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ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 3450–3456

Coumarin base-pair replacement as a fluorescent probe of ultrafast DNA dynamics

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Received 31 July 2006; revised 18 December 2006; accepted 18 December 2006
Available online 2 February 2007

Abstract—The design and synthesis of a novel coumarin C-riboside are described, and is based on the well-known photoprobe Coumarin 102. A diastereofacial selective Heck coupling between a furanoid glycal and a coumarin triflate provided a method for glycoside formation. The coumarin C-glycoside was incorporated synthetically into DNA oligomers, and was used to probe ultrafast dynamics of duplex DNA using time-resolved Stokes shift methods.

1. Introduction

In studying the dynamic properties of biomolecules using ultrafast photophysical techniques,[1] it is oftentimes useful to appropriately localize a reporter molecule within the interior of the system. Time-resolved Stokes shift (TRSS) measurements[2] are a well-established method for measuring molecular motion in a variety of non-biological systems, and although this method can be extended to proteins using native chromophores,[3] it is non-trivial to extend these studies to DNA. The native purine and pyrimidine bases have excited state lifetimes on the sub-picosecond time scale, which make them unsuitable for studying dynamic events that occur on picosecond or slower time-scales. These problems led us to develop a collaborative research program to design, synthesize, characterize, and apply new fluorescent probes for use in TRSS measurements of DNA dynamics.

In an effort to understand molecular origins of the unique dynamics of DNA, we use an adaptation of the time-resolved Stokes shift technique, where the local structural relaxation of DNA in response to the instantaneously altered dipole moment in the excited state of a probe molecule is measured. As the nearby components of the DNA molecule relax, the electric field at the dipole is increased, resulting in a lowered energy of the probe molecule and an accompanying red-shift of the fluorescence. The time dependence of the shift in the fluorescence can be related with the time-dependent relaxation of the surrounding charged groups (i.e., the bases and sugar–phosphate backbone of DNA).

In early work by Murphy and co-workers, time-resolved Stokes shift spectroscopy was introduced as a method to study ultrafast DNA dynamics, and acridine orange was used as an intercalating probe molecule.[4] Temperature-dependent Stokes shift measurements showed that fluctuations in the local environment of an intercalated probe molecule are reduced by only about one-half as it moves from aqueous solution to the interior of DNA. Steady-state fluorescence excitation and emission spectra were used to measure the Stokes shift of acridine orange, randomly intercalated into calf thymus DNA, at low temperatures. The results suggested that DNA has a surprisingly polar and fluid interior. These results led to the design of more sophisticated experiments in which the DNA would be well-defined (i.e., oligonucleotides of known sequence), the fluorescent probe would be in a fixed position within the duplex (i.e., covalently attached to the DNA backbone), and picosecond, or even femtosecond, lasers would be required to do time-resolved Stokes shift experiments at room temperature.

2. Probe design

The problem with using probes such as acridine orange is largely related to their potential for multiple modes of binding and/or orientation. In addition, with intercalating agents such as acridine orange, the DNA is distorted and hence any information about the native state of DNA is unavailable from such experiments. Incorporation of reporter molecules into DNA for use in TRSS dynamics studies of native DNA must meet three conditions: (1) the reporter must occupy a single, well-defined position in the DNA helix analogous to the position of a native base, and it must do so without distorting the native DNA structure; (2) the orientation of the

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0040–4020/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.tet.2006.12.096

probe must be fixed relative to the helix axis in a known manner; and (3) the photophysical properties of the probe must be appropriate for the experiment. In addition, the reporter must be compatible with oligonucleotide synthesis so it can be placed in an arbitrary position within the DNA sequence or positioned relative to a specific lesion or active site base pair.

Existing DNA fluorophores fail to meet at least one of these criteria. For example, fluorescent tags attached to the exterior of the DNA helix only report on the solvent dynamics, not on the conditions inside the helix. Intercalated fluorophores can serve as TRSS probes,[4,5] but distort the DNA structure and cannot be accurately positioned along the helix. Some modified DNA bases are fluorescent[6] and show a TRSS response,[7] however, their photophysics are complex and possibly influenced by excitonic mixing with the native bases.[8] Completely artificial fluorescent bases[9] are an attractive means of satisfying the above criteria, but existing examples have not been optimized for the photophysical properties needed in TRSS experiments. It should be noted that the requirements of a TRSS probe and a fluorescence-intensity probe[10] are different. In a TRSS probe, changes in the fluorescence position induced by the environment are desired. Changes in the fluorescence quantum yield or lifetime are undesirable complications.

With this said, then, the issue becomes one of looking at the photophysical properties of candidate probe molecules, deciding on their suitability to serve as base-pair replacements in DNA with respect to size and shape, estimating of the suitability of methods for incorporation of such probe molecules synthetically into DNA, and finally, coming up with a synthetic plan. Using these criteria, we selected the well-known Coumarin 102 as our first generation probe (Fig. 1).

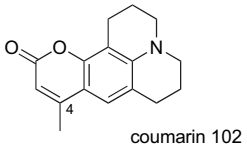


Figure 1. Structure of photoprobe Coumarin 102.

2.1. Coumarin 102

The well-characterized Coumarin 102 probe is advantageous because of the well-established photophysical properties of this fluorophore.[11] Coumarin 102 and related dyes have been demonstrated to be nearly ideal probes for solution-phase TRSS experiments.[12–14] In general, coumarins have simple photophysics, uncomplicated by competing processes, with no low lying electronic states that interact with their first excited states.[14,15] Non-radiative relaxation and intersystem crossing are typically weak, which results in high quantum yields.[16] The amine nitrogen of **1** is rigid because of the two ethylene tethers, and cannot twist or pyramidalize in the excited state.[16] Finally, the absorption maximum of **1** is near 400 nm, a wavelength long enough to prevent energy transfer to or electronic mixing with the transitions of the normal DNA bases.

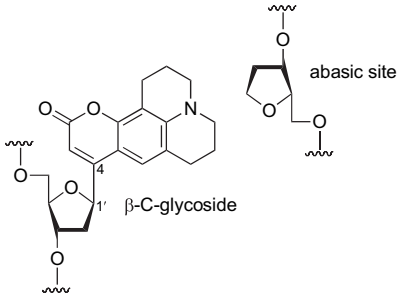


Figure 2. Coumarin C-glycoside/abasic-site tetrahydrofuran.

2.2. Molecular modeling

Molecular modeling of coumarin 102 (with the C4 methyl group deleted) within the B-DNA double helix demonstrated that the coumarin could effectively replace a normal purine–pyrimidine base pair. In what can best be described as the maximally intercalated geometry, the volume of the coumarin ring system completely filled the cavity left by deletion of one base pair in silico. In addition, this intercalated positioning placed coumarin's C3 and C4 within bonding distance of the abasic C1′ position of the deoxyribose, suggesting a possible covalent linkage between the probe and DNA.

When the coumarin ring was covalently attached in silico via C4 to the C1′ position of an opposed abasic-site tetrahydrofuran analog (Fig. 2), there was no significant distortion of the canonical double helix as measured by C1′ to C1′ distances[17] in the energy minimized structure (Fig. 3). Because Coumarin 102 has a low solubility in water relative to organic solvents, hydrophobic forces should favor the coumarin unit occupying the DNA interior as opposed to extend into the aqueous medium.

2.3. Synthetic design

Perhaps the most attractive aspect of this proposed design was the synthetic accessibility of C-glycoside **1** through the application of a palladium-catalyzed Heck coupling[18] of suitably protected glycal **2** with a halocoumarin **3** (X=I, OSO_2CF_3) following the strategy developed by Daves[19] and Cabri (Fig. 4).[20] This variation of the Heck reaction is known for the high degree of stereoselection for formation of the β-C-glycoside, where coordination of the intermediate palladium species is directed away from the 3-alkoxy group to the β-face of glycal **2**.[21]

3. Synthetic results

3.1. Glycal synthesis

Furanoid glycals related to coupling partner **2** had been reported in the literature by Ireland and co-workers,[22] using a straightforward synthetic route starting with D-ribono-γ-lactone. This route was later modified by Daves and co-workers to encompass selectively protected systems.[23] This route affords **4**, with the primary 5-hydroxyl group selectively protected as the TBS ether. This allowed us to introduce silyl and alkyl ethers for protection of the 3-hydroxyl

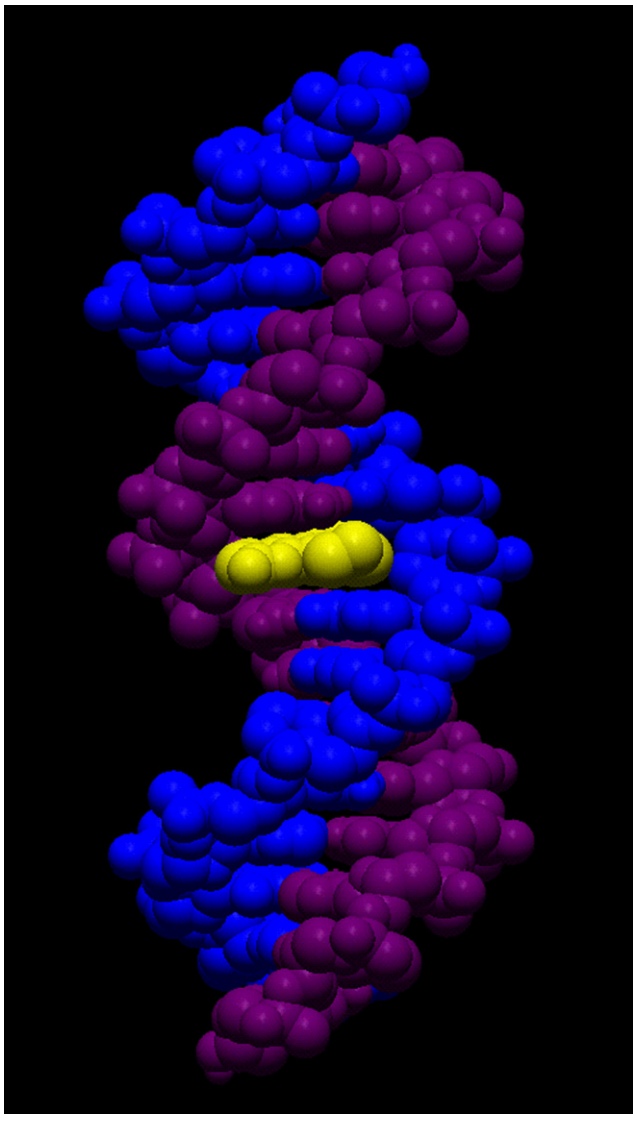


Figure 3. Energy minimized DNA B-form double helix containing the coumarin C-glycoside (purple)/abasic-site tetrahydrofuran (blue) system.

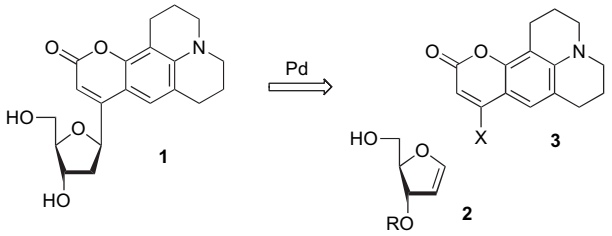
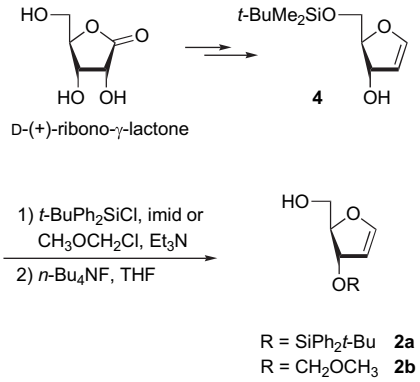


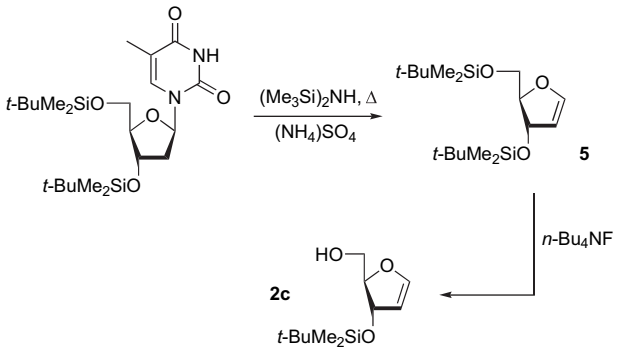
Figure 4. Heck coupling strategy for C-glycoside synthesis.

group. The primary hydroxyl group does not need to be protected for the subsequent Heck coupling.

More recently, Hammer and co-workers[24] had reported the synthesis of these same systems by thermolysis of thymidine in the presence of hexamethyldisilazane, a reaction originally reported by Pedersen and co-workers.[25] It was this latter protocol that proved most expedient in our hands, particularly because it was so amenable to production of selectively protected systems. (Many excellent references to

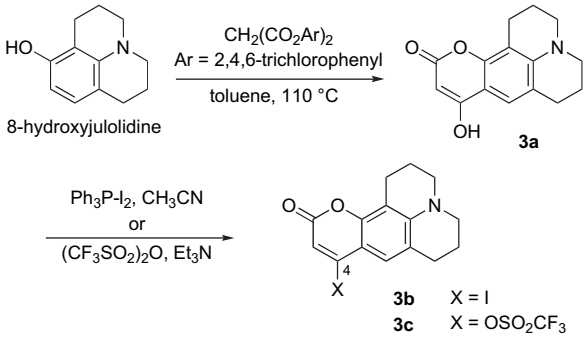


furanoid glycal chemistry can be found in these works.) In practice, warming bis-TBS protected thymidine in the presence of hexamethyldisilazane and ammonium sulfate at reflux affected conversion to the bis-TBS protected glycal **5**. Monodeprotection[26] of the primary silyl ether of **5** was easily accomplished with 1 equiv of fluoride, to afford **2c**.



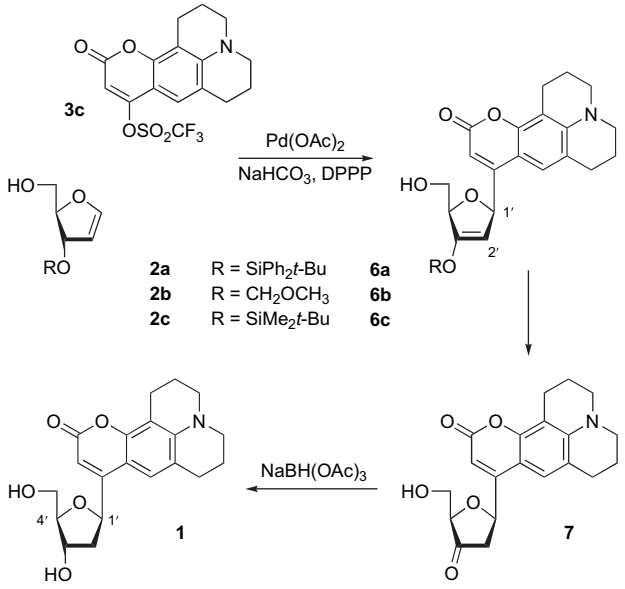
3.2. Coumarin synthesis

Coumarin **3a** (X=OH) was prepared from 8-hydroxyjulolidine following literature procedures[27] by reaction with bis(2,4,6-trichlorophenyl) malonate in refluxing toluene, which effected annulation of the α-pyrone ring system to afford **3a** in excellent yields (94%). The hydroxyl group of **3a** could be transformed to the iodide by treatment with a preformed complex of triphenylphosphine and iodine (Ph_3P, I_2, CH_3CN, 82 °C).[28] Alternatively, the hydroxyl group could be acylated with trifluoromethanesulfonic anhydride (Tf_2O, Et_3N, CH_2Cl_2, 0 °C)[29] to afford triflate **3c** in 87% yield. These systems were examined in the Heck coupling reaction with glycals **2a**–**c**.



3.3. Heck coupling

Our initial experiments on palladium-catalyzed coupling of vinylic iodide **3b** with *t*-$BuPh_2Si$ protected glycal **2a** were unsuccessful in providing any of the coupled product **6a** under a variety of reaction conditions. The predominant product formed under these conditions was the corresponding reduced coumarin (**3d**, X=H), and on occasion a dimeric product. The glycal **2a** was always recovered unchanged. These results indicated that we were forming the requisite palladium Ar–I insertion product from reaction with **3b**, but that this was taking an unproductive pathway (e.g., reduction or dimerization) in preference to reacting with the sterically encumbered, electron-rich glycal **2a**. This is consistent with Cabri's mechanistic insights where dissociation of the strong Pd–I bond must occur prior to coordination of the Pd with an electron-rich alkene.

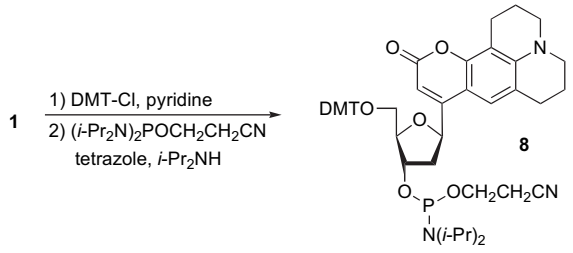


As a result of these unsuccessful experiments, two groups in this coupling reaction were changed: (1) the iodide of **3b** was changed to the more reactive trifluoromethanesulfonate **3c**, accompanied by the introduction of the chelating phosphine 1,3-(diphenylphosphino)propane (dppp); and (2) the more bulky *t*-$BuPh_2Si$ ether of **2a** was changed to the smaller methoxymethyl acetal of **2b**. In this instance, reaction of triflate **3c** with glycal **2b** in the presence of $Pd(OAc)_2$ (5–10 mol %), dppp, and $NaHCO_3$ afforded the desired Heck product **6b**, although in low yield. Coupling conversions were substantially improved by performing the reaction by sequential addition of aliquots of $Pd(OAc)_2$ as the reaction progressed. In the end, we found optimal reaction conditions for this system to be 40 mol % $Pd(OAc)_2$, 5 mol % dppp, 3 equiv $NaHCO_3$ in CH_3CN at reflux, and under these conditions, Heck product **6b** was produced in 75% yield. Under these conditions, dissociation of the Pd–OTf bond in the oxidative insertion product to form an intermediate cationic palladium species facilitates coordination to the electron-rich olefin of glycal **2b**.

Hydrolysis of the enol ether of **6b** under acidic conditions (HCl, CH_3OH, 25 °C) afforded the corresponding dimethyl acetal of ketone **7**, and this difficulty combined with the lengthy preparation of **2b** from D-ribono-γ-lactone led us to an additional tactical change. *tert*-Butyldimethylsilyl ether **2c** is easily available using the protocol of Hammer and co-workers,[24] and when **2c** was used as the glycal partner in the Heck coupling reaction, silyl enol ether **6c** could be produced in 79% yield. Fluoride-promoted cleavage of the silyl ether (HF·pyridine) afforded ketone **7** in excellent yields.[30] The carbonyl group of **7** could be reduced stereoselectively to *ribo*-glycoside **1** with sodium triacetoxyborohydride[31] following literature conditions.

The stereochemistry around the dihydrofuran ring of **6b** and **6c** was determined to exist as shown by observation of the expected coupling constant between C1′–H and C2′–H (J=3.7 Hz). The stereochemistry of the C1′ glycosidic center was confirmed by the observation of a nuclear Overhauser enhancement of the C4′–H when C1′–H was irradiated (300 MHz, $CDCl_3$).

Incorporation of this fluorophore into an oligonucleotide proceeded by protection of the 5′-hydroxyl group as the dimethoxytrityl (DMT) ether followed by phosphitylation of the 3′-hydroxyl group to afford phosphoramidite **5**. This reagent was incorporated directly into DNA oligomers using standard automated oligonucleotide synthetic protocols.



4. Time-resolved spectroscopic experiments

The initial question that was addressed with coumarin-containing DNA duplex was whether the coumarin correctly incorporated into the DNA helix and preserved the native DNA structure. Substantial shifts in the absorption and fluorescence spectra of coumarin incorporated into DNA relative to free in aqueous solution clearly demonstrated that the coumarin is incorporated within the interior the DNA helix.[32] Melting of coumarin-containing oligonucleotides is simultaneous for the native bases and the coumarin, showing that there is little tendency to expel the coumarin from the helix. The coumarin is also inaccessible to the aqueous phase, as judged from an absence of quenching by an added quencher.[33] Most importantly, coumarin-containing oligonucleotides are still selectively bound and cleaved by the APE1 endonuclease system, which acts on the abasic site directly opposite to the coumarin. Any distortions of the DNA structure from the coumarin are not large enough to disrupt the biological activity of the DNA. These results demonstrated that our criteria for probe design are satisfied.

The desirable photophysical properties of coumarin are also retained. The excited-state lifetime is almost unchanged (actually increased slightly) when the coumarin is in DNA,

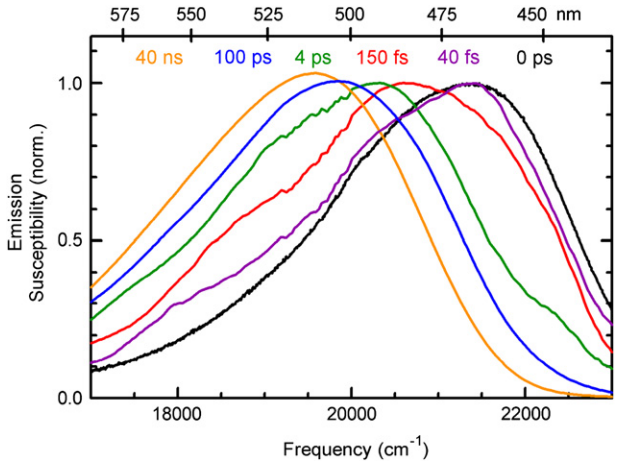


Figure 5. The emission spectrum of coumarin in DNA shifts as a function of time after excitation. This shift is a reflection of the relaxation dynamics of the DNA and solvent surrounding the coumarin. Adapted from Ref. 34.

so quenching by the native bases is negligible. Figure 5 shows the time-resolved emission of the coumarin in DNA. The response is strong, giving the TRSS experiment a good dynamic range.

Additional TRSS studies using coumarin in DNA have provided new and oftentimes surprising insights into DNA dynamics. The effective polarity within the interior of the DNA helix is high, similar to the polarity of ethanol.[33] The effective range of the electrostatic interactions causing this polarity is about 15 Å, and is caused by a combination of the nearby water, counterions, and the DNA itself.[35] As suggested in Figure 5, the time needed for this polarity response to develop is spread over a very wide time range of at least six decades spanning the range from 40 fs to 40 ns.[32,34,36] This unusual behavior has been duplicated in simulations,[35] but was not anticipated in previous theoretical work, and remains unexplained. Additional dynamics occur near the end of the helix or near a deleted base,[36,37] and bulky counterions induce extra dynamics that are not fully understood at this point.[37] The extension of these methods to DNA–protein complexes has also been demonstrated.[33] The fact that many of these results were unanticipated prior to the experiments and are still unexplained illustrates the poorly developed state of knowledge of the dynamics in complex biomolecules. Novel tools such as the coumarin probe described here provide a route to remedying this situation.

5. Experimental section

5.1. 4-Hydroxycoumarin 3a

8-Hydroxyjulolidine (3.7 g, 19.5 mmol) and bis(2,4-dichlorophenyl) malonate (9.05 g, 19.5 mmol, 1 equiv) were dissolved in dry toluene (60 mL) and the reaction mixture was warmed at reflux for 2 h. The light brown suspension was cooled, filtered, and the solids were washed with hexanes (3×30 mL) to afford **3a** as a light brown solid (4.7 g, 94%) that was used without further purification: ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.70 (br s, 1H), 7.17 (s, 1H), 5.24 (s, 1H), 3.43 (m, 4H), 3.24 (m, 2H), 2.72 (m, 2H), 1.90 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.8, 163.1, 151.1, 146.2, 120.0, 117.5, 105.5, 103.2, 101.5, 85.9, 49.6, 48.8, 27.1, 21.1, 20.2. EI-HRMS, *m/z* calcd for $C_{15}H_{15}NO_3$: 257.1052; found: 257.1048.

5.2. 4-Iodocoumarin 3b

A solution of I_2 (1.12 g, 4.4 mmol, 1.1 equiv) in dry CH_3CN (50 mL) under argon was treated with Ph_3P (1.15 g, 4.4 mmol, 1.1 equiv) and the resulting yellow precipitate of $Ph_3P{\cdot}I_2$ was stirred at 24 °C for 15 min. Triethylamine (0.6 mL, 4.4 mmol, 1.1 equiv) and 4-hydroxycoumarin **3a** (1.03 g, 4.0 mmol) were added and the reaction mixture was heated at reflux for 15 h. Evaporation of the volatiles in vacuo and purification of the residue by flash chromatography (silica gel, 7:3 hexane/acetone) afforded **3b** as a yellow solid (885 mg, 60%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00 (s, 1H), 6.71 (s, 1H), 3.28 (m, 4H), 2.83 (m, 4H), 1.97 (m, 4H); EI-HRMS, *m/z* calcd for $C_{15}H_{14}NO_2I$: 367.0069, found: 367.0091.

5.3. 4-Trifluoromethylsulfonyl coumarin 3c

4-Hydroxycoumarin **3a** (257 mg, 1 mmol) and triethylamine (0.2 mL, 1.45 mmol, 1.45 equiv) were dissolved in dry CH_2Cl_2 (10 mL) under N_2. The mixture was cooled to −10 °C (acetone/ice bath) and trifluoromethanesulfonic anhydride (0.22 mL, 1.3 mmol, 1.3 equiv) was added dropwise via syringe. The dark green reaction mixture was stirred at −10 °C for 1 h, and diluted with a mixture of ether and hexane (1:1, 30 mL). The reaction was passed through a silica column (4×10 cm), and the bright yellow product was washed from the column with 1:1 ether/hexane until the solvent came out colorless. The combined effluent was concentrated in vacuo to afford **3c** as a bright yellow solid (338 mg, 87%) that was used without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (s, 1H), 5.99 (s, 1H), 3.30 (m, 4H), 2.86 (m, 2H), 2.78 (m, 2H), 1.96 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.6, 158.3, 151.2, 147.6, 120.6, 119.2, 116.4, 106.7, 101.7, 97.6, 50.0, 49.6, 27.7, 21.0, 20.4, 20.2; IR (neat) ν_{max} 2943, 2856, 1720, 1616, 1523, 1430, 1404, 1365, 1310 cm^{-1}. EI-HRMS, *m/z* calcd for $C_{16}H_{14}NO_5F_3S$: 389.0545; found: 389.0558.

5.4. C-Riboside 6b

A solution of triflate **3c** (262 mg, 0.725 mmol) in dry CH_3CN (15 mL) in a Teflon-capped vial was treated with glycal **2b** (348 mg, 2.18 mmol, 3 equiv), $Pd(OAc)_2$ (64 mg, 0.29 mmol, 0.4 equiv), 1,3-bis(diphenylphosphino)propane (14 mg, 0.036 mmol, 0.05 equiv), and $NaHCO_3$ (153 mg, 2.175 mmol, 3 equiv). The reaction mixture was stirred for 3 h at 24 °C. The mixture was concentrated in vacuo and the residue was purified by flash chromatography (silica gel, ether) to afford **6b** as a yellow oil (227 mg, 79%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 (s, 1H), 6.20 (s, 1H), 6.00 (m, 1H), 5.08 (app t, J=1.7 Hz, 1H), 5.01 (m, 2H), 4.82 (m, 1H), 3.78 (ddd, J=12.0, 4.6, 3.0 Hz, 2H), 3.44 (s, 3H), 3.25 (m, 4H), 2.88 (m, 2H), 2.78 (m, 2H), 1.97 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.1, 155.6, 153.4, 151.5, 145.8, 121.1, 118.2, 107.0, 106.4, 104.5, 95.8, 83.0, 80.4, 63.5, 56.5, 49.9, 49.5, 33.4, 30.6, 22.7, 20.6, 20.5; IR (neat) ν_{max} 3442, 2932, 2856, 1700,

1662, 1601, 1556, 1518, 1431, 1370, 1311 cm^{-1}. EI-HRMS, *m/z* calcd for $C_{22}H_{25}NO_6$: 399.1682; found: 399.1696.

5.5. C-Riboside 6c

A solution of triflate **3c** (375 mg, 1 mmol) in dry CH_3CN (20 mL) in a Teflon-capped vial was treated with glycal **2c** (700 mg, 3 mmol, 3 equiv), $Pd(OAc)_2$ (90 mg, 0.4 mmol, 0.4 equiv), 1,3-bis(diphenylphosphino)propane (20.6 mg, 0.050 mmol, 0.05 equiv), and $NaHCO_3$ (252 mg, 3 mmol, 3 equiv). The reaction mixture was stirred for 5 h at 24 °C. The mixture was concentrated in vacuo and the residue was purified by flash chromatography (silica gel, 4:1 ether/hexane) to afford **6c** as a yellow oil (227 mg, 79%): ^1H NMR (250 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.23 (s, 1H), 5.95 (d, J=3.7 Hz, 1H), 4.87 (dd, J=2.0, 1.7 Hz, 1H), 4.66 (m, 1H), 3.72 (m, 2H), 3.25 (m, 4H), 2.86 (m, 2H), 2.78 (m, 2H), 1.97 (m, 4H), 0.93 (s, 9H), 0.11 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.0, 155.6, 152.3, 151.4, 145.7, 121.2, 118.1, 106.9, 106.6, 104.8, 98.3, 83.6, 79.7, 63.4, 49.8, 49.4, 27.7, 25.3, 21.4, 20.5, 20.4, 17.9, −4.9, −5.1; IR (neat) ν_{max} 3444, 2932, 2856, 1703, 1654, 1600, 1556, 1518, 1431, 1370, 1311 cm^{-1}. EI-HRMS, *m/z* calcd for $C_{26}H_{35}NO_5Si$: 469.2284; found: 469.2284.

5.6. 3′-Keto-2′-deoxy C-riboside 7

Enol ether **6c** (235 mg, 0.5 mmol) was dissolved in dry THF (13 mL) under argon and was treated with HF·pyridine (70% HF by weight, 0.25 mL, 17.5 equiv). The reaction mixture was stirred for 14 h at 24 °C. Concentration in vacuo and purification of the residue by flash chromatography (silica gel, ether) afforded **7** as a bright yellow solid (135 mg, 76%): ^1H NMR (300 MHz, $CDCl_3$) δ 6.79 (s, 1H), 6.43 (s, 1H), 5.36 (dd, J=11.0, 6.1 Hz, 1H), 4.08 (dd, J=3.4, 3.3 Hz, 1H), 3.94 (m, 2H), 3.20 (m, 4H), 2.97 (dd, J=18.0, 6.1 Hz, 1H), 2.81 (m, 2H), 2.70 (m, 2H), 2.38 (dd, J=18.0, 11.0 Hz, 1H), 1.91 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.2, 162.9, 154.3, 151.1, 145.8, 120.5, 118.3, 106.8, 105.5, 103.6, 82.2, 72.6, 61.3, 49.7, 49.3, 44.4, 27.6, 21.3, 20.4, 20.3; IR (neat) ν_{max} 3412, 2921, 2845, 1758, 1698, 1616, 1600, 1556, 1518, 1436, 1311 cm^{-1}. EI-HRMS, *m/z* calcd for $C_{20}H_{21}NO_5$: 355.1420; found: 355.1429.

5.7. 2′-Deoxy C-riboside 1

Ketone **7** (135 mg, 0.38 mmol) was dissolved in dry CH_3CN (10 mL) and glacial acetic acid (10 mL) under argon. The reaction mixture was cooled to 0 °C and sodium triacetoxyborohydride (106 mg, 0.5 mmol, 1.3 equiv) was added. After 10 min at 0 °C, the volatiles were removed in vacuo and the orange-brown residue was purified by flash chromatography (silica gel, 4:1 $CHCl_3$/MeOH) to afford **1** as a yellow solid (105 mg, 77%): ^1H NMR (250 MHz, $CDCl_3$) δ 6.90 (s, 1H), 6.29 (s, 1H), 5.36 (dd, J=9.6, 6.1 Hz, 1H), 4.44 (m, 1H), 4.08 (m, 1H), 3.72 (m, 2H), 3.23 (m, 4H), 2.85 (m, 2H), 2.73 (m, 2H), 2.46 (ddd, J=13.2, 6.1, 2.4 Hz, 1H), 1.94 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.4, 156.7, 151.2, 145.8, 120.9, 118.3, 106.9, 106.1, 102.8, 87.2, 75.2, 73.2, 63.1, 49.9, 49.5, 42.6, 27.7, 21.5, 20.6, 20.4; IR (neat) ν_{max} 3401, 2943, 2556, 1687, 1605, 1551, 1519, 1438, 1301 cm^{-1}. EI-HRMS, *m/z* calcd for $C_{20}H_{23}NO_5$: 357.1576; found: 357.1568.

5.8. Coumarin dimethoxytrityl ether

A solution of **1** (100 mg, 0.28 mmol), co-evaporated with pyridine (2×3 mL), dissolved in dry pyridine (4 mL) under N_2 at 25 °C was treated with bis-(4-methoxyphenyl)phenylmethyl chloride (114 mg, 0.34 mmol, 1.2 equiv). After 3 h at 25 °C, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried (Na_2SO_4), concentrated, and the residue was purified by flash chromatography (2.5×15 cm Et_3N deactivated silica, Et_2O) to afford the corresponding DMT ether (164 mg, 89%) as a yellow foam: ^1H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 9H, ArH), 6.91 (s, 1H, ArH), 6.83 (m, 4H, ArH), 6.26 (s, 1H, ArH), 5.34 (dd, J=6.4, 9.5 Hz, 1H, C1′–H), 4.33 (m, 1H, C3′–H), 4.11 (m, 1H, C4′–H), 3.71 (s, 6H, 2 OCH_3), 3.35 (m, 1H, C5′–H), 3.15 (m, 5H, 2 CH_2+C5′–H), 2.85 (m, 2H, CH_2), 2.71 (m, 2H, CH_2), 2.40 (m, 1H, C2′–H), 1.85 (m, 5H, 2 CH_2+C2′–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.8, 158.5, 156.3, 151.2, 145.6, 144.7, 135.7, 130.0, 129.9, 128.1, 127.8, 126.8, 120.9, 117.9, 113.1, 106.9, 106.2, 104.0, 103.4, 86.3, 85.9, 75.2, 64.3, 58.5, 55.2, 49.8, 49.4, 21.5, 20.6, 20.4; FABMS, *m/z* 659 (M^+): 303, 118.

5.9. Coumarin phosphoramidite 8

A solution of the above ether (100 mg, 0.15 mmol) in anhydrous CH_2Cl_2 (3 mL) under N_2 at 25 °C was treated sequentially with tetrazole (6 mg, 75 μmol, 0.5 equiv), dry diisopropylamine (11 μL, 75 μmol, 0.5 equiv), and (2-cyanoethyl)-*N*,*N*,*N*′,*N*′-tetraisopropyl phosphorodiamidite (55 mg, 0.18 mmol, 1.2 equiv). After 3 h at 25 °C, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and dried (Na_2SO_4). The solvent was removed in vacuo and the residue was purified by flash chromatography (2×12 cm Et_3N deactivated silica, 5% CH_3OH/CH_2Cl_2) to afford **8** (85 mg, 71%) as a yellow foam: ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 9H, ArH), 6.9 (s, 1H, ArH), 6.8 (m, 4H, ArH), 6.32 (s, 1H, ArH), 5.3 (dd, J=6.2, 9.5 Hz, 1H, C1′–H), 4.45 (m, 1H, C3′–H), 4.10 (m, 1H, C4′–H), 3.80 (s, 6H, 2 OCH_3), 3.65 (m, 6H, CH_2CH_2CN and 2 $CH(CH_3)_2$), 3.4 (dd, J=5.2, 9.8 Hz, 1H, C5′–H), 3.2 (m, 5H, CH_2+C5′–H), 2.8 (m, 2H, CH_2), 2.7 (m, 2H, CH_2), 2.40 (ddd, J=2.4, 6.2, 13.1 Hz, 1H, C2′–H), 1.9 (m, 5H, 2 CH_2+C2′–H) 1.19 (d, J=3.1 Hz, 6H, $CH(CH_3)_2$) 1.17 (d, J=3.1 Hz, 6H, $CH(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.7, 158.5, 156.1, 151.2, 145.6, 144.7, 135.7, 130.0, 129.9, 128.1, 127.8, 126.8, 120.9, 117.9, 117.6, 113.2, 106.9, 106.2, 103.5, 86.3, 85.8, 75.1, 74.1, 64.3, 59.2, 58.2, 51.2, 49.8, 49.4, 42.9, 42.1, 24.6, 24.5, 21.5, 20.6, 20.4, 20.3, 20.2, 16.9, 16.8.

Acknowledgements

This work was funded by a grant from the National Institutes of Health (R01GM61292).

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