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Efficient microwave-assisted solid phase coupling of nucleosides, small library generation and mild conditions for release of nucleoside derivatives

Hanumantharao Paritala^a, Yuta Suzuki^b, and Kate S. Carroll^{a,*}

^aDepartment of Chemistry, The Scripps Research Institute, Jupiter, Florida, 33458, USA

^bDepartment of Chemistry, University of Michigan, Ann Arbor, Michigan, 48109, USA

Abstract

Nucleosides are essential bio-molecules that participate in a wide array of biological processes involved in maintaining physiologic homeostasis. Recent efforts geared towards the synthesis of nucleoside analogues and development of nucleoside combinatorial libraries using solid phase synthesis has contributed invaluable information towards drug design and development. These studies have provided information concerning the structural requirements of substrate binding pockets of enzymes and evaluation of enzyme kinetics. However, the synthesis of nucleosides and its corresponding analogues remains a challenging and time consuming process. Herein, we report an efficient, microwave assisted solid phase coupling of nucleosides, combinatorial chemistry on the coupled nucleosides to generate small library and mild cleavage conditions to release nucleoside derivatives from its solid support. We anticipate these findings will accelerate the development of synthetic methods or combinatorial library design of nucleoside analogues in similar settings.

Keywords

solid phase synthesis; microwave assisted; nucleoside; combinatorial library

Introduction

Nucleosides and its derivatives are important components and hold key roles in numerous biological processes and signal transduction. These bio-molecules are utilized as genomic building blocks, enzyme substrates, and cellular energy sources¹. Additionally, nucleoside analogues have been successfully utilized in anti-cancer, -bacterial, and -viral therapeutics². In this context, the synthesis and testing of bio-mimetic nucleoside derivatives has emerged as a widely used approach in drug discovery campaigns. The design of nucleoside analogues and combinatorial libraries using solid-phase based techniques has garnered large momentum due to the importance of their therapeutic applications³. However, the synthesis of nucleosides remains a challenge due to long reaction times, harsh cleavage conditions, and poor product yields⁴. Redwan *et al.* recently reported a novel solid-phase strategy that

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*Corresponding author: Address: Department of Chemistry, The Scripps Research Institute, 130 Scripps Way, Jupiter, Florida, 33458, USA, kcarroll@scripps.edu, Phone: (561) 228-2460, Fax: 561-228-2919.

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enables the synthesis of structurally different 5-*O*-[*N*-(acyl)-sulfamoyl] adenosines from a protected ribo-purine starting material. This approach applied cleavage conditions using trifluoroacetic acid (TFA) and ammonium formate, which resulted in poor yields of the nucleoside derivatives⁵. In a separate study, Caroline *et al.* reported the synthesis of solid phase poly-phosphorylated nucleoside analogues with decent yields and mild cleaving conditions, but extremely slow reaction times⁶. Therefore, we have explored microwave assisted solid phase nucleoside synthesis in order to optimize reaction conditions and to create a platform to perform combinatorial chemistry on solid phase nucleosides. Herein, we describe an efficient microwave assisted solid phase coupling of nucleosides, small chemical library generation on the resin bound nucleosides and the mild cleavage conditions to release the nucleoside derivatives from its resin support.

Results and Discussion

For the synthesis of the adenosine scaffold, we initially used an existing protocol developed for generating inosine and uridine analogs^{3a}. This procedure required purification after formation of the benzylidene intermediate **4**, gave low yields of the scaffold (10 – 25 %), and was insufficient for further solid-phase synthesis of adenosine analogs. Collectively, this synthetic procedure was time consuming and required 8–10 days to achieve the final product. Optimization of the reported literature conditions would result in a facile synthesis of nucleoside derivatives with improved yield and decreased reaction times. The following key factors were included during the development of our approach: 1) employ microwave conditions to enhance reaction rates, and 2) utilize mild reaction conditions (Scheme 1). Generation of the poly styrene bound nucleoside scaffold started with synthesis of Aldehyde **2** as previously reported^{3a}, and activated by the formation of dimethylacetal **3**. Compound **3** was immediately converted to benzylidene **4** *via* transacetylation to prevent reversion back to compound **2**. The acetal forming reaction produced a mixture of diastereomers due to non-selective formation of the acetal carbon⁷. Compound **4** was hydrolyzed to the carboxylic acid **5** with 48 % overall yield in four steps. Finally, compound **5** was coupled to aminomethyl resin using O-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluroniumtetrafluoroborate (HBTU) activation to obtain the corresponding solid phase nucleoside scaffolds **6**. When compared to solution chemistry, MW irradiation provides an overall higher yield and shorter reaction times.

Using the resin bound nucleosides **6** as platform we started exploring possible chemistry on the aromatic amine and 5'-hydroxyl functionalities to construct four member library of resin bound nucleosides. In order to achieve this 5'-hydroxyl group of the resin bound nucleosides **6** was protected by using *tert*-butyldimethylsilyl chloride and imidazole with quantitative yields and this protection allowed us to selectively explore the alkylation reactions on the aromatic amine of **7**. We have tested variety of conditions to install propargyl group on the aromatic amine as shown in Table 1. The ideal conditions to conduct this reaction using microwave is achieved by using two equivalents of propargylbromide and two equivalents of *N,N*-diisopropylethylamine at 150°C with reasonable yields. Using the alkyne functional group on **8** we have conducted click reaction with benzyl azide and methyl 2-azidoacetate to create a four member library of solid phase nucleoside derivatives **9a**, **10a**, **9b**, **10b**, as shown in scheme 2 and scheme 3. This chemistry demonstrated the facile synthesis of solid phase nucleoside derivatives using microwave conditions.

Our next objective was to determine the mildest conditions required to cleave the polystyrene bound adenosine moiety (Table 2). We envisioned the development of cleavage conditions that would efficiently release the resin-bound nucleoside, but be able to tolerate the stability of nucleoside derivatives. This approach would contribute to the development of combinatorial libraries composed of nucleoside analogues with diverse structural motifs. We

tested various cleavage conditions against resin **6a** and **7a** to yield adenosine as shown in Table 2. The product yield of each reaction condition was determined by NMR using alanine as an internal standard, which was chosen based on its solubility and stability⁸. HPLC was used to analyze the integrity of the nucleoside after cleavage since adenosine can undergo decomposition to adenine and ribose sugar^{3a}. In order to identify optimal cleavage conditions, we screened various conditions beginning with TFA as shown in Table 2. TFA is commonly used as a reagent to cleave the ribose acetal linker, and was found to efficiently cleave the nucleoside in 15 minutes with 80% TFA. Extending the reaction time resulted in cleavage of the glycosidic bond and formation of free adenine and ribose moieties. Mild acidic conditions (5% TFA, 24 h) were tested in hopes of improving adenosine cleavage while maintaining the glycosidic bond. These conditions reduced product yield and did not prevent adenosine decomposition. Oxidative cleavage conditions (*e.g.* 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)⁹, 3 equiv.) afforded 41% yield of nucleoside coupled with the formation of adenine. Furthermore, catalytic cleavage of the acetal linker with indium trifluoromethanesulfonate (In(OTf)₃)¹⁰ did not prove as efficient as acidic or oxidative conditions, but preserved the glycoside bond. Finally, nucleoside release from the resin was tested using acetic acid to cleave the acetal bond. Acetic acid (10% v/v) in water cleaved the nucleoside with simultaneous 5'-TBDMS deprotection with 89 % yield without any detectable decomposition.

In conclusion, we have developed a microwave-assisted synthetic route for coupling of nucleosides to aminomethylated polystyrene resin and showed that using microwave conditions generations of four member solid phase nucleoside library coupled with mild cleavage conditions. This procedure offers decent product yields, shorter reaction times, and bypasses lengthy purification steps in comparison to previous reports. We anticipate that the present synthetic scheme should increase the overall efficiency of ribose-based combinatorial nucleoside libraries and is amendable to the synthesis of other derivatives.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Berg, JM.; Tymoczko, JL.; Stryer, L. Biochemistry. W.H. Freeman; Basingstoke: 2012.
2. (a) Damaraju VL, Damaraju S, Young JD, Baldwin SA, Mackey J, Sawyer MB, Cass CE. *Oncogene*. 2003; 22(47):7524–7536. [PubMed: 14576856] (b) De Clercq E. *Journal of Clinical Virology*. 2004; 30(2):115–133. [PubMed: 15125867] (c) Winn M, Goss RJM, Kimura K-i, Bugg TDH. *Natural Product Reports*. 2010; 27(2):279–304. [PubMed: 20111805]
3. (a) Epple R, Kudirka R, Greenberg WA. *J Comb Chem*. 2003; 5(3):292–310. [PubMed: 12739947] (b) de Champdore M, De Napoli L, Di Fabio G, Messere A, Montesarchio D, Piccialli G. *Chemical Communications*. 2001; (24):2598–2599. (c) Crimmins MT, Zuercher WJ. *Org Lett*. 2000; 2(8): 1065–1067. [PubMed: 10804555] (d) Bozzoli A, Kazmierski W, Kennedy G, Pasquarello A, Pecunioso A. *Bioorg Med Chem Lett*. 2000; 10(24):2759–2763. [PubMed: 11133085]
4. (a) Aucagne V, Berteina-Raboin S, Guenot P, Agrofoglio LA. *J Comb Chem*. 2004; 6(5):717–723. [PubMed: 15360206] (b) Ramasamy KS, Amador RB, Habib Q, Rong F, Han X, Li DY, Huang J, Hong Z, An H. *Nucleosides, Nucleotides and Nucleic Acids*. 2005; 24(10–12):1947–1970. (c) Rodenko B, Detz RJ, Pinas VA, Lambertucci C, Brun R, Wanner MJ, Koomen GJ. *Bioorganic & Medicinal Chemistry*. 2006; 14(5):1618–1629. [PubMed: 16249090] (d) Oliviero G, Amato J, D'Errico S, Borbone N, Piccialli G, Mayol L. *Nucleosides, Nucleotides and Nucleic Acids*. 2007;

- 26(10–12):1649–1652.(e) Poon KWC, Liang N, Datta A. Nucleosides, Nucleotides and Nucleic Acids. 2008; 27(4):389–407.
5. Redwan IN, Ingemyr HJ, Ljungdahl T, Lawson CP, Grøtli M. European Journal of Organic Chemistry. 2012; 2012(19):3665–3669.
6. Tonn VC, Meier C. Chemistry – A European Journal. 2011; 17(35):9832–9842.
7. (a) Douglass JG, Patel RI, Yerxa BR, Shaver SR, Watson PS, Bednarski K, Plourde R, Redick CC, Brubaker K, Jones AC, Boyer JL. Journal of Medicinal Chemistry. 2008; 51(4):1007–1025. [PubMed: 18232657] (b) Bakthavachalam V, Lin LG, Cherian XM, Czarnik AW. Carbohydrate Research. 1987; 170(1):124–135. [PubMed: 3435902]
8. Hamper BC, Kolodziej SA, Scates AM, Smith RG, Cortez E. The Journal of Organic Chemistry. 1998; 63(3):708–718. [PubMed: 11672064]
9. (a) McDonald CE, Dugger RW. Tetrahedron Lett. 1988; 29(20):2413–2415.(b) Oikaw Y, Yoshioka T, Yonemitsu O. Tetrahedron Lett. 1982; 23(8):889–892.
10. Gregg BT, Golden KC, Quinn JF. The Journal of Organic Chemistry. 2007; 72(15):5890–5893. [PubMed: 17595139]

Scheme 1. Synthesis of solid-supported nucleoside derivatives

Conditions: (i) Ethyl 6-bromohexanoate, K₂CO₃, DMF, microwave 150°C (ii)

Trimethylorthoformate, *p*-toluenesulfonic acid, MeOH, microwave 60°C (iii) adenosine, *p*-toluenesulfonic acid, dimethylformamide, microwave 130°C (iv) NaOH, MeOH, H₂O, microwave 100°C (v) aminomethylated polystyrene HL, *O*-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU), *N,N*-Diisopropylethylamine, DMF, microwave 60°C (vi) *tert*-butyldimethylsilyl chloride, imidazole, microwave 40°C (vii) cytidine, *p*-toluenesulfonic acid, dimethylformamide, microwave 130°C.

Scheme 2. Click chemistry on the solid-supported adenosine derivative and simultaneous 5'-TBDMS deprotection and release of nucleoside from solid phase

Conditions: (i) Propargylbromide, dimethylformamide, *N,N*-Diisopropylethylamine, microwave 150°C (ii) Benzylazide, CuI, *N,N*-Diisopropylethylamine, microwave 100°C (iii) Methyl 2-azidoacetate, CuI, *N,N*-Diisopropylethylamine, microwave 100°C (iv) Acetic acid (10% v/v) in water, rt.

Scheme 3. Click chemistry on the solid-supported cytidine derivative and simultaneous 5'-TBDMS deprotection and release of nucleoside from solid phase

Conditions: (i) Propargylbromide, dimethylformamide, *N,N*-Diisopropylethylamine, microwave 150°C (ii) Benzylazide, CuI, *N,N*-Diisopropylethylamine, microwave 100°C (iii) Methyl 2-azidoacetate, CuI, *N,N*-Diisopropylethylamine, microwave 100°C (iv) Acetic acid (10% v/v) in water, rt.

Table 1

Optimization of reaction conditions for alkylation of aromatic amine on the nucleosides:

S. No	Microwave temperature (°C)	Time (min)	Equivalents of propargylbromide	Equivalents of base (DIEA) ²	% Yield ¹
1	80	10	1	1	0
2	100	15	1	1	0
3	150	10	1	1	45
4	150	15	2	2	90

¹ % yield was determined by NMR using alanine as an internal standard.

² N,N-Diisopropylethylamine

Table 2

Cleavage conditions to cleave adenosine from solid phase adenosine scaffold

Type	Condition	Yield (%) ¹	% of adenosine 7 ²
Acidic	TFA 80%, 15 min, RT ⁴	91	88
Acidic	TFA 80%, 30 min, RT ⁴	67	80
Acidic	TFA 5%, 30 min, RT ⁴	24	90
Oxidation	DDQ 1.5 eq, 3 h, RT ⁴	11	78
Oxidation	DDQ 3 eq, 3 h, RT ⁴	14	77
Oxidation	DDQ 3 eq, 24 h, RT	41	93
Catalyst	8 mol% In(OTf) ₃ , 6 min, 100°C (MW assisted) ⁴	2	99
Catalyst	8 mol% In(OTf) ₃ , 30 min, 100°C (MW assisted) ⁴	6	99
Catalyst	8 mol% In(OTf) ₃ , 30 min, 130°C (MW assisted) ⁴	NA ³	-
Catalyst	8 mol% In(OTf) ₃ , 30 min, 150°C (MW assisted) ⁴	NA ³	-
Catalyst	40 mol% In(OTf) ₃ , 30 min, 100°C (MW assisted) ⁴	15	91
Catalyst	8 mol% In(OTf) ₃ , 1 hr, 50 °C (MW assisted) ⁴	6	99
Catalyst	8 mol% In(OTf) ₃ , 24hr, RT ⁴	5	99
Catalyst	40 mol% In(OTf) ₃ , 24hr, RT ⁴	22	98
Acidic	Acetic acid : TBAF (1:1), DMSO : water, rt, 2 hours ⁵	88	82
Acidic	Acetic acid 10% v/v, 2 hours, RT ⁵	89	99

¹ % yield was determined by NMR using alanine as an internal standard.

² % of adenosine is the ratio between adenosine and adenine (decomposed product from adenosine).

³ NA: adenosine was not observed.

⁴ R = OH

⁵ R = TBDMS