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High-Throughput Five Minute Microwave Accelerated Glycosylation Approach to the Synthesis of Nucleoside Libraries

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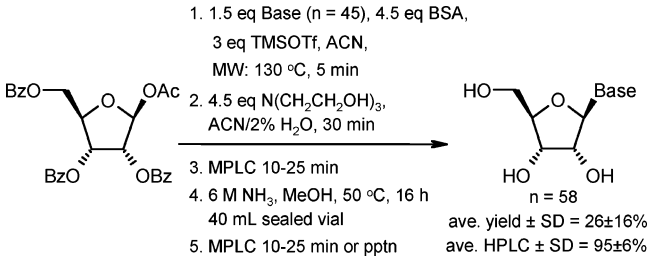

The Vorbrüggen glycosylation reaction was adapted into a one-step 5 min/130 °C microwave assisted reaction. Triethanolamine in acetontrile containing 2% water was determined to be optimal for the neutralization of trimethylsilyl triflate allowing for direct MPLC purification of the reaction mixture. When coupled with a NH_3/methanol deprotection reaction, a high-throughput method of nucleoside library synthesis was enabled. The method was demonstrated by examining the ribosylation of 48 nitrogen containing heteroaromatic bases that included 25 purines, four pyrazolopyrimidines, two 8-azapurines, one 2-azapurine, two imidazopyridines, two benzimidazoles, three imidazoles, three 1,2,4-triazoles, two pyrimidines, two 3-deazapyrimidines, one quinazolinedione, and one alloxazine. Of these, 32 yielded single regioisomer products, and six resulted in separable mixtures. Seven examples provided inseparable regioisomer mixtures of −two to three compounds (16 nucleosides), and three examples failed to yield isolable products. For the 45 single isomers isolated, the average two-step overall yield ± SD was 26 ± 16%, and the average purity ± SD was 95 ± 6%. A total of 58 different nucleosides were prepared of which 15 had not previously been accessed directly from glycosylation/deprotection of a readily available base.

Introduction

Nucleosides and nucleotides have provided a productive area of chemical and biological research for over 100 years.[1,2] The monomeric units of DNA and RNA are involved in the regulation of a myriad of cellular metabolic pathways and have been the subject of intense areas of research seeking to identify therapeutic agents for a variety of diseases including viral infections, cancer, cardiovascular diseases, CNS diseases, etc. However, few drugs have resulted from those efforts,[3] which were focused primarily in areas other than viral[4] diseases and leukemias.[5]

To more thoroughly pursue therapeutic areas that might be responsive to treatment with nucleosides, as well as to provide more building blocks for oligonucleotide synthesis, it would be beneficial to have a practical method for high-throughput synthesis of these molecules. Traditional methods of nucleoside analogue synthesis involve either a glycosylation reaction

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(1) The pioneering work of Emil Fischer in the fields of purines, carbohydrates, and proteins set the foundation for later advances in the field of nucleosides. (a) Fischer, E. *Chem. Ber.* **1897**, *30*, 1846−1859. (b) Fischer, E. *Chem. Ber.* **1898**, *31*, 104−122. (c) Fischer, E. *Chem. Ber.* **1899**, *32*, 267−273.

(2) The term nucleoside was introduced in 1909 by Levene and Jacobs. Levene, P. A.; Jacobs, W. A. *Chem. Ber.* **1909**, *42*, 2474−2478.

(3) (a) Isono, K. *Pharmacol. Ther.* **1991**, *52*, 269−286. (b) Perigaud, C.; Gosselin, G.; Imbach, J.-L. *Nucleosides Nucleotides* **1992**, *11*, 903−945. (c) Jacobson, K. A.; van Galen, P. J. M.; Williams, M. *J. Med. Chem.* **1992**, *35*, 407−422. (d) Appleman, J. R.; Erion, M. D. *Expert Opin. Invest. Drugs* **1998**, *7*, 225−243. (e) Williams, M.; Jarvis, M. F. *Biochem. Pharmacol.* **2000**, *59*, 1173−1185.

(4) (a) Robins, R. K.; Revankar, G. R. In *Antiviral Drug Development: A Multidisciplinary Approach*; De Clercq, E., Walker, R. T., Eds.; Plenum Press: New York, 1988; p 11. (b) Koszalka, G. W.; Daluge, S. M.; Boyd, F. L. *Annu. Rep. Med. Chem.* **1998**, *33*, 163−171. (c) De Clercq, E. *J. Clin. Virol.* **2004**, *30*, 115−133.

(5) (a) Plunkett, W.; Gandhi, V. *Cancer Chemother. Biol. Response Modif. Annu.* **2001**, *19*, 21−45. (b) Johnson, S. A. *Expert Opin. Pharmacother.* **2001**, *2*, 929−943.

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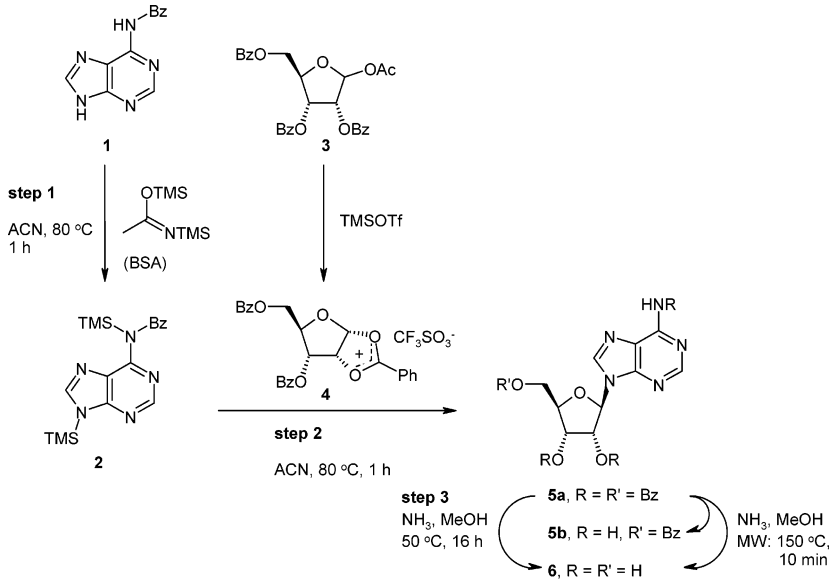
SCHEME 1. Standard Vorbrüggen Glycosylation Reaction Conditions



between a nitrogenous aromatic base and an activated sugar intermediate or derivatization of a preformed nucleoside. Only methods employing the latter strategy have been reported for nucleoside library synthesis.[6−8] These methods have been used to produce large numbers of nucleosides where the core base and ribofuranose moieties have been limited to only a few options.

The glycosylation reaction has remained undeveloped as a high-throughput process, even though over a period of more than 90 years, it has been applied to the synthesis of a vast array of diverse nucleosides.[9] Glycosylation protocols include the silver salt,[10] the chloromercury,[11] the fusion,[12] the sodium salt,[13] the phase transfer,[14] and the boron trifluoride etherate[15] methods. Vorbrüggen combined elements from some of these methods and introduced the use of Friedel−Crafts catalysts to promote the glycosylation.[16] The broad applicability of the Vorbrüggen reaction made it desirable for development into a high-throughput reaction.

As illustrated in Scheme 1 with the synthesis of adenosine (**6**) as an example, nucleoside synthesis can be considered as a three-step operation. The typical Vorbrüggen reaction requires presilylation of the base **1** (step 1) and then reaction with trimethylsilyl triflate activated sugar **3** to form the desired acyl-protected nucleoside (step 2). One advantage of this method is that frequently one nitrogen of the base is regioselectively glycosylated because of thermodynamic equilibration.[17] Conveniently, this often produces the same regioisomer as preferred by the natural product. Also, with α-2′-*O*-acyl-substituted sugar coupling partners, acyl-oxonium ion[18] stabilization of the anomeric cation, as depicted in formula **4**, directs glycosylation stereospecifically to the β-face of the sugar producing the natural β-configuration. Furthermore, while the Vorbrüggen reaction is frequently conducted as a two-step operation, it is possible to combine steps 1 and 2 and perform this reaction by combining all reagents and heating.[19] Finally, deprotection of the acyl-protecting groups of **5a** by ammoniolysis (step 3) can be performed in a simple operation to provide the nucleoside. The synthesis of adenosine (**6**) depicted in Scheme 1 exemplifies the utility of this method as it has been obtained in 81% overall yield when base silylation, glycosylation, and deprotection were each performed as distinct steps.[16]

Despite the appeal of being able to produce a wide array of these highly desirable molecules in a stereospecific and regioselective manner, several factors have precluded the adoption of the Vorbrüggen reaction as a general high-throughput method.

(6) Amine substitution of nucleoside heteroaryl-halides: (a) Rodenko, B; Wanner, M. J.; Koomen, G.-J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1247−1252. (b) Varaprasad, C. V.; Habib, Q.; Li, D. Y.; Huang, J.; Abt, J. W.; Rong, F.; Hong, Z.; An, H. *Tetrahedron* **2003**, *59*, 2297−2307. (c) Ding, Y.; Habib, Q.; Shaw, S. Z.; Li, D. Y.; Abt, J. W.; Hong, Z.; An, H. *J. Comb. Chem.* **2003**, *5*, 851−859. (d) Gunic, E.; Amador, R.; Rong, F.; Abt, J. W.; An, H.; Hong, Z.; Girardet, J.-L. *Nucleosides, Nucleotides, Nucleic Acids* **2004**, *23*, 495−499. (e) Koh, Y.-h.; Landesman, M. B.; Amador, R.; Rong, F.; An, H.; Hong, Z.; Girardet, J.-L. *Nucleosides, Nucleotides, Nucleic Acids* **2004**, *23*, 501−507.

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(11) Davoll, J.; Lowy, B. A. *J. Am. Chem. Soc.* **1951**, *73*, 1650−1655.

(12) Ishido, Y.; Sato, T. *Bull. Chem. Soc. Jpn.* **1961**, *34*, 1347−1348.

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(14) (a) Seela, F.; Winkeler, H.-D. *J. Org. Chem.* **1982**, *47*, 226−230. (b) Ugarkar, B. G.; Castellino, A. J.; DaRe, J. M.; Kopcho, J. J.; Wiesner, J. B.; Schanzer, J. M.; Erion, M. D. *J. Med. Chem.* **2000**, *43*, 2894−2905.

(15) Cottam, H. B.; Petrie, C. R.; McKernan, P. A.; Goebel, R. J.; Dalley, N. K.; Davidson, R. B.; Robins, R. K.; Revankar, G. R. *J. Med. Chem.* **1984**, *27*, 1119−1127.

(16) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234−1255.

(17) Vorbrüggen, H.; Hofle, G. *Chem. Ber.* **1981**, *114*, 1256−1268.

(18) Baker, B. R.; Joseph, J. P.; Schaub, R. E.; Williams, J. H. *J. Org. Chem.* **1954**, *19*, 1786−1792.

(19) Vorbrüggen, H.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1279−1286.

These include the following: (1) times reported for conducting the reaction generally vary from 1 to 24 h depending on the base; (2) lack of solubility of some bases in the reaction solvent often results in reactions with poor reproducibility; (3) a necessary basic aqueous workup to neutralize the acidic mixture is needed; (4) there is lack of solubility of some products in the extraction solvent; and (5) there is a necessity for chromatographic purification. This paper will explain how several modern technologies, especially microwave assisted synthesis and automated silica gel MPLC, have been applied to resolve these issues and convert this into a high-throughput process.

Chemistry

Elimination of the aqueous workup became an early priority. The polarity of some bases and products restricted their solubility and made conducting extractions in parallel cumbersome and inconsistent across a wide variety of substrates. As an alternative to extraction, neutralization of the trifluoromethanesulfonic acid (TfOH)[20] generated from hydrolysis of trimethylsilyl triflate was performed prior to direct silica gel chromatographic purification of the reaction mixture. Triethylamine was found to be inadequate since the resulting TfOH triethylammonium salt contaminated chromatographed products (as observed from ^{1}H NMR). Next, the ion-exchange resin Amberlite IRA-400 OH^- was found to effectively sequester TfOH as a resin bound salt, but it was also found to retain certain hydrophobic products as well. This resulted in decreased yields with some substrates and a complete loss of the glycosylation product for one example (entry 4, Table 1). Finally, triethanolamine (TEOA) in acetonitrile containing 2% water was determined to be the most effective acid quenching reagent since the polar nature of the ammonium salt produced resulted in a clean chromatographic separation from the glycosylation reaction product in all examples studied.

To accelerate the chromatographic process, a commercially available automated medium-pressure liquid chromatography (MPLC) instrument with UV based fraction collection was utilized for the purification of both the glycosylation and the deprotection products.[21] Moreover, for some very polar nucleosides, MPLC was only necessary for the glycosylation product. In these examples, the ammoniolysis products precipitated directly from the reaction solution (entries 5, 7, and 8 in Table 1).

We investigated conducting the Vorbrüggen glycosylation in the one-step format[19] using elevated temperatures generated by microwave heating[22] and observed that reactions reported to require up to 24 h in refluxing acetonitrile (82 °C) could be conducted satisfactorily in 5 min at 130 °C (see Table 1 for examples and the Supporting Information for a more extensive list).[23, 24] At this elevated temperature, even very polar bases were solubilized as their silylated derivatives. This resulted in more consistent reactivities across a diverse group of bases. Repeating this glycosylation with an oil bath heating apparatus with an internal reaction temperature of 130 °C (150 °C bath temperature) for 5 min produced **5a** in 65% yield as compared to a 61% yield obtained using microwave heating. Thus, the microwave advantage is chiefly the safe application of homogeneous heat to the reaction process as well as the automation capabilities inherent within the instrument. Deprotection of the acyl groups with 6 M ammonia in methanol (50 °C, 16 h) completed the nucleoside synthesis. Interestingly, the latter reaction could not be accelerated effectively with the application of higher microwave induced temperatures. When the ammoniolysis was conducted in the microwave reactor at 150 °C, the pressure limit of the microwave reaction vial (20 bar) was reached. After 10 min under these conditions, the deprotection of **5a** was incomplete, and 5′-*O*-benzoyladenosine (**5b**)[25,26] and adenosine (**6**) were isolated in 22 and 46% yields, respectively (Scheme 1).

Results and Discussion

In the original report of the Vorbrüggen one-step method,[19] adenosine (**6**), guanosine (**15**), cytidine (**44**), and compound **49** were prepared in 63, 44, 59, and 50% yields, respectively. By comparison, using the 5 min/130 °C microwave Vorbrüggen glycosylation reaction followed by deprotection, nucleosides **6**, **15** (as part of a N9/N7 mixture with **16**), **44**, and **49** were prepared in 45, 26, 50, and 51% yields, respectively (Table 1). Other natural nucleosides such as inosine (**8** as part of an N9/N7 isomer mixture with **9**) and uridine (**46**) were also readily obtained by this method, each in 36% yield. The generality and high-throughput of the method greatly compensate for the lower yields observed in certain cases. When coupled with a simple NH_3/methanol deprotection reaction and short automated MPLC purification processes, a high-throughput synthesis of nucleoside libraries was realized (see Scheme 2 for method details).

The breadth of the method is evident from the large variety of bases that was successfully ribosylated (see Table 1 and Supporting Information). The 48 bases tested as glycosylation substrates included 25 purines, four pyrazolopyrimidines, two 8-azapurines, one 2-azapurine, two imidazopyridines, two

(20) Direct evaporation of the reaction mixture prior to chromatographic purification decomposed some reaction products, resulting in decreased yields.

(21) The automated silica gel MPLC process purified most glycosylation products to homogeneity. However, a few contained impurities related to excess sugar byproducts or excess base. These were conveniently removed with automated silica gel MPLC or precipitation of the nucleoside product after the deacylation reaction so that the final nucleoside product could be obtained in high purity.

(22) Review: Kappe, C. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250−6284.

(23) A silica supported fusion glycosylation in a standard kitchen microwave is the only report of a microwave assisted glycosylation: Andrzejewska, M.; Kaminski, J.; Kazimierczuk, Z. *Nucleosides, Nucleotides, Nucleic Acids* **2002**, *21*, 73−78.

(24) Use of microwave heating for nucleoside functional group manipulations: (a) Varma, R. S.; Lamture, J. B.; Varma, M. *Tetrahedron Lett.* **1993**, *34*, 3029−3032. (b) Kumar, P.; Gupta, K. C. *Chem. Lett.* **1996**, *8*, 635−636. (c) Kumar, P.; Gupta, K. C. *Nucleic Acids Res.* **1997**, *25*, 5127−5129. (d) Tschamber, T.; Rudyk, H.; Nouen, D. L. *Helv. Chim. Acta* **1999**, *82*, 2015−2019. (e) Gorska, A.; Andrzejewska, M.; Kaminski, J.; Kazimierczuk, Z. *Nucleosides, Nucleotides, Nucleic Acids* ***2003***, *22*, 13−19. (f) Paolini, L.; Petricci, E.; Corelli, F.; Botta, M. *Synthesis* **2003**, 1039−1042. (g) Grunefeld, P.; Richert, C. *J. Org. Chem.* **2004**, *69*, 7543−7551.

(25) Identified based on comparison of ^{1}H NMR spectrum (300 MHz, DMSO-d_6) with reported values: (a) Ishido, Y.; Nakazaki, N.; Sakairi, N. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2088−2098. (b) Maury, G.; Daiboun, A.; Elalaoui, A.; Genu-Dellac, C.; Perigaud, C.; Bergogne, C.; Gosselin, G.; Imbach, J.-L. *Nucleosides Nucleotides* **1991**, *10*, 1677−1692.

(26) Other reports have documented the selective deprotection of tri- and *tetra*-benzoyl-protected nucleosides to yield 5′-*O*-benzoyl-protected nucleosides using hydrazine hydrate, ammonia, sodium methoxide, or lithium 2,2,2-trifluoroethoxide as the deacylating reagent. See ref 25a and (a) Ferris, J. P.; Devadas, B.; Huang, C.-H.; Ren, W.-Y. *J. Org. Chem.* **1985**, *50*, 747−754. (b) Nishino, S.; Rahman, A.; Takamura, H.; Ishido, Y. *Tetrahedron* **1985**, *41*, 5503−5506. (c) Zerrouki, R.; Roy, V.; Hadj-Bouazza, A.; Krausz, P. *J. Carbohydr. Chem.* **2004**, *23*, 299−303. (d) Nowak, I.; Jones, C. T.; Robins, M. J. *J. Org. Chem.* **2006**, *71*, 3077−3081.

SCHEME 2. High-Throughput Nucleoside Synthesis Method Summary

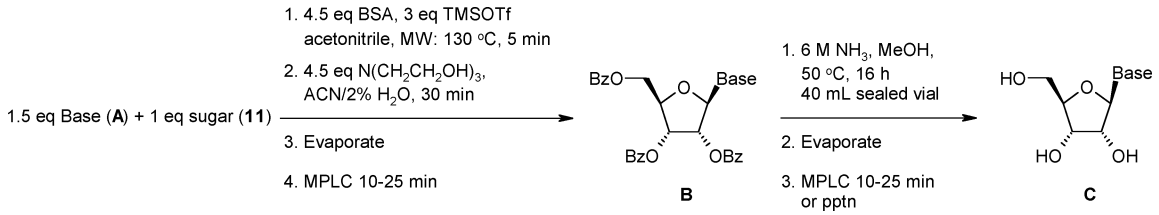

TABLE 1. Examples of High-Throughput Nucleoside Synthesis

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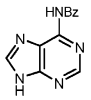
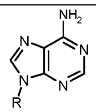
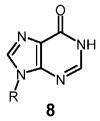
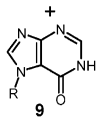
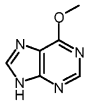
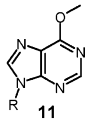
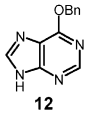
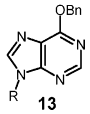
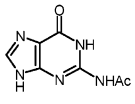
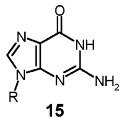
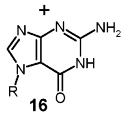
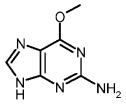
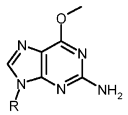
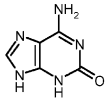
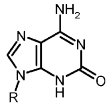
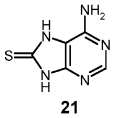
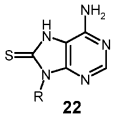
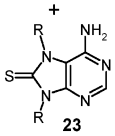
1	 1	 6	TEOA	100%	45%	Y
2	 7	 8 +  9	resin	*e*	36%	Y
				e	5%	Y
3	 10	 11	TEOA	97%	43%	N
4	 12	 13	TEOA[f]	94%	4%	Y
5	 14	 15 +  16	TEOA	*g*	26%[h]	Y
				g	7%[h]	Y
6	 17	 18	TEOA	100%	37%	N
7	 19	 20	TEOA	79%	38%[h]	N
8	 21	 22 +  23	TEOA	96%	19%[h]	N
				91%	3%	N

Table 1 (Continued)

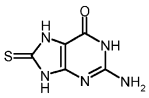
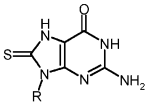
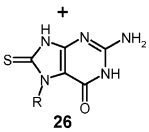
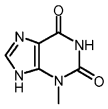
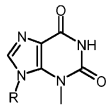
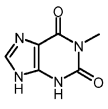
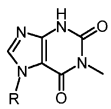
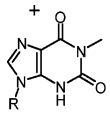
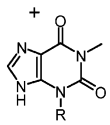
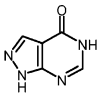
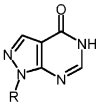
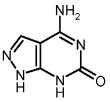
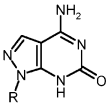
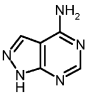
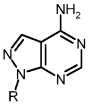
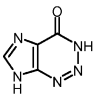
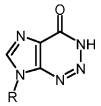
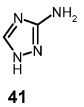
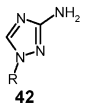
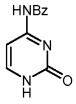
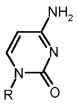
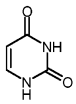
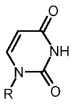
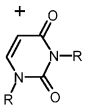
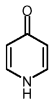
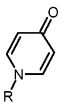
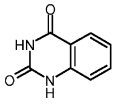
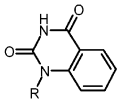
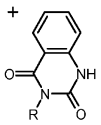
entry	base	products[a]	quench method[b]	HPLC purity	2-step yield[c]	lit. prep by glyc[d]
9	 **24**	 **25**	TEOA	*i*	7%	N
		 **26**		*i*	3%	N
10	 **27**	 **28**	TEOA	98%	10%	N
11	 **29**	 **30**	TEOA	*j*	31%	N
		 **31**		*j*	26%	N
		 **32**		*j*	4%	N
12	 **33**	 **34**	resin	*k*	0%	Y
13	 **35**	 **36**	TEOA	99%	29%	N
14	 **37**	 **38**	TEOA	*k*	0%	Y
15	 **39**	 **40**	TEOA	*k*	0%	N
16	 **41**	 **42**	TEOA	98%	19%	N

Table 1 (Continued)

entry	base	products[a]	quench method[b]	HPLC purity	2-step yield[c]	lit. prep by glyc[d]
17	 **43**	 **44**	TEOA	95%	50%	Y
18	 **45**	 **46**	TEOA	95%	36%	Y
		 **47**		91%	14%	Y
19	 **48**	 **49**	resin	95%	51%	Y
20	 **50**	 **51**	TEOA	*l*	10%	Y
		 **52**		*l*	6%	N

[a] R = β-D-ribofuranos-1-yl. [b] The quench method involves addition of an acid quenching reagent: TEOA = triethanolamine and resin = Amberlite IRA-400 OH^-. [c] Yields were weight based. To determine the yield for a compound represented as part of an isomer mixture, the overall weight based yield for the mixture was divided into individual component yields based on integral analysis of characteristic protons within the ^1H NMR spectrum of the mixture. [d] As indicated, these nucleosides and their ribofuranose and glucopyranose analogues have or have not previously been reported as products of a glycosylation/deprotection sequence conducted with the respective base according to a search of American Chemical Society SciFinder and Beilstein Crossfire databases. See Supporting Information for complete literature. [e] Compounds **8** and **9** were obtained as an inseparable mixture in 88% combined overall purity (two major HPLC peaks summed). [f] No glycosylation product was isolated from resin workup method. [g] Compounds **15** and **16** were obtained as an inseparable mixture in 96% combined overall purity (two major HPLC peaks summed). [h] Compound isolated by collecting precipitate from NH_3/methanol deprotection reaction mixture. [i] Compounds **25** and **26** were obtained as an inseparable mixture in 96% combined overall purity (two major HPLC peaks summed). [j] Compounds **30**−**32** were obtained as an inseparable mixture in 100% combined overall purity (three major HPLC peaks summed). [k] Complex mixture. Products not isolated. [l] Compounds **51** and **52** were obtained as an inseparable mixture in 75% combined overall purity (two major HPLC peaks summed).

benzimidazoles, three imidazoles, three 1,2,4-triazoles, two pyrimidines, two 3-deazapyrimidines, one quinazolinedione, and one alloxazine. Notably, 15 examples (**11**, **18**, **20**, **22**, **23**, **25**, **26**, **28**, **30**−**32**, **36**, **40**, **42**, and **52**) have not been reported as direct products of glycosylation but were previously synthesized from multistep base modification of preformed nucleosides (for background literature, see Supporting Information). Thus, not only does this method provide faster access to these specific nucleosides, but it provides an impetus to check if other novel nucleosides not previously considered as obtainable as direct products of glycosylation might be prepared in this simple manner.

Among bases tested as potential novel Vorbrüggen glycosylation substrates, three purine aza-isomers failed to yield isolable nucleosides (entries 12, 14, and 15 in Table 1). Complex mixtures were produced that were not amenable to purification by the general method, even though mass spectral evidence of product formation (**34**, **38**, and **40**, respectively) was obtained in each case. This is not entirely surprising since one of the bases that failed, **33**, has been documented to produce a mixture of five glycosylation products.[27] Another, **37**, has only been reported to work by a sodium hydride mediated glycosylation.[28] Finally the third base, **39**, does not appear in the literature as a glycosylation substrate.

(27) Lichtenthaler, F. W.; Cuny, E. *Chem. Ber.* **1981**, *114*, 1610−1623.

The products were identified by ^{1}H NMR and HPLC comparison with authentic samples when available and by comparison with literature ^{1}H NMR values otherwise. Purity was determined by HPLC and ^{1}H NMR (see Supporting Information for identification method, chromatograms, and spectra). Of the 48 examples studied, 32 glycosylations yielded a single regioisomer product, and six (one of which is included in Table 1, entry 8; see Supporting Information for others) resulted in separable mixtures of regioisomers. The isolated single products (totaling 45) were obtained in an average two-step overall yield ± SD of 26 ± 16% and an average HPLC purity ± SD of 95 ± 6%.

Mixtures of −two to three regioisomers were obtained from seven examples (five of which are included in Table 1: entries 2, 5, 9, 11, and 20). In the case of entry 20, base **50** was previously glycosylated by a different method at lower temperatures where only the N1 isomer **51** was produced.[29] The N3 isomer **52** was prepared previously by a more lengthy route.[30] Other examples have been previously documented to produce mixtures or are novel glycosylation reactions (see Supporting Information for complete literature).

The purine series was studied the most thoroughly and includes examples with base substitution combinations containing one or more of the following groups at every available position: H, NH_2, NH-alkyl, =O, O-alkyl, SH, S-alkyl, and methyl (see Supporting Information). A few polar purine bases such as 2-*N*-acetylguanine (**14**), isoguanine (**19**), and adenine-8-thiol (**21**) were essentially insoluble in the reaction medium at room temperature but became solubilized as trimethylsilyl derivatives at 130 °C. This indication of a solubility limitation was used as an advantage. The intermediate glycosylation products were soluble and readily purified by MPLC. Upon NH_3/methanol deprotection, the respective product nucleosides (**15**, **16**, **20**, and **22**) precipitated out of the reaction medium, thereby simplifying their isolation.

Conclusion

A general, unified two-step nucleoside synthesis method was developed, which is amenable to the combination of various bases with various sugars for the rapid preparation of structurally diverse nucleoside libraries.[31] Key features of the method are the following: (1) a one-step 5 min/130 °C microwave Vorbrüggen glycosylation reaction; (2) single-phase quenching of TfOH with triethanolamine; and (3) 10−25 min generic, normal phase silica gel MPLC purification protocols. The method has been demonstrated with the preparation of a diverse nucleoside library that contained 15 examples not previously obtained directly from a glycosylation/deprotection sequence on a commercially available base. Thus, this method provides rapid access to a collection of nucleosides that would have required considerably more effort were it constructed based only on previously published methods.

Experimental Procedures

***N*^{6}-Benzoyladenosine 2′,3′,5′-Tri-*O*-benzoate (5a). General Method for Microwave Mediated Vorbrüggen Glycosylation**. To a 5 mL microwave reaction vial with a stir bar was added 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**3**) (150 mg, 0.30 mmol), *N*^{6}-benzoyladenine (**1**) (107 mg, 0.45 mmol), 3 mL of acetonitrile, *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (0.328 mL, 1.34 mmol), and trimethylsilyl triflate (TMSOTf) (0.161 mL, 0.90 mmol). The vial was sealed with a mechanically crimped septum cap and heated in a Biotage microwave reactor at 130 °C for 5 min. The contents were transferred to a 40 mL glass vial and rinsed with 2 mL of acetonitrile. To this was added 1.33 mL of a 1 M solution of triethanolamine (1.33 mmol) in acetonitrile with 2% water, and the vial was shaken at room temperature for 30 min. The volatiles were removed by evaporation, and the residue was dissolved in 4 mL of 1:1 MeOH/CH_2Cl_2 and adsorbed onto 3 g of silica gel by evaporation. This was transferred to a plastic precolumn and subjected to automated MPLC through a 12 g column of silica gel eluting at 30 mL/min with a gradient of 0−15% MeOH/CH_2Cl_2 over 30 min to provide 123 mg (61%) of **5a** as an amorphous solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.68 (dd, 1H, J = 12, 5 Hz), 4.82 (dd, 1H, J = 12, 4 Hz), 4.89 (q, 1H, J = 5 Hz), 6.30 (t, 1H, J = 6 Hz), 6.55 (dd, 1H, J = 6, 5 Hz), 6.69 (d, 1H, J = 5 Hz), 7.4−7.7 (m, 12H), 7.9−8.1 (m, 8H), 8.62 (s, 1H), 8.73 (s, 1H), 11.26 (s, 1H); HPLC purity (254 nm) = 94%.

Alternatively, instead of quenching the acid with triethanolamine, the crude reaction mixture was added to 1.5 g of Amberlite IRA-400 (OH^-) suspended in 6 mL of CH_2Cl_2, and the mixture was shaken for 30 min, filtered, and evaporated. The residue was then subjected to MPLC as described previously.

Adenosine (6). General Method for NH_3/Methanol Deprotection. The protected nucleoside **5a** (117 mg, 0.171mmol) was transferred to a 40 mL vial and dissolved in 2 mL of 6 M NH_3 in methanol. This vial was sealed with a screw cap and shaken at 50 °C for 16 h. The solvent was evaporated, and the residue was dissolved in 1:1 MeOH/CH_2Cl_2 and adsorbed onto 1 g of silica gel by evaporation. This was transferred to a plastic precolumn and subjected to automated MPLC through a 12 g column of silica gel eluting at 30 mL/min with a gradient of 0−20% MeOH/CH_2Cl_2 over 30 min to provide 34 mg (74%, 45% overall for two steps) of adenosine (**6**) as a solid that had HPLC R_t (purity at 254 nm = 100%) and ^{1}H NMR values identical to an authentic sample.

Compounds **15**, **16**, **20**, and **22** were isolated by filtration from the crude reaction mixture as precipitated solids.

Acknowledgment. This work was supported in part by NIH SBIR Grant AI050278.

Supporting Information Available: Extended table of examples including those presented here, with a method of product identification and related synthesis literature and ^{1}H NMR, HPLC, and MS data for all deprotected nucleosides. This material is available free of charge via the Internet at http://pubs.acs.org.

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(28) Eldrup, A. B.; Prhavc, M.; Brooks, J.; Bhat, B.; Prakash, T. P.; Song, Q.; Bera, S.; Bhat, N.; Dande, P.; Cook, P. D.; Bennett, C. F.; Carroll, S. S.; Ball, R. G.; Bosserman, M.; Burlein, C.; Colwell, L. F.; Fay, J. F.; Flores, O. A.; Getty, K.; LaFemina, R. L.; Leone, J.; MacCoss, M.; McMasters, D. R.; Tomassini, J. E.; Von Langen, D.; Wolanski, B.; Olsen, D. B. *J. Med. Chem.* **2004**, *47*, 5284−5297.

(29) (a) Stout, M. G.; Robins, R. K. *J. Org. Chem.* **1968**, *33*, 1219−1225. (b) Schweizer, M. P.; Banta, E. B.; Witkowski, J. T.; Robins, R. K. *J. Am. Chem. Soc.* **1973**, *95*, 3770−3778. (c) Dunkel, M.; Pfleiderer, W. *Nucleosides Nucleotides* **1992**, *11*, 787−819. (d) Dunkel, M.; Pfleiderer, W. *Nucleosides Nucleotides* **1993**, *12*, 125−137. (e) Bhattacharya, B. K.; Chari, M. V.; Durland, R. H.; Revankar, G. R. *Nucleosides Nucleotides* **1995**, *14*, 45−63.

(30) Girniene, J.; Apremont, G.; Tatibouet, A.; Sackus, A.; Rollin, P. *Tetrahedron* **2004**, *60*, 2609−2619.

(31) This process, known as *Nucapalooza* in our labs, has been applied successfully to the production of more than 400 nucleosides from the reaction of six different acylated ribofuranosyl derivatives with a set of bases which included those listed in Table 1 and the Supporting Information Table S1.