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SHORT COMMUNICATION

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Short-Step Anodic Access to Emissive RNA Homonucleosides

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Emissive RNA homonucleosides were added to the growing library of synthetic probes. The emissive nucleobases were constructed in one step from the methallyl *C*-glycoside of D-ribofuranose, which was prepared stereoselectively. The homonucleosides displayed various emission colors by using a single excitation wavelength that could be fine-tuned merely by changing the carbonyl substituent on the methoxyphenol.

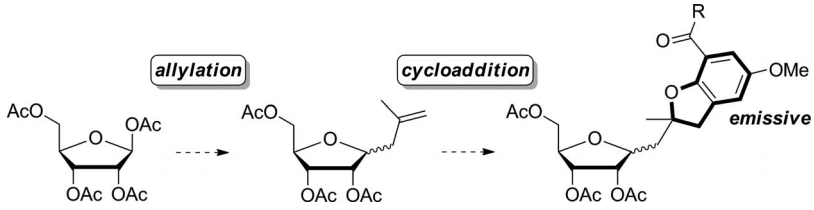
Introduction

Emissive nucleoside analogues have been widely used to monitor fundamental biochemical transformations directly, because natural nucleobases are not emissive.[1] This characteristic has led to the construction of useful libraries of synthetic emissive nucleoside analogues. Typically, these synthetic probes are prepared through glycosylation with preformed emissive nucleobases under carefully selected conditions to control both stereoselectivity at the anomeric position and regioselectivity of the aglycon attack. Because these selectivities are affected by the interaction of the protected groups of both the sugar and aglycon, glycosylation usually requires a time-consuming trial-and-error procedure. These synthetic probes also should exhibit minimal structural perturbation, provide visible emission, and likely be resistant to nonspecific enzymatic degradation. In this context, *C*-nucleosides and homonucleosides show greater metabolic stabilities than natural nucleosides, and they have been studied extensively.[2] Although there are various practical, well-established synthetic routes for generating emissive *C*-nucleosides, the synthesis of homonucleoside[3] variants remains challenging, as alkyl-*C*-glycosylation is required instead of commonly studied aryl-*C*-glycosylation.

The early finding that allylsilanes could react with acetals in the presence of Lewis acids[4] led to the elaboration of an efficient procedure for the synthesis of an anomerically allylated *C*-glycoside of D-ribofuranose that could be a convenient precursor for the preparation of homonucleosides.[5] We have been developing anodic methods by using an $LiClO_4/MeNO_2$ electrolyte solution that facilitates intermolecular carbon–carbon bond-formation reactions.[6] In particular, unactivated olefinic nucleophiles were shown to trap anodically generated phenoxonium ions to yield emissive [3+2] cycloadducts in one step.[7] In light of previous studies, we sought to realize a short-step synthesis of emissive RNA homonucleosides assisted by anodic methods (Scheme 1). In this scenario, stereochemistry at the anomeric position could be addressed at an earlier stage, and the regioselectivity could be fully controlled.

Results and Discussion

The present work began with the allylation of β-D-ribofuranose 1,2,3,5-tetraacetate (**1**) with methallyltrimethylsilane (**2**). The tertiary olefinic carbon atom is required for subsequent cycloadditions. Although the direct allylation of both **1** and β-D-deoxyribofuranose 1,3,5-triacetate with simple allyltrimethylsilane has been accomplished in high yield by using TMSOTf (Tf = trifluoromethylsulfonyl) as a promoter in MeCN,[8] allylation with methallyltrimethylsilane (**2**) under the same conditions was disappointing (Table 1, Entry 1). The corresponding methallyl *C*-glycoside



Scheme 1. Anodic access to emissive RNA homonucleosides.

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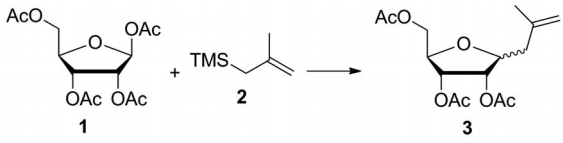
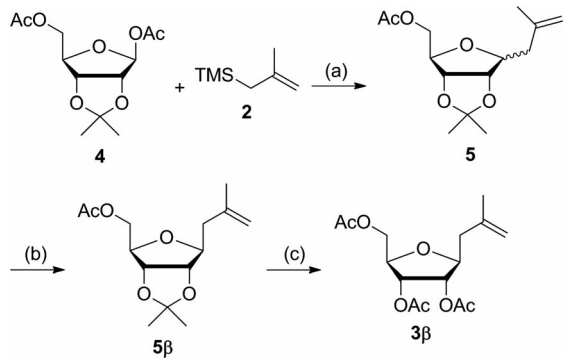
of D-ribofuranose (**3**) was obtained only in low yield, together with several byproducts that were not readily separable. Although the use of other Lewis acids in MeCN did not efficiently afford the desired product **3** (Table 1, Entries 2–5), a remarkable solvent effect was observed with THF (Table 1, Entry 8). Under these conditions, the α-methallyl *C*-glycoside of D-ribofuranose (**3α**) was selectively obtained in excellent yield. Although TMSOTf induced severe gelation of THF, the reaction took place efficiently, and the gel was readily removable upon completion of the reaction. In contrast, dioxane and Et_2O provided only modest solvent effects (Table 1, Entries 9 and 10), which suggested that the solvent effect observed with THF was not simply due to the coordinating nature of ethers. Moreover, the addition of MeCN was found to significantly impact the solvent effect of THF. For example, little α/β selectivity was observed upon using a 9:1 (v/v) mixture of THF/MeCN as the solvent (Supporting Information, Table S1). The excellent α-selectivity observed can be rationalized in terms of the stereoelectronic effects of five-membered-ring oxocarbenium ions.[9]

Table 1. Allylation of β-D-ribofuranose 1,2,3,5-tetraacetate (**1**) with methallyltrimethylsilane (**2**).



Entry	Solvent	Lewis acid	Yield [%]	α/β ratio
1	MeCN	TMSOTf	32	3:1
2	MeCN	TIPSOTf	9	1:1
3	MeCN	TBDMSOTf	trace	–
4	MeCN	$SnCl_4$	trace	–
5	MeCN	BF_3·OEt	0	–
6	CH_2Cl_2	TMSOTf	19	18:1
7	toluene	TMSOTf	23	5:1
8	THF	TMSOTf	91	>99:1
9	dioxane	TMSOTf	42	13:1
10	Et_2O	TMSOTf	33	32:1

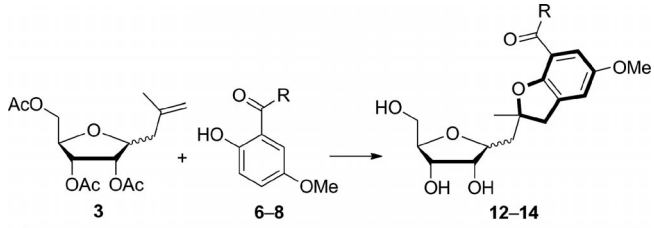
The β-methallyl *C*-glycoside of D-ribofuranose (**3β**) was prepared by using 2,3-*O*-isopropylidene diacetate (**4**), which was obtained in two steps from D-ribose (Supporting Information, Scheme S1). The allylation of **4** with **2** was performed by using $ZnBr_2$ as a promoter in $MeNO_2$ to yield the methallyl *C*-glycoside of D-ribofuranose (**5**) with high β-selectivity (Scheme 2).[10] After purification, acidic deprotection of the β-methallyl *C*-glycoside of D-ribofuranose (**5β**) followed by acetylation gave the desired **3β**. Replacement of the protecting groups was required, as isopropylidene protection was not suitable for subsequent acidic electrochemical conditions.



Scheme 2. Preparation of the β-methallyl *C*-glycoside of D-ribofuranose (**3β**). Reagents and conditions: (a) $ZnBr_2$, $MeNO_2$, r.t., 65%, α/β = 1:13. (b) The α-anomer was removed. (c) (1) 70% AcOH (aq.), 70 °C; (2) Ac_2O, pyridine, r.t., 81% over two steps.

With both **3α** and **3β** in hand, we then focused on the anodic construction of the emissive [3+2] cycloadducts. In a preliminary experiment, we found that the addition of AcOH, which might stabilize the corresponding cationic intermediates and/or function as a proton source for cathodic reduction, was effective for these reactions.[11] It was also found that the olefinic nucleophilicities of both **3α** and **3β** toward anodically generated phenoxonium ions were similar, and no epimerization at the anomeric position was observed under these electrochemical conditions. The anodic oxidation of methoxyphenols **6**–**8** in the presence of **3α** or **3β** gave the [3+2] cycloadducts **9**–**11** (Table 2). Deprotection under basic conditions afforded the desired homonucleosides **12**–**14**.

Table 2. Anodic construction of the emissive [3+2] cycloadducts by using methoxyphenols **6**–**8**.[a]



Entry	Substrate	Yield [%] Cycloaddition	Yield [%] Deprotection
1	**3α** + **6** (R = H)	50 (**9α**)	61 (**12α**)
2	**3α** + **7** (R = Me)	88 (**10α**)	quant. (**13α**)
3	**3α** + **8** (R = OMe)	46 (**11α**)	75 (**14α**)
4	**3β** + **6** (R = H)	51 (**9β**)	51 (**12β**)
5	**3β** + **7** (R = Me)	83 (**10β**)	75 (**13β**)
6	**3β** + **8** (R = OMe)	44 (**11β**)	69 (**14β**)

[a] Reagents and conditions: (1) $LiClO_4$, $MeNO_2$, carbon felt anode, platinum cathode, 1.1–1.3 V vs. Ag/AgCl, r.t.; (2) MeONa, MeOH, r.t.

The photophysical properties of homonucleosides **12**–**14** are summarized in Table 3. As expected, the anomeric configuration had little impact on the photophysics of the respective [3+2] cycloadducts. Homonucleosides **12**–**14** displayed emission bands ranging from 408 to 493 nm in EtOH or H_2O that could be fine-tuned merely by changing the carbonyl substituent on methoxyphenols **6**–**8**.

This simple change in structure yielded various emission colors by using a single excitation wavelength (Figure 1). These emission bands were blueshifted if homonucleosides **12**–**14** were dissolved in dioxane; however, the absorption bands were essentially unaffected by solvent polarity. This

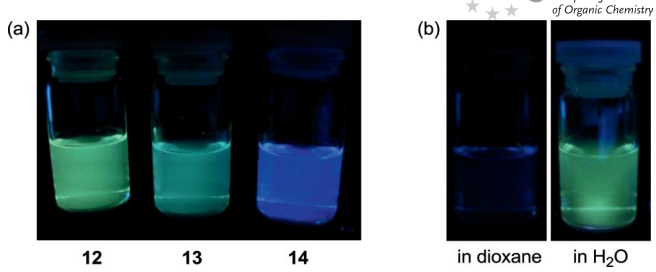
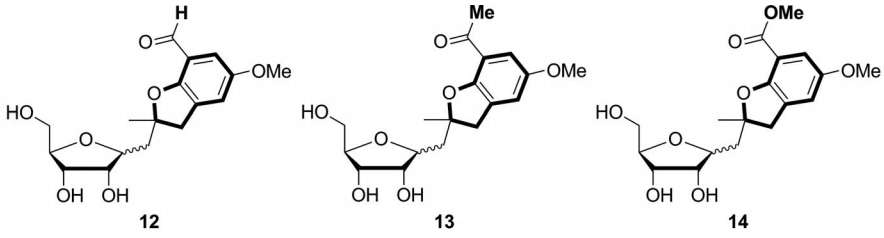


Figure 1. Fluorescence (λ_{ex} = 365 nm) of (a) homonucleosides **12**–**14** in EtOH and (b) homonucleoside **13** in dioxane or H_2O.

Table 3. Photophysical data of homonucleosides **12**–**14**.



Entry	Compound	Solvent	λ_{abs} [nm]	λ_{em} [nm]	ϕ[a]	Stokes shift[b]
1	**12α**	EtOH	370	480	0.32	6.2
2	**12α**	dioxane	368	429	0.04	3.7
3	**12α**	H_2O	371	493	0.03	6.7
4	**13α**	EtOH	369	461	0.42	5.4
5	**13α**	dioxane	365	418	0.03	3.8
6	**13α**	H_2O	369	482	0.04	6.4
7	**14α**	EtOH	350	408	0.51	4.1
8	**14α**	dioxane	344	392	0.44	3.6
9	**14α**	H_2O	340	425	0.25	5.9
10	**12β**	EtOH	370	478	0.37	6.1
11	**12β**	dioxane	366	424	0.03	3.7
12	**12β**	H_2O	371	493	0.06	6.7
13	**13β**	EtOH	360	460	0.49	6.0
14	**13β**	dioxane	354	409	0.02	3.8
15	**13β**	H_2O	360	484	0.04	7.1
16	**14β**	EtOH	341	408	0.66	4.8
17	**14β**	dioxane	339	389	0.55	3.8
18	**14β**	H_2O	337	423	0.29	6.0

[a] Absolute quantum yield. [b] 10^3 cm^{-1}.

suggested that environmental polarity could be monitored in real time by using these compounds.

Conclusions

The experimental results presented herein illustrate a short-step approach to emissive RNA homonucleosides synthesized by anodic cycloaddition in combination with stereoselective allylation. The carbonyl substituent had a large impact on the photophysical properties of the respective homonucleosides that could fine-tune the emission colors, and this enables the rational design of reaction-based emissive probes. Notably, our approach can form emissive RNA homonucleosides from nonemissive precursors in one step, and the stereochemistry at the anomeric position can be validated prospectively through allylation.

Experimental Section

General Procedure for the Allylation of Acetoxy D-Ribofuranose 1 or 4 with Methallyltrimethylsilane (2): Methallyltrimethylsilane (**2**, 3.0 equiv.) was added under argon at 0 °C to a stirred solution of β-D-ribofuranose 1,2,3,5-tetraacetate (**1**) or 2,3-*O*-isopropylidene diacetate (**4**) in the respective solvent. After 10 min, the Lewis acid (1.5–2.5 equiv.) was added dropwise. The resulting reaction mixture was then warmed to room temperature naturally and stirred for a further 6 h, followed by quenching with saturated $NaHCO_3$ (aq.). The aqueous phase was extracted with EtOAc, and the combined organic layers were washed with brine, dried with $MgSO_4$, and concentrated in vacuo. Purification by silica gel column chromatography (EtOAc/hexanes) gave the desired methallyl *C*-glycoside of D-ribofuranose (**3**).

General Procedure for the Anodic Construction of the [3+2] Cycloadducts by Using Methoxyphenols 6–8: Methoxyphenols **6**–**8**, **3** (10 equiv.), and AcOH (5%) were added to a solution of lithium perchlorate (3.0 M in $MeNO_2$). A carbon felt anode (20 mm × 20 mm) and a platinum cathode (20 mm × 20 mm) were inserted into the solution, and electrolysis was performed by using an undivided cell with stirring at a constant potential of 1.1–1.3 V versus Ag/AgCl at room temperature. An electric charge of 2.5 F was passed through the solution (also monitored by TLC), followed by dilution with EtOAc. The organic layer was washed with brine, dried with $MgSO_4$, and concentrated in vacuo. Purification by silica gel column chromatography (EtOAc/hexanes) gave acetylated homonucleosides **9**–**11**.

General Procedure for the Basic Deprotection of Acetylated Homonucleosides 9–11: MeONa (5.0 M in MeOH, 5%) was added dropwise at room temperature to a stirred solution of acetylated homonucleosides **9**–**11** in MeOH. The resulting reaction mixture was then stirred for another 1.5 h, followed by neutralizing with cation exchange resin. After filtration, the filtrate was concentrated in vacuo to give homonucleosides **12**–**14**. In some cases, purification by silica gel column chromatography ($MeOH/CH_2Cl_2$) was required.

Supporting Information (see footnote on the first page of this article): Additional general information, spectral information and copies of the ^1H and ^{13}C NMR spectra.

Acknowledgments

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