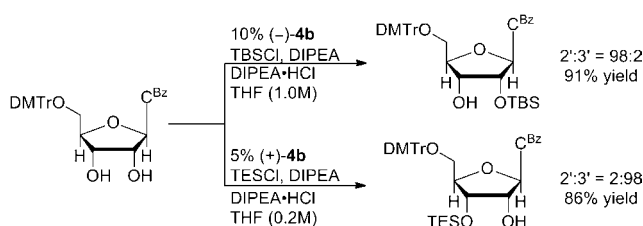# Practical Silyl Protection of Ribonucleosides

Thomas P. Blaisdell,[†] Sunggi Lee,[†] Pinar Kasaplar,[‡] Xixi Sun,[†] and Kian L. Tan*,[†]

Institute of Chemical Research of Catalonia, Av. Paisos Catalans 16, 43007 Tarragona, Spain, and Boston College, 2609 Beacon Street, Chestnut Hill, Massachusetts 02467-3860, United States

kian.tan@novartis.com

ABSTRACT

Herein we report the site-selective silylation of the ribonucelosides. The method enables a simple and efficient procedure for accessing suitably protected monomers for automated RNA synthesis. Switching to the opposite enantiomer of the catalyst allows for the selective silylation of the 3′-hydroxyl, which could be used in the synthesis of unnatural RNA or for the analoging of ribonucleosides. Lastly, the procedure was extended to ribavirin a potent antiviral therapeutic.

Site-selective catalysis[1] is the ability to differentiate a functional group, when multiple similar groups are present in the molecule. Over the past several years site-selective catalysis has emerged as a powerful and efficient means of derivatizing and analoging natural products.[2,3] To achieve site selectivity in the absence of a catalyst, synthetic chemists rely on either a significant bias in innate reactivity within the substrate (e.g., functionalizing a primary over a tertiary hydroxyl) or protecting group strategies, which mask other potential reactive sites. Beyond late stage functionalization, site-selective catalysis can also be employed in more traditional synthetic streamlining; in particular, it has been employed in the selective functionalization of monosaccharides.[4,5]

A significant challenge for site-selective catalysis is the manipulation of sites that are less or similarly reactive to other positions within the molecule. To this end, we have been pursuing catalysts that react with specific functional group motifs within complex molecules. To achieve this goal we have synthesized catalysts that have a catalytic residue appended to a substrate-binding site (Figure 1). The substrate-binding event occurs through a reversible covalent bond,[6] minimizing the molecular interactions necessary for substrate localization. Through this design,

[†] Boston College.

[‡] Institute of Chemical Research of Catalonia.

(1) Mahatthananchai, J.; Dumas, A. M.; Bode, J. W. *Angew. Chem., Int. Ed.* **2012**, *51*, 10954–10990.

(2) (a) Lewis, C. A.; Miller, S. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 5616–5619. (b) Chen, M. S.; White, M. C. *Science* **2007**, *318*, 783–787. (c) Lewis, C. A.; Longcore, K. E.; Miller, S. J.; Wender, P. A. *J. Nat. Prod.* **2009**, *72*, 1864–1869. (d) Yoshida, K.; Furuta, T.; Kawabata, T. *Tetrahedron Lett.* **2010**, *51*, 4830–4832. (e) Fowler, B. S.; Laemmerhold, K. M.; Miller, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 9755–9761. (f) Pathak, T. P.; Miller, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 6120–6123. (g) Bruckl, T.; Baxter, R. D.; Ishihara, Y.; Baran, P. S. *Acc. Chem. Res.* **2012**, *45*, 826–839. (h) Beale, T. M.; Taylor, M. S. *Org. Lett.* **2013**, *15*, 1358–1361. (i) Pathak, T. P.; Miller, S. J. *J. Am. Chem. Soc.* **2013**, *135*, 8415–8422.

(3) For examples of directed and reagent controlled functionalization of natural products, see: (a) Breslow, R.; Baldwin, S.; Flechtner, T.; Kalicky, P.; Liu, S.; Washburn, W. *J. Am. Chem. Soc.* **1973**, *95*, 3251–3262. (b) Breslow, R.; Corcoran, R. J.; Snider, B. B.; Doll, R. J.; Khanna, P. L.; Kaleya, R. *J. Am. Chem. Soc.* **1977**, *99*, 905–915. (c) Breslow, R.; Heyer, D. *J. Am. Chem. Soc.* **1982**, *104*, 2045–2046. (d) Wilcock, B. C.; Uno, B. E.; Bromann, G. L.; Clark, M. J.; Anderson, T. M.; Burke, M. D. *Nat. Chem.* **2012**, *4*, 996–1003.

(4) For a review on sugar functionalization, see: Lee, D.; Taylor, M. S. *Synthesis* **2012**, *44*, 3421–3431.

(5) (a) Griswold, K. S.; Miller, S. J. *Tetrahedron* **2003**, *59*, 8869–8875. (b) Kawabata, T.; Muramatsu, W.; Nishio, T.; Shibata, T.; Schedel, H. *J. Am. Chem. Soc.* **2007**, *129*, 12890–12895. (c) Kawabata, T.; Furuta, T. *Chem. Lett.* **2009**, *38*, 640–647. (d) Chan, L.; Taylor, M. S. *Org. Lett.* **2011**, *13*, 3090–3093. (e) Gouliaras, C.; Lee, D.; Chan, L.; Taylor, M. S. *J. Am. Chem. Soc.* **2011**, *133*, 13926–13929. (f) Lee, D.; Taylor, M. S. *J. Am. Chem. Soc.* **2011**, *133*, 3724–3727. (g) Lee, D.; Williamson, C. L.; Chan, L.; Taylor, M. S. *J. Am. Chem. Soc.* **2012**, *134*, 8260–8267.

(6) (a) Park, Y. J.; Park, J. W.; Jun, C. H. *Acc. Chem. Res.* **2008**, *41*, 222–234. (b) Rousseau, G.; Breit, B. *Angew. Chem., Int. Ed.* **2011**, *50*, 2450–2494. (c) Tan, K. L. *ACS Catal.* **2011**, *1*, 877–886.

selectivity and rate acceleration are achieved through proximity effects.[7] This is an ideal mechanism for site-selective catalysis, because acceleration is directly linked to the structural arrangement of the functional groups. Because proximity effects can result in orders of magnitude of rate acceleration, differentiation of groups that have similar reactivity and even the functionalization of inherently less reactive sites is possible.[8]
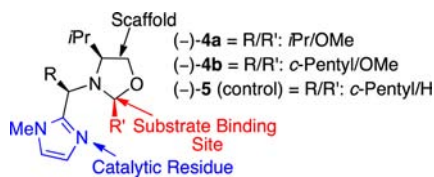

Figure 1. Design of scaffolding catalysts.

Most recently we applied scaffolding catalysts to the derivatization of monosaccharides and natural products.[9] In this manuscript we apply the catalyst to the selective functionalization of ribonucleosides.[10] Ribonucleosides are the core building blocks for RNA and also serve as templates for the development of therapeutics, in particular antiviral agents.[11] Beyond RNA's traditional role in protein expression (i.e., tRNA and mRNA), RNA has been found to be critical in gene regulation through riboswitches[12] and RNAi.[13] These discoveries have led to an explosion in the use of synthetic RNA as potential therapeutics[14] as well as tools for the biological sciences. Although the expectation would be that RNA and DNA synthesis would be similar, the additional 2′-hydroxyl adds a layer of complexity to the synthesis of RNA and derivatives, because this hydroxyl has to be differentiated from the 3′-position. Current automated methods for RNA synthesis require that the 2′-hydroxyl be protected. One of the most popular protecting groups used with the RNA monomers is a tert-butyldimethylsilyl (TBS) group due to its chemical orthogonality. The current synthesis of these

(7) (a) Page, M. I.; Jencks, W. P. Proc. Natl. Acad. Sci. U.S.A. **1971**, 68, 1678–1683. (b) Pascal, R. Eur. J. Org. Chem. **2003**, 1813–1824.

(8) Worthy, A. D.; Sun, X.; Tan, K. L. J. Am. Chem. Soc. **2012**, 134, 7321–7324.

(9) Sun, X.; Lee, H.; Lee, S.; Tan, K. L. Nat. Chem. **2013**, 5, 790–795.

(10) (a) Somoza, A. Chem. Soc. Rev. **2008**, 37, 2668–2675. (b) Zewge, D.; Gosselin, F.; Sidler, R.; DiMichele, L.; Cvetovich, R. J. J. Org. Chem. **2010**, 75, 5305–5307.

(11) (a) Butler, M. S. Nat. Prod. Rep. **2008**, 25, 475–516. (b) Leyssen, P.; De Clercq, E.; Neyts, J. Antivir. Res. **2008**, 78, 9–25.

(12) Serganov, A.; Nudler, E. Cell **2013**, 152, 17–24.

(13) Kurreck, J. Angew. Chem., Int. Ed. **2009**, 48, 1378–1398.

(14) (a) Kim, D. H.; Rossi, J. J. Nat. Rev. Genet. **2007**, 8, 173–184. (b) Keefe, A. D.; Pai, S.; Ellington, A. Nat. Rev. Drug Discovery **2010**, 9, 537–550. (c) Davidson, B. L.; McCray, P. B. Nat. Rev. Genet. **2011**, 12, 329–340.

(15) (a) Hakimelahi, G. H.; Proba, Z. A.; Ogilvie, K. K. Tetrahedron Lett. **1981**, 22, 4775–4778. (b) Hakimelahi, G. H.; Proba, Z. A.; Ogilvie, K. K. Tetrahedron Lett. **1981**, 22, 5243–5246. (c) Hakimelahi, G. H.; Proba, Z. A.; Ogilvie, K. K. Can. J. Chem. **1982**, 60, 1106–1113. (d) MatulicAdamic, J.; Beigelman, L.; Portmann, S.; Egli, R.; Usman, N. J. Org. Chem. **1996**, 61, 3909–3911. (e) Song, Q.; Wang, W.; Fischer, A.; Zhang, X.; Gaffney, B. L.; Jones, R. A. Tetrahedron Lett. **1999**, 40, 4153–4156.

Table 1. Optimization of D-Uridine Silylation[a]

entry	catalyst	equiv TBSCl/DIPEA	2a:3a[d]	conversion (%)[c]
1	20% NMI	1.5/2.0	71:29	100
2	20% (−)-**4a**	1.5/2.0	98:2	70
3	20% (−)-**4b**	1.5/2.0	98:2	72
4	20% (−)-**5**	1.5/2.0	45:55	45
5[b]	20% (−)-**4b**	2.0/1.5	98:2	100
6[b]	10% (−)-**4b**	2.0/1.5	98:2	100 (93)[e]
7[b]	5% (−)-**4b**	2.0/1.5	98:2	88 (84)[e]

[a] Reactions performed at 0.2 M of **1a** with 3 mol % DIPEA−HCl. [b] Reactions run at 1.0 M of **1a**. [c] Conversion determined by ^{1}H NMR using trimethoxybenzene as an internal standard. [d] Ratio was determined by ^{1}H NMR. [e] Isolated yield.

monomers requires either an unselective silylation of the 2′ and 3′ positions followed by separation of the isomers[15] or a multistep protecting group sequence.[16] Given the increasing importance of RNA synthesis, we have devised a simple one-step procedure that provides the desired monomers in high yield and selectivity for all the natural ribonucleosides. Moreover, switching to the opposite enantiomer of the catalyst enables the selective protection of the 3′-hydroxyl, which can be potentially used in the synthesis of unnatural ribonucleosides.

We initially investigated site-selective functionalization of uridine using TBSCl as the electrophile.[17] As a control reaction, we employed N-methylimidazole (NMI) as the catalyst, and similar to previous reports[15c] the product forms in a 71:29 ratio of the silylated C2′−OH:C3′−OH (**2a:3a**, Table 1 entry 1). Using scaffolding catalyst (−)-**4a** or (−)-**4b**[18] results in a dramatic improvement in selectivity to 98:2 with more moderate conversion (∼70%, Table 1, entries 2 and 3). To test for the necessity of covalent bonding to (−)-**4b**, an additional control reaction was

(16) Erlacher, M. D.; Lang, K.; Wotzel, B.; Rieder, R.; Micura, R.; Polacek, N. J. Am. Chem. Soc. **2006**, 128, 4453–4459.

(17) For examples of catalyzed silyl transfer, see: (a) Isobe, T.; Fukuda, K.; Araki, Y.; Ishikawa, T. Chem. Commun. **2001**, 243–244. (b) Rendler, S.; Auer, G.; Oestreich, M. Angew. Chem., Int. Ed. **2005**, 44, 7620–7624. (c) Zhao, Y.; Rodrigo, J.; Hoveyda, A. H.; Snapper, M. L. Nature **2006**, 443, 67–70. (d) Zhao, Y.; Mitra, A. W.; Hoveyda, A. H.; Snapper, M. L. Angew. Chem., Int. Ed. **2007**, 46, 8471–8474. (e) You, Z.; Hoveyda, A. H.; Snapper, M. L. Angew. Chem., Int. Ed. **2009**, 48, 547–550. (f) Weickgenannt, A.; Mewald, M.; Muesmann, T. W. T.; Oestreich, M. Angew. Chem., Int. Ed. **2010**, 49, 2223–2226. (g) Weickgenannt, A.; Mewald, M.; Oestreich, M. Org. Biomol. Chem. **2010**, 8, 1497–1504. (h) Rodrigo, J. M.; Zhao, Y.; Hoveyda, A. H.; Snapper, M. L. Org. Lett. **2011**, 13, 3778–3781. (i) Sheppard, C. I.; Taylor, J. L.; Wiskur, S. L. Org. Lett. **2011**, 13, 3794–3797. (j) Sun, X.; Worthy, A. D.; Tan, K. L. Angew. Chem., Int. Ed. **2011**, 50, 8167–8171. (k) Giustra, Z. X.; Tan, K. L. Chem. Commun. **2013**, 49, 4370–4372.

(18) Catalysts **4a** and **4b** are available at Strem Chemicals.

Table 2. Silylation of Natural Ribonucleosides

entry	nucleobase	2:3[d]	yield (%)[e]	time
1[a]	A[Bz]	95:5	82	24 h
2[b]	C[Bz]	98:2	91	24 h
3[c]	G[Ib]	97:3	75	48 h

[a] Reaction conditions: 0.5 M concentration in substrate. [b] Reaction conditions: 1.0 M concentration in substrate. [c] Reaction conditions: 20 mol % (−)-**4b**, 0.5 M concentration in substrate. [d] Ratio was determined by ^{1}H NMR. [e] Isolated yield

Table 3. Protection of the C3-Hydroxyl of Ribonucleosides

nucleobase	entry	catalyst	5:6[a]	yield (%)[b]
U (**1a**)	1	(+)-**4a**	7:93	80
	2	(−)-**4b**	>98:<2	86
A[Bz](**1b**)	3	(+)-**4a**	4:96	85
	4	(−)-**4b**	98:2	88
C[Bz](**1c**)	5	(+)-**4b**	2:98	86
	6	(−)-**4b**	98:2	86
G[Ib](**1d**)	7[c]	(+)-**4a**	14:86	78
	8[d]	(−)-**4b**	92:8	71

[a] Ratio was determined by ^{1}H NMR. [b] Isolated yield. [c] 20 mol % (+)-**4a**. [d] 10 mol % (−)-**4b**.

Figure 2. Functionalization of ribavirin.

performed with (−)-**5**, which lacks a substrate binding site; both the reactivity and selectivity (**2a**:**3a** = 45:55, Table 1, entry 4) decreased as expected. By both increasing the number of equivalents of TBSCl and the concentration of the reaction (1.0 M in substrate), complete conversion was achieved without loss of selectivity (Table 1, entry 5). Moreover, the catalyst loading could be lowered to 10% **4b** affording an isolated yield of 93% and a site selectivity of 98:2 (**2a**:**3a**, Table 1, entry 6). Lowering the catalyst loading to 5% results in a modest decrease in yield (84%) with no change in selectivity (98:2, Table 1, entry 7).
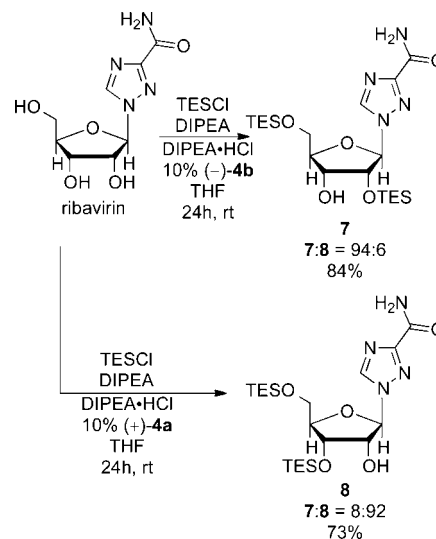
With the optimal conditions in hand, we surveyed the remaining naturally occurring ribonucleosides. To our delight all the ribonucleosides yielded the desired TBS protected product in excellent selectivity and yield (Table 2). The most problematic substrate was guanosine which required an increase in the catalyst loading (20 mol %) and a decrease in substrate concentration (0.5 M) to obtain the optimal selectivity (**2d**:**3d** = 97:3) and yield (75%, Table 2, entry 3).

A way to view the current silylation reaction is as a pseudodesymmetrization of a *cis*-1,2-diol. This would

imply that switching to the opposite enantiomer of catalyst would result in silylation of the C3′-hydroxyl. Initial efforts to TBS protect the 3′ position resulted in poor yield and low selectivity in the reaction. We have previously reported that for inherently less reactive positions within molecules it is often necessary to use more reactive electrophiles. Using chlorotriethylsilane (TESCl) in place of TBSCl allowed for the highly selective protection of the 3′-hydroxyl of uridine (2′:3′ = 7:93, Table 3, entry 1). With the more reactive electrophile the catalyst loading can be reduced to 5 mol % with a reaction time of 4 h. Moreover, switching to (−)-**4b** affords the 2′-protected product in excellent

yield and selectivity (Table 3, entry 2). Application of the scaffolding catalysts to the other natural ribonucleosides allows for switching of the site selectivity through simply changing the enantiomer of the catalyst. In general either isomer of product can be isolated in >95:5 selectivity and >80% yield using 5% catalyst (Table 3). Guanosine proved to be the most challenging substrate, but by increasing the catalyst loading to 20 mol % the desired product was isolated in 78% yield (**5:6** = 14:86, Table 3, entry 7).

Unnatural ribonucleosides have been found to be effective antiviral agents, and so the selective modification of these compounds is critical to the development of novel therapeutics. We investigated whether the scaffolding catalysts would also be effective in the selective protection of ribavirin, which is currently used to treat Hepatitis C virus (HCV). Using unprotected ribavirin, catalyst (−)-**4b** silylates the 5′ and 2′ positions with high selectivity (**7:8** = 94:6, Figure 2), leaving the 3′ hydroxyl available for further manipulation. Switching to catalyst (+)-**4a** affords the product with the 5′ and 3′ hydroxyls protected, allowing access to the 2′-hydroxyl (Figure 2).

Using a scaffolding catalyst, site-selective functionalization of ribonucleosides was achieved enabling an efficient synthesis of appropriately protected monomers for automated RNA synthesis. Moreover, simply switching the antipode of the catalyst allows for toggling of the site selectivity to favor protection of the 3′-hydroxyl. We believe that developing catalysts that recognize specific functional group motifs will provide a practical and predictable method for manipulating polyfunctional molecules.

Supporting Information Available. Experimental procedures, characterization data, and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.