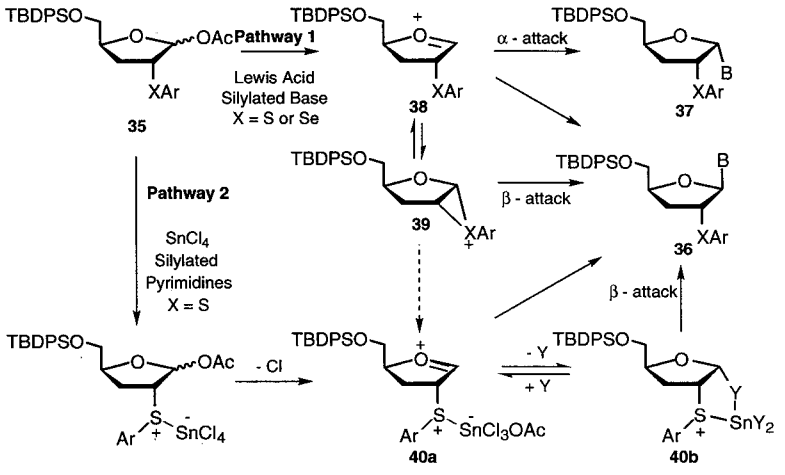
Table 3. Results for the Coupling of **35** and **8**

Entry	XAr	Lewis Acid/Solvent	Yield (%)	Ratio (**36**:**37**)
1	PhSe	$TMSOTf/ClCH_2CH_2Cl$	78	99:1
2	PhS	$SnCl_4/ClCH_2CH_2Cl$	87	92:8
3	PhS	$SnCl_4/CH_2Cl_2$	83	96:4
4	$(2\text{-}NO_2)PhS$	$SnCl_4/CH_2Cl_2$	76	89:11
5	PhS	$TMSOTf/CH_2Cl_2$	91	80:20

5′-*O*-*tert*-Butyldiphenylsilyl-2′-α-phenylthio-3′-deoxythymidine; Typical Procedure:
To acetate **35** (XAr = SPh) (506 mg, 1 mmol) dissolved in CH_2Cl_2 (10 mL) at 0 °C was added dropwise over 15 min a mixture containing: (i) silylated thymine **8** (311 mg, 1.15 mmol); (ii) $SnCl_4/CH_2Cl_2$ solution (1.35 mL, 1.35 mmol, 1 M, Aldrich) and (iii) CH_2Cl_2 (4 mL). The mixture was then warmed to r.t. and stirred for 2 h. The reaction mixture was then quenched with CH_2Cl_2 (15 mL), conc. NH_4OH (5 mL), and abs. EtOH (5 mL). The quenched mixture was then filtered over a 5 cm plug of silica gel with EtOAc. The solvent was removed in vacuo to yield a pale yellow foam. Recrystallization from hexanes/Et_2O (1:1) gave a white powder; yield: 427 mg (75%); (β/α) 24:1.

Two groups published almost simultaneously on the use of the 2-α-phenylthio group (**35**, XAr = PhS) to control the glycosylation reaction.[24,25] It was found that use of tin(IV) chloride and silylated thymine **8** (entries 2–5, Table 3) provided very good selectivity favoring the β isomer **36** (X = PhS). The phenylsulfenyl group was then removed via oxidative elimination to give D4T **(4)**. In similar fashion, silylated N^4-acetyl cytosine and uracil were also coupled, with similar selectivities being observed (89:11 and 93:7, respectively).[25]

The 2-phenylsulfenyl lactone **34** (XAr = SPh) was initially formed as a mixture of isomers [(trans/cis) 2-1:1] through formation of the enolate of **33a**. However, it was later discovered that the *trans* lactone **34** could be formed with much larger selectivity (9-22:1) and in better yields by using the silyl ketene acetal and *N*-phenylthio-



Scheme 11

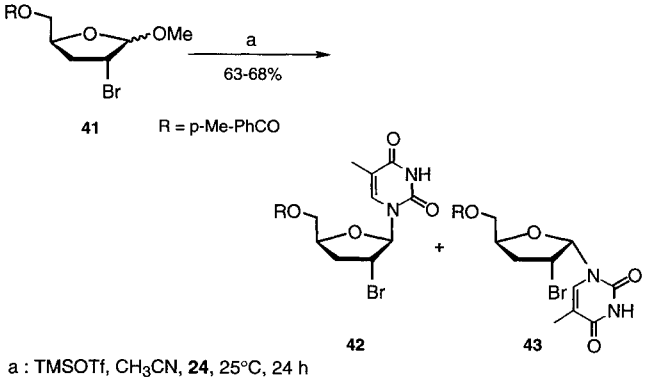
amides.[24b] This further improved the overall stereoselectivity of the process.

The selectivity gained by the use of 2-phenylthio and selenenyl substituents can be rationalized through neighboring group participation (Scheme 11). In the case of the phenylselenenyl group, a pathway which operates by episelenenium ion formation (Pathway 1, **39**) provides the β selectivity, since TMSOTf is utilized. However, the phenylthioribosides operate via a Lewis acid dependent pathway.

When the reaction is carried out with tin(IV) chloride (entries 2–4), the selectivity is much higher than when TMSOTf is used (entry 5). These reactions proceeded through complexation of the sulfur atom and tin Lewis acid (Pathway 2, Scheme 11). Two possible complexed intermediates **40a** and **40b** would promote selective β attack of the silylated base for steric and electronic reasons. Use of TMSOTf involves oxonium ion formation **38** and results in lower selectivity. Also, the electron withdrawing 2-nitrophenylthio substituent (entry 4) gives a third of the selectivity of the phenylthio (entry 3) ribose, which supports the complexation hypothesis.

The final variation of this methodology involves utilization of a furan glycal in an in situ sulfenylation/glycosylation sequence (Scheme 10, **33b**).[24c] Reduction of the lactone **33a**, followed by dehydration readily provides glycal **33b**. Treatment of this compound with phenylsulfenyl chloride and silylated thymine **8** in the presence of a Lewis acid shows similar results to those observed for the reaction with α-phenylsulfenyl acetate **35**. When trimethylsilyl trifluoromethanesulfonate is utilized with **33b**, the result is identical (i.e. 4 : 1 ratio of 36 : 37, 50% yield). However, the selectivity with tin(IV) chloride is slightly higher in the reaction of phenylsulfenyl chloride, **33b**, and silylated thymine **8** (42 : 1 ration of 36 : 37; 68% yield) than that involving acetate **35** (24 : 1 ratio, entry 3, Table 3). The results are similar for silylated uracil and *N*-acetylcytosine,[24c,d] and this concept has been extended to phenylselenenyl chloride[24e] and *N*-iodosuccinamide[24f] with good to excellent selectivities. The mechanism of the glycal transformations follows those discussed in Scheme 11 (i.e. formation of intermediates **38**, **39**, **40a**).

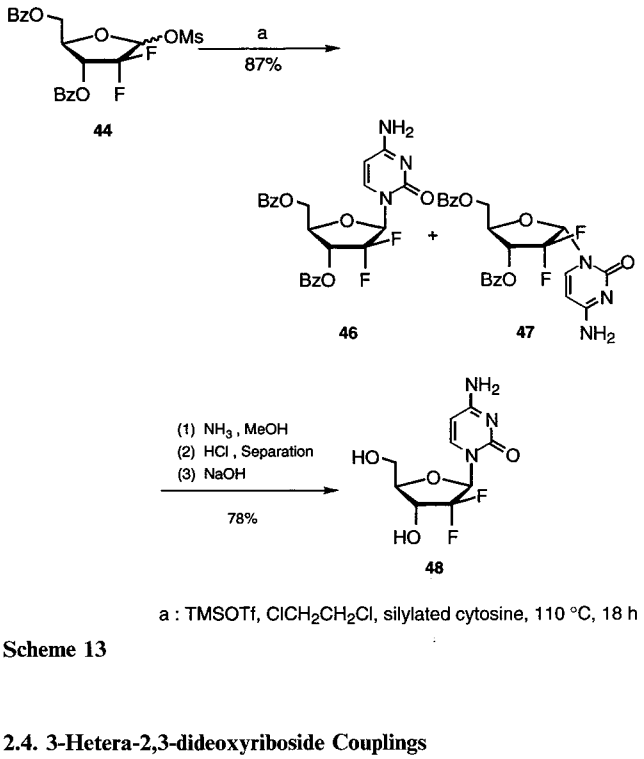
When a halogen atom (bromine, **41**, Scheme 12 or fluorine, **44**, Scheme 13) is used in the 2-α position, much lower selectivity results.[24f,26] This lowered selectivity in the bromine case indicates this atom is failing to act as a neighboring group through a bromonium ion. Also, it seems that the bromine atom is not sterically demanding enough to prevent attack of the silylated base on the α face. The β compound **42** is separated, exposed to elimination conditions, and deprotected to give D4T **(4)**.



Scheme 12

1-[2-Bromo-2,3-dideoxy-5-*O*-(4-methylbenzoyl)-D-*erythro*-pentofuranosyl]uracil; Typical Procedure:

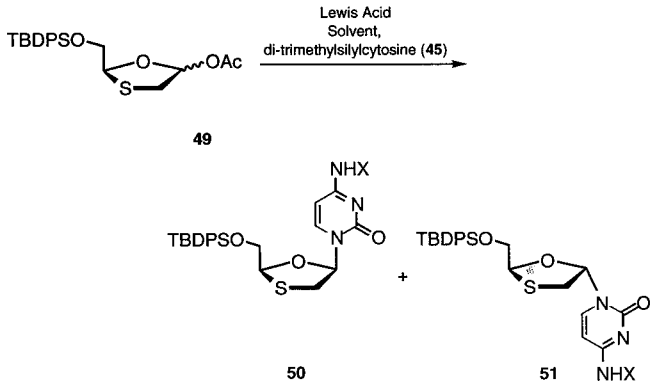
To a stirred solution of **41** (4.9 g, 0.015 mol) and *O,O'*-bis(trimethylsilyl)uracil (0.017 mol) in dry MeCN (70 mL) is added dropwise TMSOTf (5.4 mL, 0.03 mol) in MeCN (10 mL) at 0 °C. After addition, the mixture is stirred for 24 h at r.t. The mixture is then diluted with CH_2Cl_2 (300 mL) and exracted with ice cold sat. $NaHCO_3$ (300 mL). The aqueous solution is extracted with CH_2Cl_2 (2 × 150 mL). The organic layer is washed with cold H_2O, dried (Na_2SO_4) and evaporated under reduced pressure to give the desired nucleoside as crude product which is chromatographed on silica gel (100 g, 0.04–0.063 mm) using Et_2O/petroleum ether (2 : 1) to afford the title compound as a solid; yield: 68%.



Scheme 13

2.4. 3-Hetera-2,3-dideoxyriboside Couplings

In the cases where a 3′ hetero atom (oxygen and sulfur) replaces the C-3′ in the nucleoside ring (**5** and **6**, Scheme 1), there are no naturally occurring nucleosides to use as starting materials, and therefore a convergent approach is necessitated. The reactions of silylated bases with oxathiolanyl (Scheme 14) and dioxolanyl (Scheme 15) ring systems show a Lewis acid dependent trend in the stereoselectivity. In the sulfur-substituted case, **49** (Scheme 14), the coupling with either form of silylated cytosine **45** (X = H or Ac) has been performed. When using TMSOTf as the Lewis acid, a 2:1 mixture of **50**:**51** (X = Ac; 64% yield) was obtained with silylated N^4-acetylcytosine in 1,2-DCE.[27] However, when tin(IV) chloride was employed with silylated cytosine **45** (X = H), only the β isomer **50** (X = H) resulted (88% yield).[28]

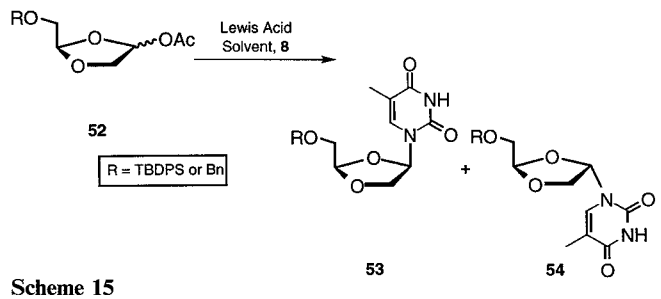


Scheme 14

5′-Butyryl-2′,3′-dideoxy-3′-thia-5-iodouridine; Typical Procedure: 5-Iodouracil (4.03 g, 0.0170 mol) was silylated with 10 eq of HMDS (0.170 mL, 10 equiv). After silylation was completed, the excess of HMDS was evaporated under vacuum to yield a translucent yellow oil, which was then dissolved in anhydr. CH_2Cl_2 (80 mL). To this solution was added a 1 M soln of tin(IV) chloride in anhydr. CH_2Cl_2 (20 mL, 0.0255 mol, 1.5 equiv) which was allowed to complex with the silylated base for 20 min before being cannulated into a flame dried, Ar charged 250 mL flask in which 4.0 g (0.016 mol, 1.0 eq) of 1-*O*-acetyl-2-deoxy-3-thia-5-butyrylfuranosyl was dissolved in anhydr. CH_2Cl_2 (80 mL). This reaction was monitored by TLC using a 5% MeOH/95% CH_2Cl_2 solvent system. After completion in 3 h, the reaction was diluted with CH_2Cl_2 (50 mL) and quenched with conc. aq NH_3 (10 mL), which caused the formation of tin salts. The reaction mixture was filtered through Celite and the solvent removed under reduced pressure to give a brown foaming solid which was recrystallized from MeOH to give the desired product as a white solid; yield: 6.11 g (89.6%).

Exclusive formation of the β isomer **50** is explained by in situ complexation of the tin and the sulfur atom on the oxathiolanyl ring. In the first example, the starting material **49** was made optically active (+)-**49** from D-mannose in 14 steps; (−)-**49** from L-gulose in 12 steps. The authors also discovered that contrary to TMSOTf, the products were racemized when tin(IV) chloride was employed. The coupled prodct **50** was deprotected in each case to provide the free nucleoside BCH-189 **(6)** in racemic (+), and (−) forms.

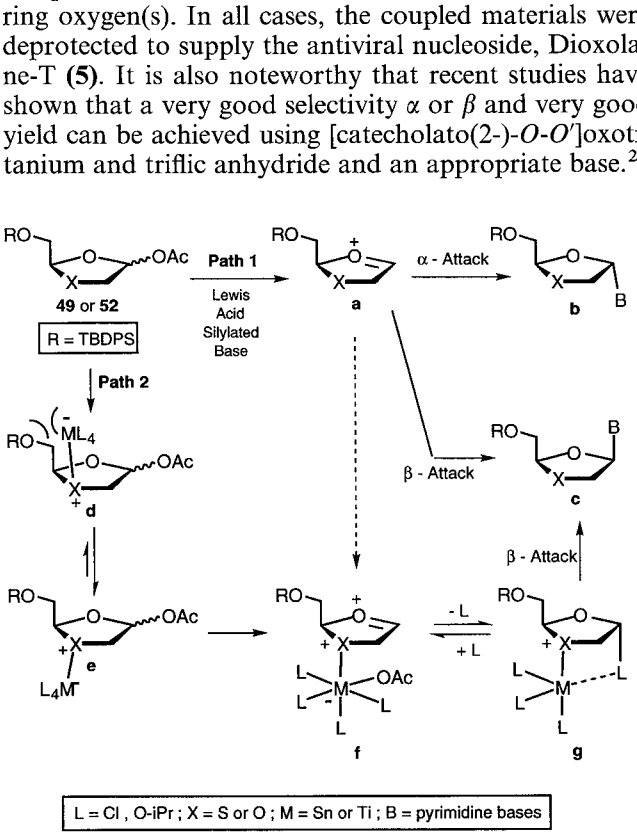
In the case of the dioxolanyl derivative, Dioxolane-T **(5)**, there are three reported syntheses which all converge on the usage of the *O*-acetyldioxolane (**52**, Scheme 15).[28,29,30] Here too, a Lewis acid selectivity is observed in the coupling reaction. In the first reported synthesis, Norbeck coupled **52** (R = Bn) with TMSOTf and silylated thymine **8**, and reported a 1:1 mixture of anomers **53** and **54** (R = Bn, 76% yield).[29] The second synthesis in which the (+) form of **52** (R = TBDPS, made from 1,6-anhydro-D-mannose in 9 steps) was also reacted with **8** and TMSOTf, resulted in a 1.5:1 mixture of **53** and **54** (R = TBDPS, 74% yield).[30]



Scheme 15

5′-*O*-*tert*-Butyldiphenylsilyl-3′-oxa-2′,3′-dideoxythymidine; Typical Procedure:
The acetate **52** (185.4 g, 0.465 mmol) was dissolved in dry CH_2Cl_2 (8 mL) with **8** (144 mg, 1.15 equiv) and stirring under Ar at r.t. Next, a freshly prepared solution of $TiCl_3$(O-*i*-Pr) in CH_2Cl_2 [0.57 mL, 1.15 equiv, 1 M soln prepared from 2 equiv of $TiCl_4$ and 1 equiv of $TiCl(O)$-*i*-$Pr)_3$] was added dropwise over 25 min. After 2.5 h, a $TiCl_4/CH_2Cl_2$ soln (0.07 mL, 0.15 equiv, 1 M, Aldrich) was added and the reaction was stirred for an additional hour. Then EtOH (3 mL) and aq $NaHCO_3$ (5 mL) were added, stirred for 10 min, followed by extraction with additional aq $NaHCO_3$. The aqueous layer was separated, washed twice with CH_2Cl_2 (100 mL), and the organic layers were combined and dried ($MgSO_4$). Filtration, solvent removal, column chromatography (1:2 hexanes/EtOAc) gave 190 mg (88%) of a white foam. Recrystallization (hexanes/Et_2O 1:1) gave 160 mg (74%) of **53** as a white powder; yield: 160 mg (74%).

The third synthesis used the racemic form of **52** (R = TBDPS), but selectivity was gained in the coupling by using titanium Lewis acids.[28] When titanium trichloroisopropoxide and silylated thymine **8** were used, a 10:1 mixture of **53** and **54** (R = TBDPS, 74% yield) resulted. Again, the selectivity can be explained through in situ complexation between the titanium Lewis acid and the ring oxygen(s). In all cases, the coupled materials were deprotected to supply the antiviral nucleoside, Dioxolane-T **(5)**. It is also noteworthy that recent studies have shown that a very good selectivity α or β and very good yield can be achieved using [catecholato(2-)-*O*-*O*′]oxotitanium and triflic anhydride and an appropriate base.[28]



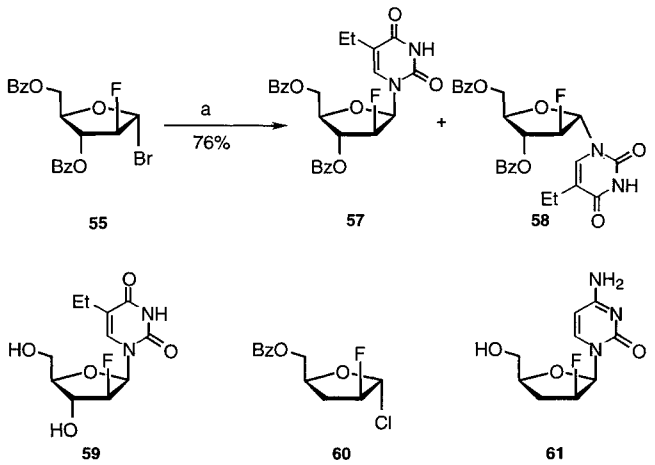
Scheme 16

3. S_N2 Displacements

3.1. Involving 2-β-Fluoro-1-α-halo-2-deoxyribosides

There are two examples of the use of a 2-β-fluoro group on the riboside.[31,32] However, these reactions do not

involve a Lewis acid, and selectivity is gained by S_N2 displacement of 1-α-bromo- or chlororibosides. In one example, Howell and co-workers showed that the 1-α-bromo-2-β-fluororiboside **55** (Scheme 17) coupled with silylated 5-ethyluracil **(56)** under displacement conditions to give mainly the β isomer **57** (**57/58** 16:1).[31] The ratio of the two isomers was found to be dependent upon the polarity of the solvent used in the reaction (e.g., CCl_4 gave a 41 : 1 ratio of **57/58**).[31]



a : $CHCl_3$, silylated 5-ethyluracil (**56**), Δ,40 h

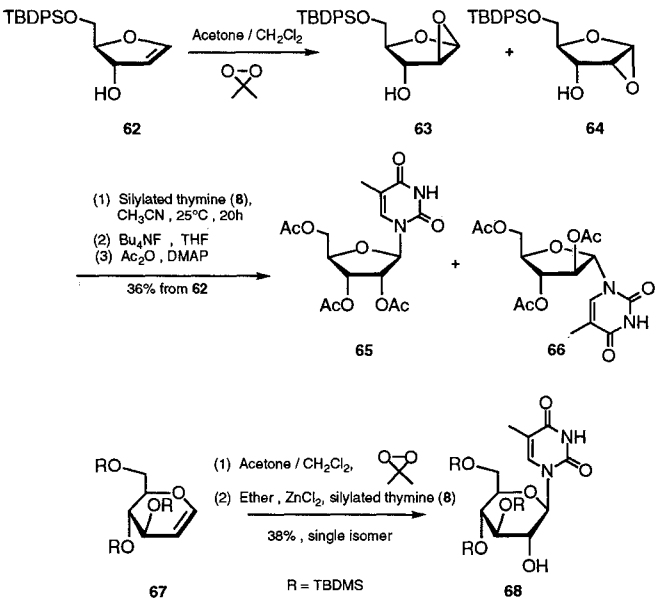
Scheme 17

1-(3′,5′-Di-*O*-benzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-ethyluracil; Typical Procedure:
A solution of 2,4-bis-*O*-(trimethylsilyl)-5-ethyluracil (116 g, 0.408 mol), 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-arabinofuranosyl bromide (145 g, 0.343 mol), and $CHCl_3$ (1.7 L) was stirred at reflux for 20 h. The cooled reaction mixture was washed with H_2O (2 × 2 L) and dried (Na_2SO_4), and the solvent was removed at reduced pressure. The solid product was recrystallized from hot absolute EtOH (1.5 L) to give the title compound; yield: 126 g (76.2%).

In another example, Okabe coupled the 1-α-chloro-3-deoxy derivative of **60** with silylated N^4-acetylcytosine **45** and obtained a similar result.[32] The β fluorine atom in these cases not only serves in a biochemical role, but also as a stereochemical template allowing stereoselective incorporation of the halogen atom at the 1 α position and subsequent displacement reaction. These compounds were then deprotected to provide the promising anti-HIV agents 2′-β-fluoro-2′-deoxy-5-ethyluridine (FEAU, **59**, Scheme 17) and 2′-β-fluoro-2′,3′-dideoxycytidine (F-ddC, **61**).

3.2. Opening of Glucal Epoxides

Another method in this discussion involves the use of a 1,2-anhydrofuranoside. In this case, Danishefsky and Chow have used a 1,2-epoxyribose to allow attachment of the pyrimidine base with some stereoselectivity (Scheme 18).[33] The appropriate starting material for this methodology is 3(*S*)-hydroxy-4(*R*)-*tert*-butyldiphenylsiloxymethyl-3,4-dihydrofuran **(62)**, which was epoxidized using 3,3-dimethyldioxirane.



Scheme 18

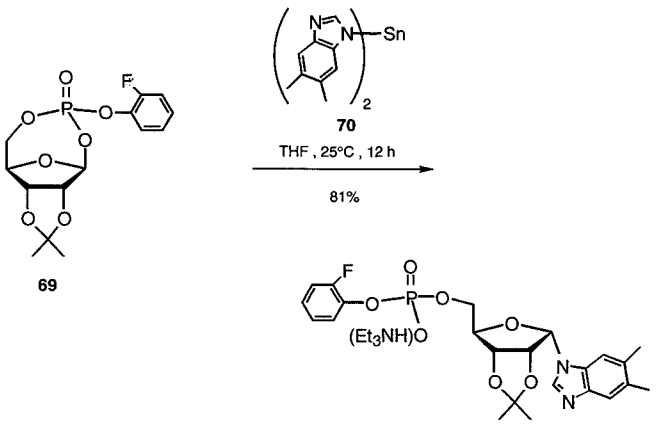
1-(2′,3′,5-Tri-*O*-acetyl-α/β-D-ribofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione; Typical Procedure:
Furanoid glycal **62** (76 mg, 0.21 mmol) was dissolved in CH_2Cl_2 (30 mL). The solution was cooled to 0 °C, whereupon 3,3-dimethyldioxirane (5.1 mL, 0.32 mmol, 63 mM soln in acetone) was added. The reaction mixture was stirred at 0 °C for 40 min before being concentrated to 2 mL by passing a steam of N_2 over the solution. MeCN (10 mL) was added, and the stream of N_2 continued until the volume was reduced to 5 mL. To the resultant mixture of the epoxy sugar was added silylated thymine **8** (0.17 g, 0.63 mmol). The reaction mixture was stirred at r.t. for 19.5 h before being quenched with 5% aq $NaHCO_3$ (10 mL). This was followed by extraction with EtOAc (3 × 15 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in THF (3 mL); TBAF (1.0 mL, 1 M solution in THF) was added. After being stirred overnight the reaction mixture was concentrated and the residue was subjected to chromatography (silica gel, $CHCl_3$/MeOH 10 : 1). The product obtained was dissolved in THF (10 mL); pyridine (0.34 mL), Ac_2O (0.40 mL), and a catalytic amount of DMAP were added. After being stirred at room temperature for 12 h, the reaction mixture was concentrated and the residue was subjected to chromatography (silica gel, hexanes/EtOAc 1 : 1) to afford a 4 : 1 mixture of **65/66**; yield: 29 mg (36%).

The resulting mixture of epoxides (**63/64** 1 : 9) was opened under S_N2 conditions with silylated thymine **8**, and without the use of a Lewis acid. The result was a 4 : 1 mixture of **65/66**, two of the four possible isomers that could be formed in this series of reactions. The approach is more selective with 1,2-pyranosides, resulting in formation of a single pyranonucleoside isomer (**67** to **68**, Scheme 18). Further studies in this area may provide a more selective method of nucleoside base incorporation for furanoside sugar derivatives.

3.3. Displacement of Cyclic Phosphonates

The second reaction type involves a S_N2 displacement of a cyclic phosphonate and preferential production of a nucleoside isomer. Mukaiyama and co-workers assembled riboside derivative **69** (Scheme 19) with the idea that the cyclic C_1/C_5 phosphonate would act as a β leaving group and promote α attack by a nucleoside base.[34] When ribofuranoside **69** was exposed to the 5,6-dimethylbenz-

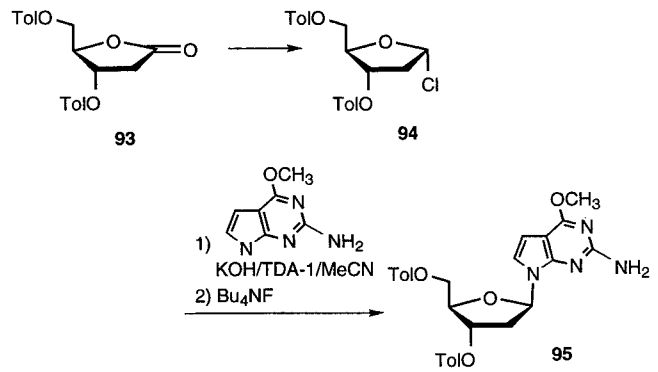
imidazole/tin(II) complex **70**, only the α isomer **71** was formed in 81% yield. Other bases gave slightly lower selectivity and/or yields, and the generality of this method has not been fully explored. However, other variants of this concept may provide a potentially useful glycosylation method.



Scheme 19

3.4. Involving a Solid–Liquid Phase-Transfer Glycosylation

Considering the availability of the 1-chloro-2-deoxy-3,5-di-*O*-tolyl-D-erythropentofuranose,[35a] many groups had effected the coupling of this sugar with different bases via S_N2 displacement of the chlorine atom. Another methodology using solid–liquid phase transfer reaction was realized. Reaction of the sugar moiety with the heterocycles in a biphasic mixture of benzene/50% aqueus sodium hydroxide in the presence of Aliquat 336 under the conditions of phase transfer catalysis resulted in a complex mixture[35b] After treatment of the contents of the organic phase with concentrated aqueous ammonia the number of the reaction products was reduced to one main product. After chromatography the desired product was isolated in 48% yield.



Scheme 20

2-Amino-7-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine; Typical Procedure:

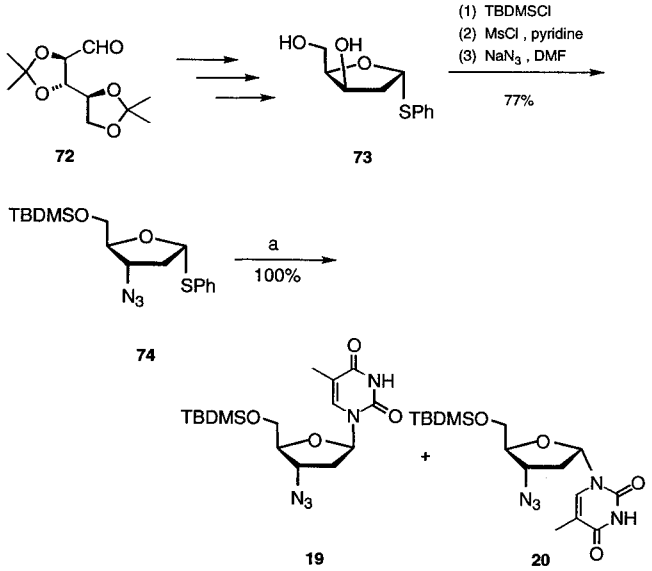
A suspension of O^6-methyl-7-deazaguanine (0.5 g, 3.05 mmol) and Aliquat 336 (methyltrioctylammonium chloride, 133 mg, 0.3 mmol) in benzene/dimethoxyethane (4:1), 20 mL) and an equal volume of 50% aq NaOH was stirred with a Vibromixer for 30 min. During this time the chlorosugar (1.66 g, 4.26 mmol) dissolved in hot benzene (30 mL) was added in small portions. After this the suspension was diluted with H_2O (100 mL) and CH_2Cl_2 (100 mL). The organic phase was separated and evaporated in vacuo. The resulting syrup was dissolved in MeOH (20 mL), treated with conc. NH_3, and stirred for 24 h at r.t. The solvent was removed in vacuo to give a light brown syrup (520 mg). The aqueous phase was acidified with conc. aq HCl to pH 4. Precipitated toluic acid was filtered off and the filtrate was evaporated to dryness. The residue was suspended in MeOH (100 mL) and heated under reflux for 10 min. Inorganic salt was filtered off, and the solvent was removed in vacuo to leave a brown amorphous residue (9.65 g). The contents of both phases were combined and dissolved in MeOH (30 mL), adsorbed on silica gel (5 g), and evaporated in vacuo. The residue was suspended in CH_2Cl_2 (50 mL) and applied to a silica gel column (2.5 × 20 cm). A prerun with CH_2Cl_2 gave only nonnitrogenous material, which was discarded. Further elution with solvent system (gradient of MeOH in CH_2Cl_2) gave the desired nucleosides as colorless solids; yield: 48%.

Seela[35b] proposed that a 1′-3′ or 1′-5′-*ortho*-amide structure could be formed as an intermediate. As they had shown earlier, *ortho*-amides are quite stable in the case of pyrrolo[2,3-*d*]pyrimidines and do not rearrange to the final glycosylation product. However, such *ortho*-amides are cleaved by aqueous ammonia to yield the glycosylated product. No trace of the α product has been isolated.

4. Other Activation Methods

4.1. Use of a 1-Phenylthio Group and *N*-Bromosuccinimide

Another variation of a displacement reaction to provide stereochemical control was attempted in the synthesis of AZT (**1**, Scheme 21). Sugimura and co-workers had the idea of using a phenylthio group positioned in the α configuration at the anomeric center and activating it for S_N2 displacement with an electrophile.[36,37] The actual synthesis became a variation of the earlier Chu strategy (Scheme 5), and also begins with the use of a carbohydrate derived material. Starting with diisopropylidene L-allose (**72**, Scheme 21), it was converted stereoselectively (4 steps) to the 1-α-phenylthiofuranoside **73**.[35] Protection, followed by azide incorporation at C_3 provided the appropriate azidoriboside **74**.



a : NBS, CH_2Cl_2, silylated thymine (8)

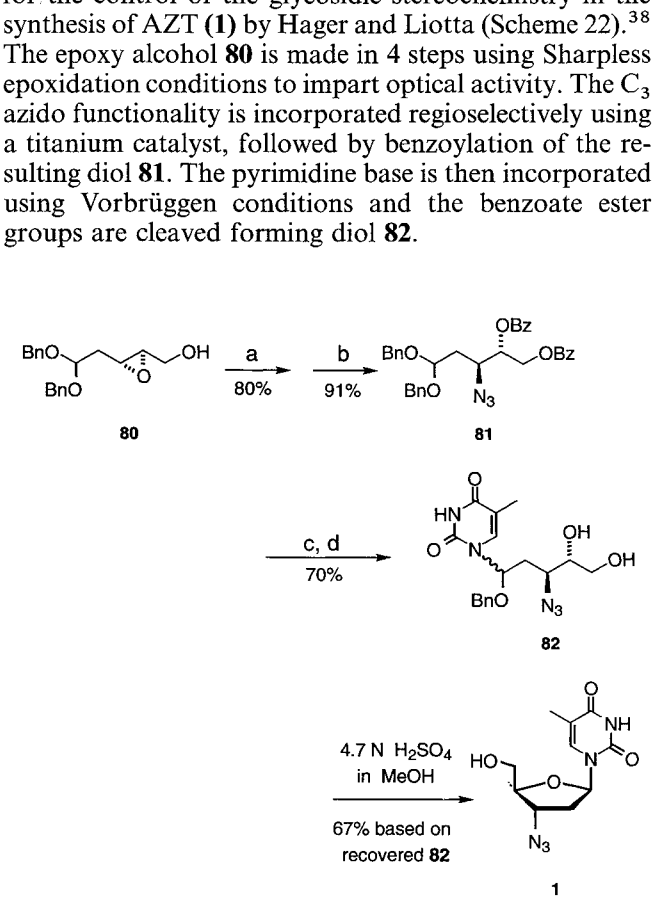
Scheme 21

Activation of the sulfur group with NBS was successful, but the reaction proceeded with no stereoselectivity [(**19**/**20**) 1:2; 100% yield] in the coupling with silylated thymine **8**. It is very likely that the mechanism involves an oxonium ion **21a** (X = N_3) and, therefore, the stereochemical integrity of the α-phenylthio group is lost. This result confirms the observation that prior activation of the anomeric position by either Lewis acid or electrophile in the case of 2-deoxyribose sugars prevents facial control in the glycosylation due to an oxonium intermediate (i.e., **21a**, Scheme 6).

5. Miscellaneous Methods

5.1. From Acyclic Nucleosides

There are other convergent approaches to nucleosides involving a base coupling step which do not fit into the other categories discussed previously and are considerable contributions to this field. One example of a unique convergent route involves the use of cyclization protocols for the control of the glycosidic stereochemistry in the synthesis of AZT **(1)** by Hager and Liotta (Scheme 22).[38] The epoxy alcohol **80** is made in 4 steps using Sharpless epoxidation conditions to impart optical activity. The C_3 azido functionality is incorporated regioselectively using a titanium catalyst, followed by benzoylation of the resulting diol **81**. The pyrimidine base is then incorporated using Vorbrüggen conditions and the benzoate ester groups are cleaved forming diol **82**.



a : Ti(OiPr)$_4$, benzene, TMSN$_3$. b : PhCOCl, Et$_3$N, CH$_2$Cl$_2$, c: TMSOTf, ClCH$_2$CH$_2$Cl, silylated thymine (**8**). d : NaOH, MeOH

Scheme 22

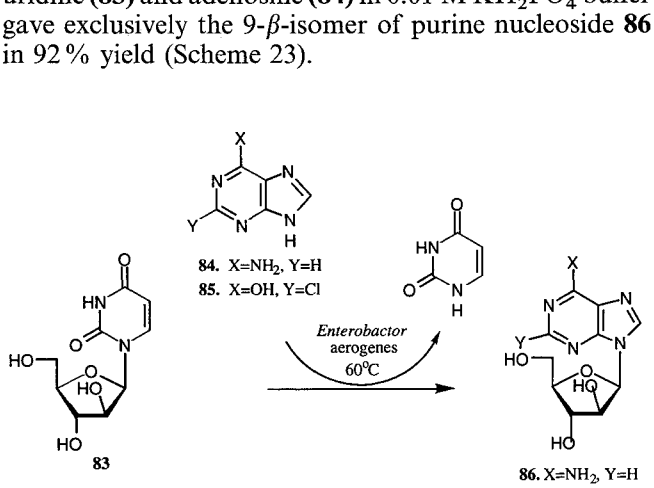
The acyclic diol **82** is then exposed to protic conditions and the result is iminium ion formation by displacement of the benzyloxy moiety, followed by cyclization to provide AZT **(1)** in a stereochemically pure form. The selectivity in the cyclization step is explained through a "gauche" effect. Presumably the ammonium ion of **82** adopts a gauche conformation in which the spatial arrangement at carbons 1, 3, and 4 are restricted in the desired conformation before cyclization takes place. This route may prove quite useful in the stereoselective synthesis of other 3′-substituted nucleosides.

5.2. Intramolecular Glycosylations

Another variation for the control of the stereoselectivity in the coupling reaction is the intramolecular glycosylation. In this case the O-2 linked pyrimidine nucleoside is delivered to the β face of the sugar using a different Lewis acid and utilizing the configuration at the C_4 position of the pseudosugar.[39] This approach has been applied for the synthesis of biologically active molecules (AZT, FLT) with good yields[40] but its use has been limited to the 2,4-oxopyrimidine derivatives.

5.3. Enzyme-Regulated Glycosylations

The enzymatic transfer of a ribofuranoside between purine and pyrimidine bases is an efficient method that offers complete stereocontrol in the formation of the glycosidic linkage in nucleoside analogs. The synthesis of 9-β-D-arabinofuranosyladenine **(86)** by Morisawa and co-workers illustrates the stereo- and regioselectivity of an enzymatic transglycosylation from a pyrimidine to a purine nucleoside.[41a] With intact cells of *Enterobacter* aerogenes AJ 11125, a mixture of 1-β-arabinofuranosyluridine **(83)** and adenosine **(84)** in 0.01 M KH_2PO_4 buffer gave exclusively the 9-β-isomer of purine nucleoside **86** in 92% yield (Scheme 23).

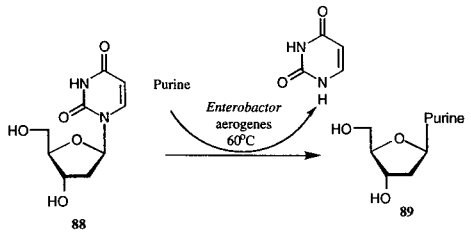


Scheme 23

The incubation mixture from the enzymatic reaction also contained a small amount of arabinofuranose-1-α-phosphate, a key intermediate responsible for retention of the β-configuration in the purine nucleoside **86**. In the absence of inorganic phosphate, *Enterobactor* aerogenes and other bacterial cells were unable to catalyze the transglycosylation reaction. The overwhelming evidence for the phosphate intermediate clearly indicates that the reaction is catalyzed by a nucleoside phosphorylase.

Enzymatic conditions similar to the transarabinosylation from uracil to adenosine were applied to the synthesis of 9-β-D-arabinofuranosyl-2-chlorohypoxanthine. However, only 34% of 2-chlorohypoxanthine **(85)** was converted to the purine nucleoside **87** (Scheme 23).[41b] In a

similar case, Holy and Votruba converted 2′-deoxyuridine **(88)** to various functionalized purine 2′-deoxy-β-D-ribonucleosides **89** using bacterial cells encapsulated in permeable alginate gel.[42] Although this enzymatic transdeoxyribosylation is amenable to a variety of purine derivatives, a general trend for substrate activity could not be established according to the substitution pattern of the purine bases (Table 4).



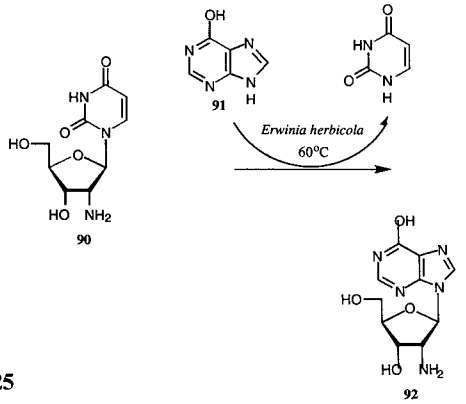
Scheme 24

Table 4. Conversion of 2′-Deoxyuridine **(88)** to Purine 2′-Deoxy-β-ribonucleosides **89**

Purine Base	Conversion (%)
adenine	63.5
6-methyladenine	64.5
6-dimethyladenine	64.5
2-methyladenine	67.0
2-aminoadenine	46.0
8-aminoadenine	21.0
6-mercaptopurine	0
6-chloropurine	63.5
2,6-dichloropurine	33.0
hypoxanthine	38.5
2-hydroxypurine	0
8-aza-7-deazaadenine	11.0

Under similar conditions, the transdeoxyribosylation from uracil to substituted pyrimidines proved to be less effective than the case with substituted purines. Uracil derivatives possessing small substituents at C_5 (e.g., thymine and 5-fluorouracil) appeared to be the only pyrimidines that were substrates for the enzymatic transdeoxyribosylation. Derivatives with larger substituents such as 5-ethyl- and 5-acetyluracil were converted to the corresponding 2′-deoxy-β-ribonucleosides in modest yields of 36% and 31%, respectively, while 5-(1-propyl)- and 5-(3-butenyl)uracil proved to be completely inactive toward the enzymatic glycosylation reaction.

The synthesis of purine 2′-amino-2′-deoxynucleosides via an enzymatic transaminoribosylation reaction was accomplished with *Erwinia herbicola* AJ 2803. Under similar conditions to Morisawa's transarabinosylation (0.01 M phosphate buffer), the enzymatic reaction of 2′-amino-2′-deoxyuridine **(90)** with hypoxanthine **(91)** gave 2′-amino-2′-deoxyinosine **(92)** in 52% yield (Scheme 25).[43] Since *Erwinia herbicola* exhibited higher activity for the transaminoribosylation than *Enterobacter* aerogenes and other bacterial strains, Morisawa suggested that these cells contain different types of nucleoside phosphorylases that have different substrate specificities. However, the transaminoribosylation catalyzed by *Erwinia herbicola* is still less efficient than transarabinosylation, which indicates that minor alterations at the $C_{2'}$ position reduces a carbohydrate's ability to be a substrate of nucleoside phosphorylases. Mikhailopulo and co-workers further explored substrate specificities of various modified carbohydrate moieties with nucleoside phosphorylases.



Scheme 25

Simple modifications at the $C_{2'}$ and $C_{3'}$ positions of uracil nucleosides showed a dramatic decrease in the ability of nucleoside phosphorylases in *Escherichia coli* BM-11 cells to catalyze transglycosylation reactions. While the transglycosylation of uridine to adenosine gave 100% conversion, various $C_{2'}$ derivatives such as 2′-deoxyuridine and 2′-amino-2′-deoxyuridine afforded lower yields of corresponding adenosine derivatives (Table 5). Uridine derivatives with alterations at the $C_{3'}$ position showed the lowest substrate activity with the nucleoside phosphorylases. Derivatives such as 3′-deoxyuridine and 3′-aminouridine were converted to the corresponding adenosine nucleosides in 4.8% and 0.2%, respectively, while 3′-fluoro and 3′-methyl analogs were completely inactive toward the enzymatic transglycosylation.[44]

Table 5. Substrate Specificity of Purine Nucleoside Phosphorylases in the Conversion of Uracil Nucleosides to Adenine Nucleosides

Substrate	Relative Activity (%)
uridine	100
2′-deoxyuridine	78.8
3′-deoxyuridine	4.8
2′-amino-2′-deoxyuridine	2.7
3′-amino-3′-deoxyuridine	0.2
3′-fluoro-3′-deoxyuridine	0
2′-methyl-2′-deoxyuridine	0
3′-methyl-3′-deoxyuridine	0

The phosphorylases in *Lactobacillus heveticus* cells, which are very effective in catalyzing the transglycosylation of 2′-deoxynucleosides, effectively catalyze the ribosyl exchange between pyrimidine and purine 2′,3′-dideoxynucleosides. Even though 2′,3′-dideoxyribosyl exchange was

15 times less efficient than the corresponding 2′-deoxyribosyl exchange with *Lactobacillus heveticus*, the transglycosylation of 2′,3′-dideoxycytidine with adenine gave 20% conversion to 2′,3′-dideoxyadenosine. Carson and Wasson also found that the 2′,3′-dideoxyribosyl exchange was amenable to a wide variety of 2,6-substituted purine bases when 2′,3′-dideoxycytidine and 2′,3′-dideoxythymidine were used as 2′,3′-dideoxyribosyl donors.[45]

The bacterial strain *Escherichia coli* BMT-1D/1A, which possesses high activity for thymidine phosphorylases, shifts the enzymatic glycosylation reaction toward the formation of pyrimidine nucleosides. Zinchenko and coworkers showed that 2′-deoxyguanidine and 2′-deoxyadenosine in the presence of excess thymine and *Escherichia coli* BMT-1D/1A cells were converted to 2′-deoxythymine in 56 and 32% yield, respectively. Even though 2′-deoxycytidine is not a substrate for thymidine phosphorylases, the cytidine deaminases in *Escherichia coli* BMT-1D/1A cells convert the nucleoside to the highly active substrate 2′-deoxyuridine. In fact, the transglycosylation of 2′-deoxycytidine with thymine and whole cells of *Escherichia coli* BMT-1D/1A gives 2′-deoxythymine in 76% yield. With a wide range of substrate specificity, this enzymatic glycosylation proved to be an effective method in converting a crude mixture of 2′-deoxynucleosides from DNA to 2′-deoxythymidine. After DNA hydrolysis and subsequent dephosphorylation with *Spicaria violacea*, the resulting mixture of 2′-deoxynucleosides and excess thymine were incubated with whole cells of *Escherichia coli* BMT-1D/1A to give a mixture that exhibited a twofold increase in the concentration of 2′-deoxythymidine.[46]

6. Conclusions

As has been shown, nucleoside analogs have frequently been the aim of convergent synthesis. A high degree of structural variation has been shown to be attainable with this approach allowing the nucleosides synthesized according to a traditional linear strategy (divergent synthesis) to be supplemented. Thus both D and L forms have been produced in conjunction with all possible configurations of $C_{1'}$, $C_{2'}$ and $C_{3'}$, e.g., all 8 isomers of AZT are now known.

The syntheses have focused on the formation of a carbohydrate intermediate suitable for condensations with activated bases, and great ingenuity in the selection of ultimate precursors as well as in their transformations to key intermediates have been exhibited.

A serious problem is the formation of mixtures of the desired biologically active β-anomer and the undesired α-anomers which are often very difficult to separate. Therefore the most recent efforts have concentrated on the introduction of auxiliary groups at the 2-position of the furanose to improve stereoselectivity in the coupling reaction with the activated bases. In this way, almost complete stereoselectivity has been attained.

Whatever the detailed structure of the synthetic route, we anticipate the number of nucleoside analogs obtained by a convergent synthesis to increase dramatically in the future.

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