



HAL
open science

The sulfo-click reaction and dual labeling of nucleosides

Guillaume Clavé, J.J. Vasseur, Michael Smietana

► **To cite this version:**

Guillaume Clavé, J.J. Vasseur, Michael Smietana. The sulfo-click reaction and dual labeling of nucleosides. *Current Protocols in Nucleic Acid Chemistry*, 2020, 83 (1), 10.1002/cpnc.120 . hal-03027722

HAL Id: hal-03027722

<https://hal.science/hal-03027722v1>

Submitted on 27 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The sulfo-click reaction and dual labeling of nucleosides

AUTHOR(S) AND CONTACT INFORMATION:

Guillaume Clavé – Université de Montpellier, CNRS, ENSCM 34090 Montpellier, France; orcid.org/0000-0001-6401-0935; Email: guillaume.clave@cnrs.fr (Corresponding author)

Jean-Jacques Vasseur – Université de Montpellier, CNRS, ENSCM 34090 Montpellier, France ; orcid.org/0000-0002-4379-6139; Email: jean-jacques.vasseur@umontpellier.fr

Michael Smietana – Université de Montpellier, CNRS, ENSCM 34090 Montpellier, France; orcid.org/0000-0001-8132-7221; Email: michael.smietana@umontpellier.fr (Corresponding author)

ABSTRACT:

This unit contains detailed synthetic procedures for the implementation of the sulfo-click reaction to nucleoside derivatives. First, 3'-O-TBDMS-protected nucleosides are converted to their corresponding 4'-thioacid derivatives in three steps. Then, various conjugates are synthesized via a biocompatible and chemoselective coupling procedure using sulfonyl-azides partners. Finally, to illustrate the potential of the sulfo-click reaction a nucleoside bearing two orthogonal azido groups is synthesized and engaged in a one-pot dual labelling through a sulfo-click / copper-catalyzed azide-alkyne cycloaddition (CuAAC) cascade. The high efficiency of the sulfo-click reaction applied to nucleosides opens up new possibilities in the context of bioconjugation.

Basic Protocol 1: General protocol for the synthesis of 4'-thioacid-nucleoside derivatives

Basic Protocol 2: Implementation of the sulfo-click reaction

Basic Protocol 3: Synthesis of 3'-azido-4'-(carboxamido)ethane-sulfonyl azide-3'-deoxythymidine

Basic Protocol 4: Detailed synthetic procedure for one-pot double-click conjugations

KEYWORDS:

nucleoside, sulfo-click reaction, bioconjugation, thioacid, sulfonyl azide

INTRODUCTION:

Click reactions that combine chemoselectivity, bioorthogonality and high efficiency are required to functionalize nucleosides in biocompatible conditions for therapeutic or imaging applications. Moreover, multiple conjugations may be needed in order to access highly functionalized nucleosides. For this purpose orthogonal reactions are required. Although several examples of multiple labelling of nucleic acids have been described in the literature (Meyer et al., 2010; Reisacher et al., 2019; Schoch, Staudt, Samanta, Wiessler, & Jäschke, 2012; Winz, Linder, Becker, & Jäschke, 2018), new orthogonal click reactions are needed to expand the potential of multiple labelling strategies. The sulfo-click reaction is an emergent alternative click reaction which involves a thioacid and a sulfonyl azide. The reaction leads to an amide linkage through a stepwise addition-cycloaddition-retro [3+2] cycloaddition sequence, generating elemental sulfur and dinitrogen as byproducts. Thus, the implementation of the sulfo-click reaction on nucleosides requires first the introduction of the thioacid (or sulfonyl azide) function on the nucleosides. In this unit, we report detailed synthetic protocols for the synthesis of 4'-thioacid nucleosides derivatives (Basic Protocol 1). Then, an aqueous biocompatible procedure is described for the implementation of the sulfo-click reaction to these modified nucleosides (Basic Protocol 2). Finally, the synthesis of a nucleoside bearing two orthogonal azides (i.e. a sulfonyl azide and an aliphatic azide) at the 5' and 3' positions respectively is described (Basic Protocol 3). The latter undergoes a sequential one-pot dual conjugation procedure *via* the sulfo-click and the CuAAC reactions leading to a bis-conjugate with high yield (Basic Protocol 4). This protocol based on previously published work (Clavé, Dursun, Vasseur, & Smietana, 2020) opens the way to a new efficient and easy to implement route to synthesize various doubly functionalized nucleoside derivatives.

STRATEGIC PLANNING

The protocols presented in this unit are based on several compounds which are not yet commercially available or which are extremely expensive and should therefore be synthesized. Thus, before carrying out the different basic protocols described in this unit, it is necessary to prepare the required reagents according to literature procedures (i.e. compounds **1a-d**, **5a-c**).

BASIC PROTOCOL 1

GENERAL PROTOCOL FOR THE SYNTHESIS OF THIOACID NUCLEOSIDE DERIVATIVES

Introductory paragraph:

This section provides details of a three-step procedure for the preparation of 4'-thioacid-nucleosides **4a-d** from protected nucleoside derivatives **1a-d** (Fig. 1). First, 5'-OH nucleosides **1a-d** are oxidized with TEMPO/BAIB in aqueous media to obtain carboxylic acids **2a-d** by adapting a procedure described by Epp and Widlanski. (Epp & Widlanski, 1999). Then, coupling the carboxylic acids **2a-d** to 9-fluorenylmethyl thiol (Fm-SH) using BOP as activating reagent in DMF led to the desired 4'-thioesters **3a-d**. Finally, both the 9-fluorenylmethyl and the TBDMS groups are cleaved in a single step using TBAF in THF leading to 4'-thioacid-nucleosides **4a-d** under the form of tetrabutylammonium (TBA) thioacetate salts.

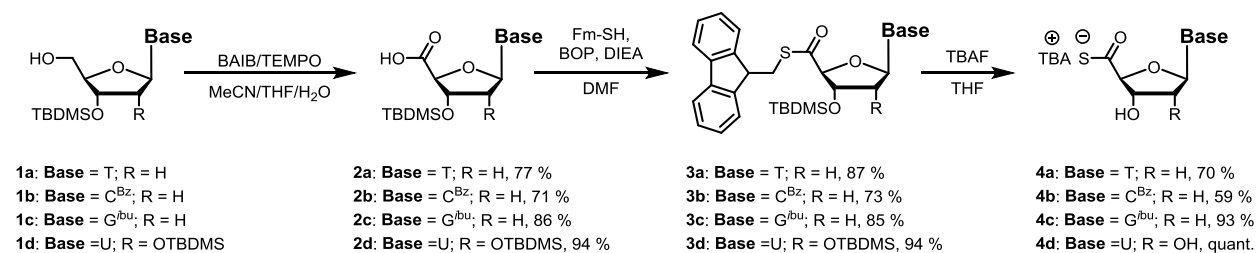


Figure 1 Synthesis of 4'-tetrabutylammonium thioacetate nucleoside derivatives **4a-d**.

Materials:

- 3'-O-TBDMS-thymidine **1a** (Patil, Mane, & Salunkhe, 1994)
- 3'-O-TBDMS-N-Bz-cytidine **1b** (Patil et al., 1994)
- 3'-O-TBDMS-N-*i*Bu-guanosine **1c** (Patil et al., 1994)
- 2',3'-O-TBDMS-uridine **1d** (Zhu, Williams, & Scott, 2000)
- (Diacetoxyiodo)benzene (BAIB, Sigma-Aldrich, CAS Number 3240-34-4)
- 2,2,6,6-Tetramethylpiperidine 1-oxyl (TEMPO, Sigma-Aldrich, CAS Number 2564-83-2)
- 9-fluorenylmethanethiol (Fm, Sigma-Aldrich, CAS Number 957753-00-3) (Crich, Sana, & Guo, 2007)
- (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, Sigma-Aldrich, CAS Number 56602-33-6)
- N,N*-Diisopropylethylamine, dry (DIEA, Sigma-Aldrich, CAS Number 7087-68-5)
- Tetrabutylammonium fluoride solution, 1M in THF (TBAF, Sigma-Aldrich, CAS Number 429-41-4)
- Acetonitrile, analytical grade (MeCN, Sigma-Aldrich, CAS Number 75-05-8)
- Dimethyl formamide, dry (DMF, Sigma-Aldrich, CAS Number 68-12-2)
- Dichloromethane, analytical grade (DCM, Sigma-Aldrich, CAS Number 75-09-2)
- Methanol, analytical grade (MeOH, Sigma-Aldrich, CAS Number 67-56-1)
- Ethyl acetate, analytical grade (AcOEt, Sigma-Aldrich, CAS Number 141-78-6)
- Cyclohexane, analytical grade (Sigma-Aldrich, CAS Number 110-82-7)
- Tetrahydrofuran, analytical grade (THF, Sigma-Aldrich, CAS Number 109-99-9)
- Toluene, analytical grade (Sigma-Aldrich, CAS Number 108-88-3)

Sodium sulfate (Na₂SO₄, CAS Number 7757-82-6), anhydrous
0.1% aqueous trifluoroacetic acid buffer (aq. TFA, 0.1%, v/v, pH 2.0)
Deionized water
Milli-Q water 18.2 MΩ.cm (e.g., from Millipore filtration system)
Brine (saturated aqueous NaCl solution)
10 w% aqueous citric acid solution
Saturated aqueous NaHCO₃ solution

500-, 100-, and 50-mL round-bottom flasks with ground-glass stoppers
Rubber septa
Magnetic stirrer
Magnetic stir bars
Rotary evaporator
Lyophilizer (freeze-drier) [e.g., FreeZone[®] bench top freeze dryer]
Vacuum source (water aspirator/membrane pump; vacuum oil pump)
UV lamp, 254 nm
Thin-layer chromatography (TLC) plate (Silica gel 60 F254 aluminum sheets, Merck)
HPLC system equipped with analytical RP-C18 column (e.g., RP-HPLC Accucore™ C18 aQ column, 2.6 μm, 4.6×50 mm)
Flash chromatography system [e.g., Biotage Isolera 1[®] system]
Pre-packed silica column [e.g., Büchi FlashPure[®]]
Stainless steel spatula
Cotton
Fritted glass funnel
Argon gas

Prepare 4'-carboxylic acid-nucleosides 2a-d

1. Add 8.46 mmol nucleoside **1** to a 500-mL round-bottomed flask equipped with a magnetic stir bar.

For **1a** : 3.0 g

For **1b** : 3.8 g

For **1c** : 3.8 g

For **1d** : 4.0 g

2. Add solvent and stir until complete dissolution.

For **1a** : 100 mL MeCN

For **1b** : 100 mL MeCN and 100 mL THF

For **1c** : 100 mL MeCN

For **1d** : 100 mL MeCN and 100 mL THF

3. Add 100 mL deionized water.

4. In one portion, add 5.45 g (17 mmol, 2 equivalents) BAIB.

- In one portion, add 265.6 mg (1.7 mmol, 0.2 equivalents) TEMPO.
- Stir reaction mixture 18 hr at room temperature with an open flask.
- Monitor reaction by TLC (9:1 DCM/MeOH); TLC analysis indicates the end of the reaction by disappearance of nucleoside **1** from the reaction mixture.

*If the reaction is not complete and does not seem to progress any more, add BAIB and TEMPO again in the same proportions as many times as necessary every 6 hr until complete disappearance of nucleoside **1**. Typically, the product **2** can partially precipitate in the reaction mixture.*

- Evaporate solvents under reduced pressure using a vacuum pump.
- Add 50 mL toluene and co-evaporate using rotary evaporator (three times).

*At this stage, nucleosides **1a** and **1c** can be partially purified by trituration with 100 mL Et₂O and filtration through a fritted glass funnels. Recover and evaporate Et₂O using rotary evaporator.*

- Purify product **2** on a silica gel column. Eluent: DCM/MeOH; 120-g flash column, linear gradient 0->20% in 40 minutes. Pool fractions containing product and evaporate using rotary evaporator.
- Dry the resulting residue 2 hr in vacuo.
- Characterize product by TLC, ¹H NMR, ¹³C NMR, and HRMS.

*For 4'-carboxylic acid-3'-O-TBDMS-thymidine **2a**. Yield of white solid 2.41 g (77%). ¹H NMR (400 MHz, MeOD): δ = 0.17 (d, J = 4.7 Hz, 6H), 0.95 (s, 9H), 1.90 (d, J = 1.1 Hz, 3H), 2.01-2.08 (m, 1H), 2.23-2.28 (m, 1H), 4.41 (s, 1H), 4.7 (d, J = 4.4 Hz, 1H), 6.44 (dd, J₁ = 9.2 Hz, J₂ = 5.3 Hz, 1H), 8.30 (d, J = 1.2 Hz, 1H) ppm. ¹³C NMR (100.6 MHz, MeOD): δ = -4.9, -4.8, 12.6, 18.8, 26.2, 40.5, 77.5, 86.8, 87.9, 111.7, 136.6, 152.5, 166.4, 174.2 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₁₆H₂₆N₂O₆Si: 371.1638; found 371.1643. R_f (9:1 DCM/MeOH) = 0.10.*

*For 4'-carboxylic acid-3'-O-TBDMS-N-Bz-cytidine **2b**. Yield of white solid 2.76 g (71%). ¹H NMR (400 MHz, DMSO) δ = 0.13 (s, 6H), 0.90 (s, 9H), 1.98-2.05 (m, 1H), 2.36-2.41 (m, 1H), 4.43 (s, 1H), 4.68 (bs, 1H), 6.29 (dd, J₁ = 8.6 Hz, J₂ = 5.0 Hz), 7.39 (d, J = 7.7 Hz, 1H), 7.49-8.0 (m, 5H), 8.72 (d, J = 7.4 Hz, 1H) ppm. ¹³C NMR (100.6 MHz, DMSO) δ = -5.0, -4.9, 17.7, 25.6, 40.4, 75.5, 85.1, 87.8, 96.4, 128.4, 132.7, 133.1, 145.0, 154.3, 163.1, 167.5, 172.0 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₂₂H₃₀N₃O₆Si : 460.1912; found 460.1908. R_f (9:1 DCM/MeOH) = 0.11.*

*For 4'-carboxylic acid-3'-O-TBDMS-N-iBu-guanosine **2c**. Yield of white solid 3.29 g (86%). ¹H NMR (400 MHz, DMSO) δ = 0.14 (s, 6H), 0.90 (s, 9H), 1.12 (d, J = 6.9 Hz, 6H), 2.35-2.40 (m, 1H), 2.56-2.63 (m, 1H), 2.73-2.80 (m, 1H), 4.35 (bs, 1H), 4.75 (bs, 1H), 6.32-6.35 (m, 1H), 8.29 (s, 1H), 11.64 (bs, 1H), 12.10 (bs, 1H) ppm. ¹³C NMR (100.6 MHz, DMSO) δ = -5.0, -5.0, 17.8, 18.8, 18.9, 25.7, 34.8, 39.4, 75.6, 83.9, 85.3, 120.0, 137.5, 148.3, 148.7, 154.9, 171.8, 180.2 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₂₀H₃₂N₅O₆Si: 466.2122; found 466.2121. R_f (9:1 DCM/MeOH) = 0.02.*

For 4'-carboxylic acid-2',3'-O-TBDMS-uridine **2d**. Yield of white solid 3.87 g (94%). ¹H NMR (400 MHz, DMSO) δ = -0.07 (s, 3H), 0.02 (s, 3H), 0.09 (s, 3H), 0.11 (s, 3H), 0.81 (s, 9H), 0.89 (s, 9H), 4.17-4.20 (m, 1H), 4.28-4.31 (m, 2H), 5.78 (dd, J₁ = 8.1, J₂ = 1.9 Hz, 1H), 5.96 (d, J = 6.6 Hz), 8.18 (d, J = 8.5 Hz, 1H), 11.41 (bs, 1H) ppm. ¹³C NMR (100.6 MHz, DMSO) δ = -5.3, -5.0, -4.8, 17.6, 17.8, 25.6, 25.7, 74.3, 74.7, 82.4, 87.2, 102.6, 140.3, 150.9, 163.0, 171.9 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₂₁H₃₉N₂O₇Si₂: 487.2296; found 487.2300. Rf (9:1 DCM/MeOH) = 0.15.

Prepare 4'-(9-fluorenylmethyl thioester)-nucleosides **3a-d**

1. Add 2.0 mmol 4'-carboxylic acid-nucleoside **2** to a 100-mL round-bottomed flask equipped with a magnetic stir bar.

For **2a** : 740 mg

For **2b** : 918 mg

For **2c** : 930 mg

For **2d** : 972 mg

2. Add 20 mL dry DMF and stir until complete dissolution.

3. In one portion, add 658 mg (3.1 mmol, 1.5 equivalents) 9-fluorenylmethanethiol.

4. In one portion, add 1.37 g (3.1 mmol, 1.5 equivalents) BOP reagent.

5. In one portion, add 715 μL (4.1 mmol, 2 equivalents) dry DIEA.

6. Stir reaction mixture 2 hr at room temperature under an argon atmosphere.

7. Monitor reaction by TLC.

For **3a** : (9:1 DCM/MeOH) and (8:2 Cyclohexane/AcOEt); TLC (9:1 DCM/MeOH) analysis indicates the end of the reaction by disappearance of **2a** from the reaction mixture. TLC (9:1 Cyclohexane/AcOEt) analysis indicates the formation of **3a**.

For **3b** : (9:1 DCM/MeOH) and (1:1 Cyclohexane/AcOEt); TLC (9:1 DCM/MeOH) analysis indicates the end of the reaction by disappearance of **2b** from the reaction mixture. TLC (9:1 Cyclohexane/AcOEt) analysis indicates the formation of **3b**.

For **3c** : (9:1 DCM/MeOH) and (3:7 Cyclohexane/AcOEt); TLC (9:1 DCM/MeOH) analysis indicates the end of the reaction by disappearance of **2c** from the reaction mixture. TLC (9:1 Cyclohexane/AcOEt) analysis indicates the formation of **3c**.

For **3d** : (9:1 DCM/MeOH) and (9:1 Cyclohexane/AcOEt); TLC (9:1 DCM/MeOH) analysis indicates the end of the reaction by disappearance of **2d** from the reaction mixture. TLC (9:1 Cyclohexane/AcOEt) analysis indicates the formation of **3d**.

8. Evaporate solvent close to dryness under reduced pressure using a vacuum pump.

The evaporation of DMF require a vacuum <20 mbar.

9. Add 100 mL AcOEt to the residue and wash with 100 mL aq. 10% citric acid, then 100 mL aq. sat. NaHCO₃ and 100 mL brine. Repeat wash step with brine three times to ensure the elimination of DMF of the organic layer.

10. Dry the organic layer over anhydrous Na₂SO₄ and filter off the drying agent on cotton.

11. Purify product **3** on a silica gel column. Eluent: Cyclohexane/AcOEt; 40-g flash column, linear gradient 0->40% in 40 minutes. Pool fractions containing product and evaporate using rotary evaporator.

12. Dry the resulting residue 2 hr in vacuo.

13. Characterize product by TLC, ¹H NMR, ¹³C NMR, and HRMS.

For 4'-(9-fluorenylmethyl thioester)-3'-O-TBDMS-thymidine **3a**. Yield of white solid 982 mg (87%). Rf (8:2 Cyclohexane/AcOEt) = 0.36. ¹H NMR (400 MHz, CDCl₃): δ = 0.03 (d, 6H), 0.86 (s, 9H), 1.43-1.49 (m, 1H), 1.81 (s, 3H), 1.95-2.00 (m, 1H), 3.78-3.81 (m, 3H), 4.24 (s, 1H, H_{4'}), 4.28 (t, J = 4.3 Hz, 1H, Fm), 6.39 (dd, J₁ = 9.8 Hz, J₂ = 4.6 Hz, 1H, H_{1'}), 7.27-7.73 (m, 9H), 9.02 (s, 1H) ppm. ¹³C NMR (100.6 MHz, CDCl₃): δ = -4.8, -4.7, 12.7, 18.0, 25.0, 30.9, 38.9, 46.8, 76.0, 86.1, 91.3, 111.4, 119.9, 120.0, 124.8, 127.2, 128.0, 128.1, 135.8, 141.6, 144.6, 144.7, 150.5, 163.8, 198.7 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₃₀H₃₇N₂O₅SSi: 565.2192; found 565.2194.

For 4'-(9-fluorenylmethyl thioester)-3'-O-TBDMS-N-Bz-cytidine **3b**. Yield of colorless oil 954 mg (73%). Rf (1:1 Cyclohexane/AcOEt) = 0.52. ¹H NMR (400 MHz, CDCl₃): δ = 0.01 (d, J = 12.1 Hz, 6H), 0.85 (s, 9H), 1.27-1.32 (m, 1H), 2.25-2.29 (m, 1H), 3.76-3.87 (m, 3H), 4.30-4.32 (m, 2H), 6.39-6.42 (m, 1H), 7.29-8.10 (m, 15H) ppm. ¹³C NMR (100.6 MHz, CDCl₃): δ = -4.8, 18.0, 25.8, 30.9, 40.2, 46.9, 76.2, 88.4, 91.9, 120.0, 120.1, 124.9, 125.0, 127.2, 127.4, 127.7, 128.0, 128.2, 129.2, 133.3, 141.6, 141.7, 144.6, 144.7, 145.3, 162.2, 198.5 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₃₆H₄₀N₃O₅SiS: 654.2458; found 654.2460.

For 4'-(9-fluorenylmethyl thioester)-3'-O-TBDMS-N-iBu-guanosine **3c**. Yield of white solid 1.12 g (85%). Rf (3:7 Cyclohexane/AcOEt) = 0.31. ¹H NMR (400 MHz, CDCl₃) δ = 0.02 (s, 3H), 0.03 (s, 3H), 0.82 (s, 9H), 1.17 (dd, J = 21.1, 7.2 Hz, 6H), 1.70-1.77 (m, 1H), 1.94-1.98 (m, 1H), 2.60 (hept, J = 7.1 Hz, 1H), 3.56-3.61 (m, 1H), 3.78-3.86 (m, 2H), 4.19 (s, 1H), 4.24 (t, J = 4.4 Hz, 1H), 6.19 (dd, J₁ = 10.0, J₂ = 5.0 Hz, 1H), 7.25-7.43 (m, 4H), 7.48-7.53 (m, 3H), 7.69-7.75 (m, 2H), 8.98 (bs, 1H), 12.05 (bs, 1H) ppm. ¹³C NMR (100.6 MHz, CDCl₃) δ = -4.9, -4.8, 17.9, 18.9, 19.1, 25.7, 30.7, 36.4, 39.1, 46.9, 76.1, 84.2, 91.8, 119.8, 120.0, 124.7, 125.2, 127.0, 127.6, 128.0, 128.3, 137.0, 141.3, 141.7, 144.5, 144.8, 147.8, 148.7, 155.7, 178.7, 199.1 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₃₄H₄₂N₅O₅SiS: 660.2676; found 660.2680.

For 4'-(9-fluorenylmethyl thioester)-2',3'-O-TBDMS-uridine **3d**. Yield of white solid 1.28 g (94%). Rf (9:1 Cyclohexane/AcOEt) = 0.30. ¹H NMR (400 MHz, CDCl₃) δ = -0.09 (s, 3H), 0.01 (s, 3H), 0.02 (s, 3H), 0.04 (s, 3H), 0.83 (s, 9H), 0.86 (s, 9H), 3.58-3.61 (m, 1H), 3.66-3.73 (m, 2H), 3.84-3.89 (m, 1H), 4.27 (bs, 1H), 4.30 (t, J = 4.4 Hz, 1H), 5.65 (dd, J₁ = 8.2, J₂ = 2.2 Hz, 1H), 6.08 (d, J = 7.3 Hz, 1H), 7.30-7.48 (m, 5H), 7.58 (t, J = 7.3 Hz, 2H), 7.75 (t, J = 7.7 Hz, 2H), 8.85 (s, 1H) ppm. ¹³C NMR

(100.6 MHz, CDCl₃) δ = -4.8, -4.7, -4.6, -4.3, 17.9, 18.0, 25.7, 25.8, 30.9, 46.5, 74.0, 74.9, 87.2, 89.1, 103.2, 119.9, 120.0, 124.7, 124.9, 127.2, 127.4, 128.1, 128.2, 140.2, 141.5, 141.6, 144.5, 144.8, 150.5, 163.0, 198.5 ppm. HRMS (ESI) m/z : [M+H]⁺ Calcd for C₃₅H₄₉N₂O₆SSi₂: 681.2850; found 681.2860.

Prepare 4'-tetrabutylammonium thioacetate-nucleosides 4

1. Add 329 μ mol **3** to a 50-mL round-bottomed flask equipped with a magnetic stir bar.

For **3a** : 186 mg

For **3b** : 215 mg

For **3c** : 217 mg

For **3d** : 224 mg

2. Add 8 mL dry THF and stir until complete dissolution.

3. In one portion, add 1M TBAF in THF.

For **3a** : 823 μ L (823 μ mol, 2.5 equivalents)

For **3b** : 823 μ L (823 μ mol, 2.5 equivalents)

For **3c** : 823 μ L (823 μ mol, 2.5 equivalents)

For **3d** : 1.15 mL (1.15 mmol, 3.5 equivalents)

4. Stir reaction mixture 2 hr at room temperature under an argon atmosphere.

5. Monitor reaction by TLC (8:2 Cyclohexane/AcOEt) and RP-HPLC analysis; TLC analysis indicates the disappearance of **3** from the reaction mixture. RP-HPLC analysis indicates the formation of **4**.

RP-HPLC (Accucore™ C18 aQ column, 2.6 μ m, 4.6×50 mm) with CH₃CN and 0.1% aqueous trifluoroacetic acid (aq. TFA, 0.1%, v/v, pH 2.0) as the eluents [0% CH₃CN (2 min), followed by linear gradient from 0 to 100% (25 min) of CH₃CN] at a flow rate of 1 mL.min⁻¹. Triple UV detection was achieved at 210, 260 and 650 nm (Fig. 2).

Be aware that the fluorenylmethanethioester is the first protecting group cleaved within ten minutes. The TBDMS group is cleaved after prolonged reaction time. TLC analyzes are difficult to exploit due to the high polarity of the product and the presence of tetrabutylammonium salts. Thus, RP-HPLC analyses are required to ensure the deprotection of the TBDMS groups by observing a single nucleoside peak at 260 nM (Fig 2). Noteworthy, the peak corresponding to the fluvone is variable due to partial precipitation during sample preparation in aqueous media.

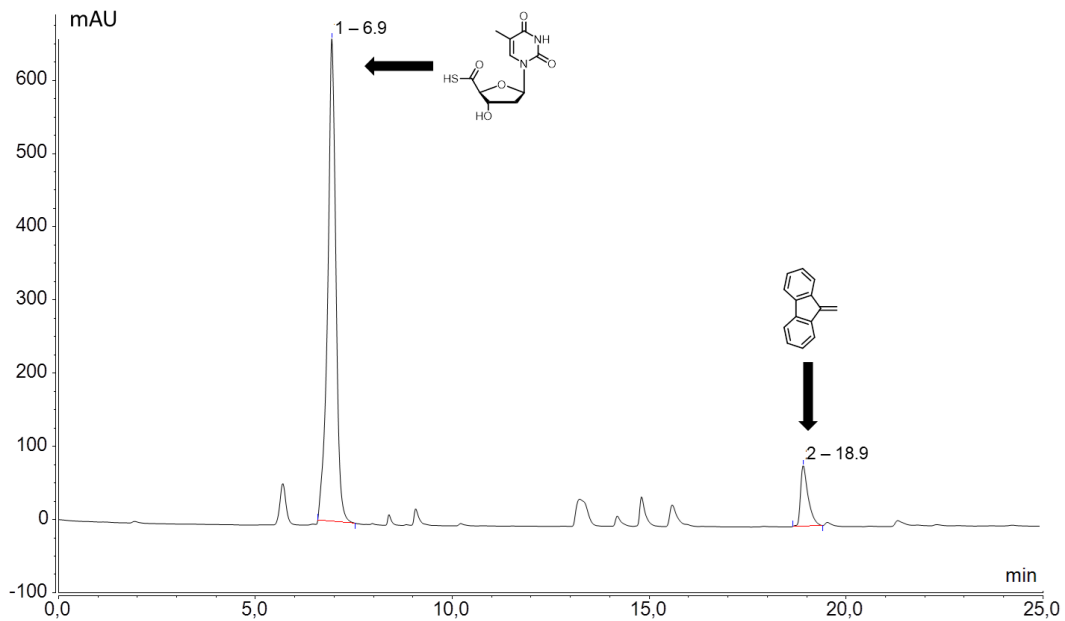


Figure 2.1 Chromatogram of crude deprotection of **3a** at 260 nm. t_R (product) = 6.9 min, t_R (fluvene) = 18.9 min.

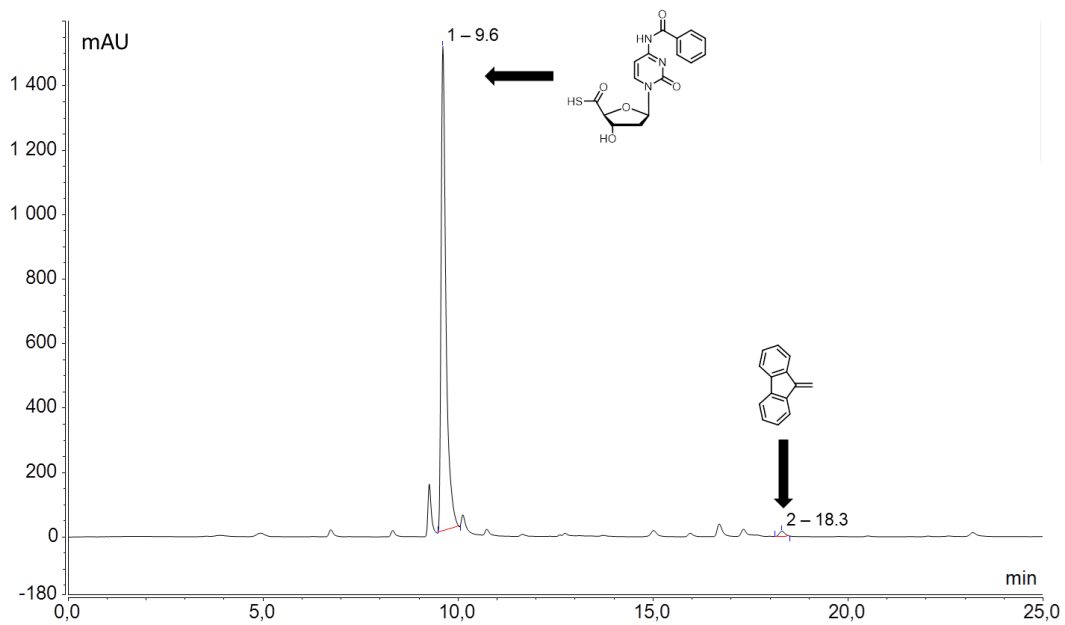


Figure 2.2 Chromatogram of crude deprotection of **3b** at 260 nm. t_R (product) = 9.6 min, t_R (fluvene) = 18.3 min.

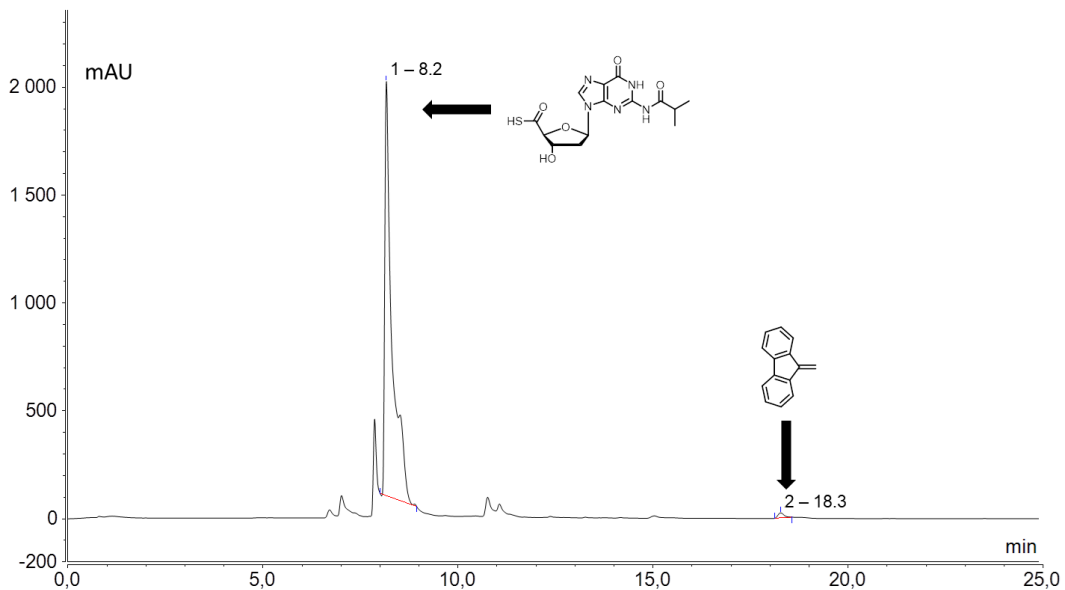


Figure 2.3 Chromatogram of crude deprotection of **3c** at 260 nm. t_R (product) = 8.2 min, t_R (fluvene) = 18.3 min.

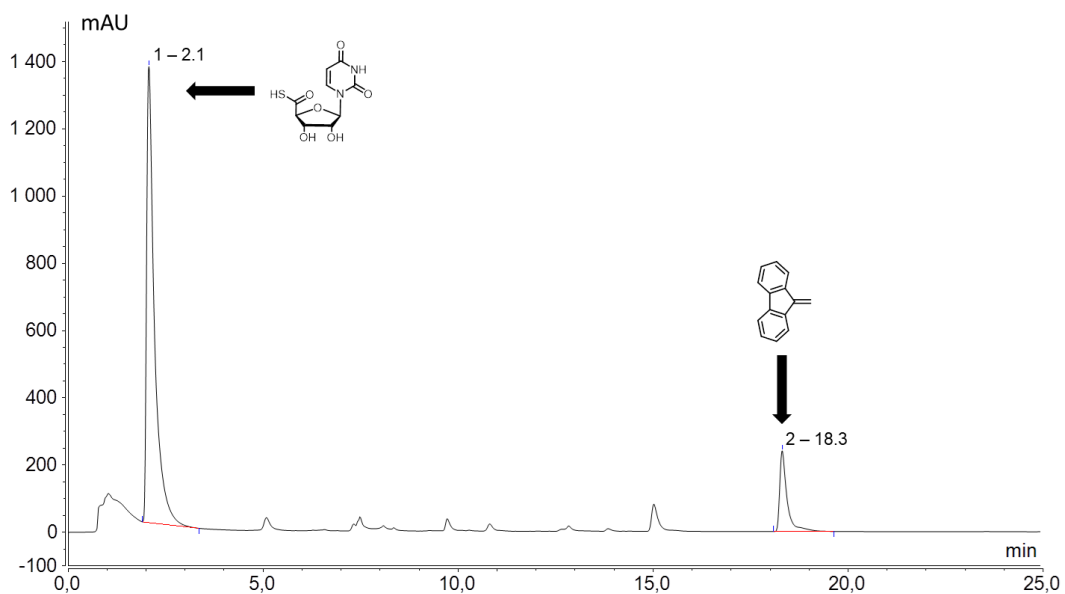


Figure 2.4 Chromatogram of crude deprotection of **3d** at 260 nm. t_R (product) = 2.1 min, t_R (fluvene) = 18.3 min.

6. Evaporate solvent under reduced pressure using a vacuum pump.

7. Purify product **4** on a silica gel column. Eluent: DCM/MeOH; 24-g flash column, linear gradient 0->20% in 40 minutes. Pool fractions containing product and evaporate using rotary evaporator.

Noteworthy, the product **4** is isolated as a tetrabutylammonium thioacetate salt. The latter is stable for month at -20°C but is likely to degrade at room temperature.

8. Dry the resulting residue 2 hr in vacuo.

9. Ad 10 mL of Milli-Q water and lyophilize.

10. Characterize product by TLC, ¹H NMR, ¹³C NMR, and HRMS.

For 4'-tetrabutylammonium thioacetate-thymidine **4a**. Yield of white amorphous powder 118 mg (70%). Rf (8:2 DCM/MeOH) = 0.10. ¹H NMR (400 MHz, CD₃CN): δ = 0.94 (t, J = 7.5 Hz, 14H), 1.29-1.38 (m, 10H), 1.56-1.64 (m, 10H), 1.83 (s, 3H), 2.02-2.16 (m, 2H), 3.10-3.15 (m, 10H), 4.26 (q, J = 4.6 Hz, 1H), 4.46 (d, J = 3.7 Hz, 1H), 6.12 (t, J = 6.0 Hz, 1H), 8.69 (s, 1H) ppm. ¹³C NMR (100.6 MHz, CD₃CN): δ = 12.8, 13.8, 20.3, 24.5, 39.9, 59.2, 75.1, 86.3, 95.1, 109.9, 139.2, 151.7, 165.4, 213.2 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₁₀H₁₁N₂O₅S: 271.0389; found 271.0393.

For 4'-tetrabutylammonium thioacetate-N-Bz-cytidine **4b**. Yield of white amorphous powder 117 mg (59%). Rf (8:2 DCM/MeOH) = 0.12. ¹H NMR (500 MHz, MeOD): δ = 1.01 (t, 12H, J = 7.4 Hz), 1.36-1.46 (m, 8H), 1.62-1.70 (m, 8H), 1.95-2.01 (m, 1H), 2.47-2.52 (m, 1H), 3.21-3.26 (m, 8H), 4.63 (bs, 1H), 4.79 (s, 1H), 6.44 (dd, 1H, J₁ = 5.4 Hz, J₂ = 8.4 Hz), 7.48-7.66 (m, 4H), 7.98 (d, J = 8.0 Hz, 2H), 9.47 (d, J = 7.4 Hz, 1H) ppm. ¹³C NMR (125.7 MHz, MeOD): δ = 14.0, 20.7, 24.8, 41.1, 59.5, 77.2, 90.0, 97.3, 98.0, 129.1, 129.8, 134.0, 134.7, 148.4, 157.9, 164.6, 169.0, 217.1 ppm. HRMS (ESI) m/z: [M-H]⁻ Calcd for C₁₆H₁₄N₃O₅S: 360.0654; found 360.0657.

For 4'-tetrabutylammonium thioacetate-N-iBu-guanosine **4c**. Yield of white amorphous powder 186 mg (93%). Rf (8:2 DCM/MeOH) = 0.10. ¹H NMR (400 MHz, CD₃CN) δ = 0.95 (t, J = 7.4 Hz, 13H), 1.15-1.18 (m, 6H), 1.34 (hex., J = 7.3 Hz, 9H), 1.55-1.63 (m, 9H), 2.30-2.52 (m, 2H), 2.78 (quint., J = 7.0 Hz, 1H), 3.07-3.11 (m, 9H), 4.45-4.49 (m, 1H), 4.56 (d, J = 4.0 Hz, 1H), 4.70 (bs, 1H), 6.22 (t, J = 6.5 Hz, 1H), 8.41 (s, 1H), 10.14 (bs, 1H), 12.01 (bs, 1H) ppm. ¹³C NMR (100.6 MHz, CD₃CN) δ = 13.8, 19.3, 20.3, 24.3, 36.5, 40.2, 59.3, 75.3, 84.5, 95.1, 121.6, 139.6, 149.0, 149.4, 156.6, 181.2, 213.2 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₁₄H₁₆N₅O₅S : 366.0872; found 366.0873.

For 4'-tetrabutylammonium thioacetate-uridine **4d**. Yield of white amorphous powder 170 mg (quant.). Rf (8:2 DCM/MeOH) = 0.35. ¹H NMR (400 MHz, CD₃CN) δ = 0.96 (t, J = 7.3 Hz, 14H), 1.34 (hex., J = 7.2 Hz, 10H), 1.56-1.64 (m, 10H), 3.08-3.12 (m, 10H), 3.87-3.88 (m, 1H), 3.92 (bs, 1H), 4.12 (t, J = 4.0 Hz, 1H), 4.54 (d, J = 5.7 Hz, 1H), 4.90 (bs, 1H), 5.59 (d, J = 8.1 Hz, 1H), 5.73 (d, J = 3.5 Hz, 1H), 8.59 (d, J = 8.1 Hz, 1H), 9.26 (bs, 1H) ppm. ¹³C NMR (100.6 MHz, CD₃CN) δ = 13.8, 20.3, 24.3, 59.3, 74.6, 75.6, 90.1, 91.5, 101.9, 142.7, 151.7, 164.5, 212.6 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₉H₁₁N₂O₆S: 275.0338; found 275.0336.

BASIC PROTOCOL 2

IMPLEMENTATION OF THE SULFO-CLICK REACTION

The 4'-tetrabutylammonium thioacetate-nucleosides **4a-d** (basic protocol 1) and the sulfonyl azide derivatives **5a-c** are used to implement the sulfo-click reaction in an aqueous buffered medium without the addition of any other reactant (Fig. 3). For examples of synthesis of sulfonyl azide derivatives see (Clavé et al., 2020; Merx, Brouwer, Rijkers, & Liskamp, 2005; Rohmer, Mannuthodikayil, & Wittmann, 2015; Yim et al., 2010).

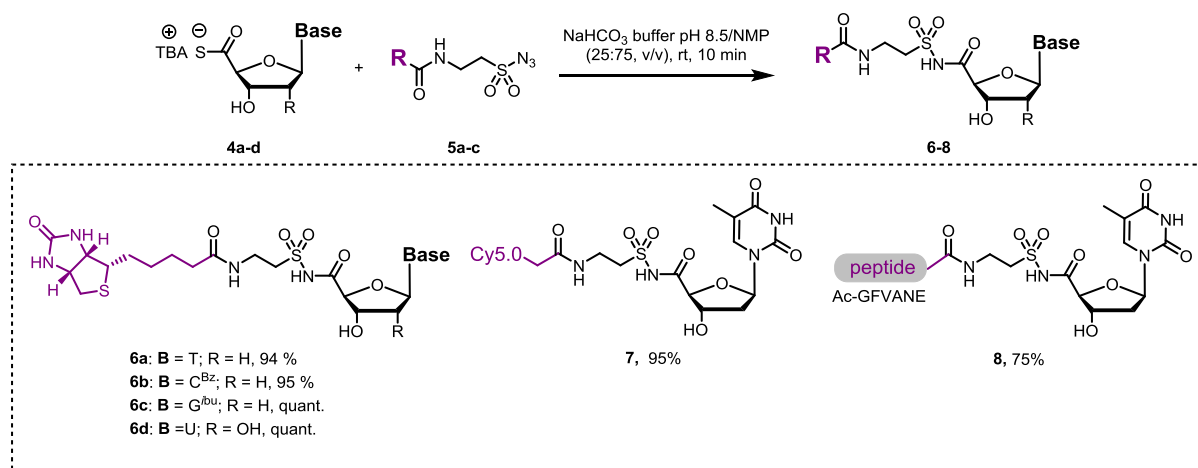


Figure 3 Scope of the sulfo-click reaction applied to nucleosides.

Materials:

4'-tetrabutylammonium thioacetate-nucleosides **4a-d** (basic protocol 1)

Sulfonyl azide derivatives **5a-c** (Clavé et al., 2020)

1-Methyl-2-pyrrolidinone, anhydrous (NMP, Sigma-Aldrich, CAS Number 872-50-4)

Acetonitrile, analytical grade (MeCN, Sigma-Aldrich, CAS Number 75-05-8)

Milli-Q water 18.2 MΩ.cm (e.g., from Millipore filtration system)

100 mM NaHCO₃ buffer, pH 8.5

0.1% aqueous trifluoroacetic acid buffer (aq. TFA, 0.1%, v/v, pH 2.0)

1.5-mL Eppendorf Safe-Lock Tubes

Vortex (e.g., Vortex-Genie[®] 2T mixer)

Microcentrifuge (Eppendorf)

Micropipet [e.g., Gilson[™] PIPETMAN Classic[™]]

HPLC system equipped with analytical RP-C18 column (e.g., RP-HPLC Accucore[™] C18 aQ column, 2.6 μm, 4.6×50 mm)

Lyophilizer (freeze-drier) [e.g., FreeZone[®] bench top freeze dryer]

Vacuum source (water aspirator/membrane pump; vacuum oil pump)

Flash chromatography system [e.g., Biotage Isolera 1[®] system]

Pre-packed RP-silica column [e.g., Büchi FlashPure[®]]

Stainless steel spatula

1. Add 15 μmol 4'-tetrabutylammonium thioacetate-nucleoside **4** to a first 1.5-mL Eppendorf Safe-Lock Tube.

For **4a** : 7.7 mg **4a**

For **4b** : 9.0 mg **4b**

For **4c** : 9.1 mg **4c**

For **4d** : 7.7 mg **4d**

2. Add 200 μL dry NMP and vortex until complete dissolution. Centrifuge the microtube.

3. Add 10 μmol sulfonyl azide derivative **5** to a second 1.5-mL Eppendorf Safe-Lock Tube.

For **5a** : 3.8 mg

For **5b** : 9.0 mg

For **5c** : 8.1 mg

4. Add 200 μL dry NMP and vortex until complete dissolution. Centrifuge the microtube.

5. Mix the two solutions using a micropipet and immediately add 100 μL of 0.1 M aq. NaHCO_3 buffer.

6. Vortex for 10 sec and place the microtube at rt without agitation for 10 min.

At this point, you can visually check that the reaction occurs after five minutes by observing nitrogen bubbles along with elemental Sulphur precipitates within the mixture.

7. Monitor the reaction by RP-HPLC analysis using the following gradient: (Accucore™ C18 aQ column, 2.6 μm , 4.6 \times 50 mm) with CH_3CN and 0.1% aqueous trifluoroacetic acid (aq. TFA, 0.1%, v/v, pH 2.0) as the eluents [0% CH_3CN (2 min), followed by linear gradient from 0 to 100% (25 min) of CH_3CN] at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. Triple UV detection was achieved at 210, 260 and 650 nm.

8. Take up the crude in a 10 mL syringe; dilute it by withdrawing 9 mL of aq. TFA, 0.1% and purify product on a RP-silica gel column. Eluent: Aq. TFA, 0.1% / MeCN; 12-g flash column, linear gradient 0- \rightarrow 40% in 40 minutes. Pool fractions containing product and lyophilize.

Alternatively, depending on the synthesis scale envisaged and the material available, the product can be purified via semi-preparative RP-HPLC using the same gradient and eluents.

10. Characterize product by RP-HPLC, ^1H NMR, ^{13}C NMR, and HRMS.

*For Biotin-thymidine conjugate **6a**. Yield of white amorphous powder 5.5 mg (94%). $T_R = 8.4$ min. ^1H NMR (400 MHz, DMSO): $\delta = 1.23\text{-}1.64$ (m, 6H), 1.79 (s, 3H), 2.04-2.15 (m, 4H), 2.57 (d, $J = 12.2$ Hz, 1H), 2.79-2.83 (dd, 1H, $J_1 = 5.2$ Hz, $J_2 = 12.6$ Hz), 3.01-3.10 (m, 1H), 3.35-3.59 (m, 4H), 4.10-4.13 (m, 1H), 4.28-4.31 (m, 1H), 4.38 (s, 1H), 4.48 (bs, 1H), 6.31-6.35 (m, 1H), 6.40 (bs, 2H), 7.91 (s, 1H), 8.01 (t, $J = 5.4$ Hz, 1H), 11.4 (s, 1H), 12.2 (bs, 1H) ppm. ^{13}C NMR (100.6 MHz, DMSO): $\delta = 12.4, 25.0, 28.0, 28.2, 33.2, 35.0, 38.1, 39.8, 51.5, 55.4, 59.2, 61.0, 73.5, 84.7, 85.8, 109.6, 136.4,$*

150.6, 162.7, 163.7, 170.9, 172.4 ppm. HRMS (ESI) m/z : $[M+H]^+$ Calcd for $C_{22}H_{33}N_6O_9S_2$: 589.1755; found 589.1750.

For Biotin-cytidine conjugate **6b**. Yield of white amorphous powder 6.4 mg (95%). $T_R = 10.9$ min. 1H NMR (500 MHz, DMSO): $\delta = 1.23$ -1.62 (m, 6H), 2.04-2.14 (m, 3H), 2.36-2.42 (m, 1H), 2.55 (d, $J = 12.4$ Hz, 1H), 2.79 (dd, $J_1 = 12.4$, $J_2 = 5.1$ Hz, 1H), 3.05-3.09 (m, 1H), 3.42-3.58 (m, 4H), 4.09-4.11 (m, 1H), 4.27-4.29 (m, 1H), 4.49 (s, 1H), 4.52 (s, 1H), 5.82 (bs, 1H), 6.29-6.32 (m, 1H), 6.37 (bs, 1H), 6.43 (bs, 1H), 7.42 (bs, 1H), 7.52 (t, $J = 7.8$ Hz, 2H), 7.63 (t, $J = 7.4$ Hz, 1H), 8.01 (d, $J = 7.5$ Hz, 2H), 8.06 (t, $J = 5.8$ Hz, 1H), 8.65 (s, 1H), 11.3 (bs, 1H), 12.3 (bs, 1H) ppm. ^{13}C NMR (125.7 MHz, DMSO): $\delta = 25.1, 28.0, 28.2, 33.2, 35.1, 39.1, 39.6, 51.4, 55.4, 59.2, 61.0, 73.8, 85.5, 88.5, 96.5, 128.5, 132.9, 133.1, 145.9, 154.6, 162.8, 163.3, 167.4, 171.0, 172.5$ ppm. HRMS (ESI) m/z : $[M+H]^+$ Calcd for $C_{28}H_{36}N_7O_9S_2$: 678.2010; found 678.2008.

For Biotin-guanosine conjugate **6c**. Yield of white amorphous powder 6.8 mg (quant.). $T_R = 9.9$ min. 1H NMR (400 MHz, DMSO) $\delta = 1.12$ (d, $J = 6.8$ Hz, 6H), 1.28-1.63 (m, 6H), 2.03-2.07 (m, 3H), 2.37-2.42 (m, 1H), 2.53-2.60 (m, 2H), 2.74-2.82 (m, 2H), 3.05-3.10 (m, 1H), 3.37-3.57 (m, 4H), 4.10-4.13 (m, 1H), 4.28-4.31 (m, 1H), 4.47 (s, 1H), 4.61-4.63 (m, 1H), 6.37-6.40 (m, 1H), 8.02 (t, $J = 5.6$ Hz, 1H), 8.34 (s, 1H), 11.7 (s, 1H), 12.1 (bs, 1H) ppm. ^{13}C NMR (100.6 MHz, DMSO) $\delta = 18.9, 25.0, 28.0, 28.2, 33.2, 34.8, 35.1, 38.9, 39.5, 51.5, 55.4, 59.3, 61.1, 73.6, 84.2, 85.3, 119.8, 137.5, 148.3, 148.6, 154.8, 162.8, 170.6, 172.5, 180.2$ ppm. HRMS (ESI) m/z : $[M+H]^+$ Calcd for $C_{26}H_{38}N_9O_9S_2$: 684.2234; found 684.2240.

For Biotin-uridine conjugate **6d**. Yield of white amorphous powder 5.9 mg (quant.). $T_R = 7.6$ min. 1H NMR (600 MHz, DMSO) $\delta = 1.23$ -1.63 (m, 6H), 2.05 (t, $J = 7.6$ Hz, 2H), 2.52 (d, $J = 9.5$ Hz, 1H), 2.57 (d, $J = 12.2$ Hz, 1H), 2.81 (dd, $J_1 = 12.6$ Hz, $J_2 = 5.2$ Hz, 1H), 3.07-3.10 (m, 1H), 3.35-3.58 (m, 4H), 4.10-4.15 (m, 3H), 4.29-4.31 (m, 1H), 4.42 (d, $J = 2.5$ Hz, 1H), 5.76 (dd, $J_1 = 8.3$ Hz, $J_2 = 2.2$ Hz, 1H), 5.92 (d, $J = 6.4$ Hz, 1H), 6.39 (bs, 1H), 8.00 (t, $J = 5.8$ Hz, 1H), 8.07 (d, $J = 8.0$ Hz, 1H), 11.38 (s, 1H) ppm. ^{13}C NMR (150.6 MHz, DMSO) $\delta = 25.0, 28.0, 28.2, 33.1, 35.0, 40.1, 51.5, 55.4, 59.2, 61.0, 72.8, 72.9, 82.3, 88.0, 102.4, 140.7, 150.9, 162.7, 163.0, 170.8, 172.5$ ppm. HRMS (ESI) m/z : $[M+H]^+$ Calcd for $C_{21}H_{31}N_6O_{10}S_2$: 591.1538; found 591.1536.

For Cy5.0-thymidine conjugate **7**. Yield of deep blue amorphous powder 10.6 mg (95%). $T_R = 10.8$ min. HRMS (ESI) m/z : $[M+H]^+$ Calcd for $C_{45}H_{57}N_6O_{14}S_3$: 1001.3095; found 1001.3095. Cy5.0-thymidine conjugate **7** was only characterized by RP-HPLC and HRMS analysis.

Hexapeptide-thymidine conjugate **8**. Yield of white amorphous powder 7.6 mg (75%). $T_R = 9.3$ min. 1H NMR (500 MHz, DMSO): $\delta = 0.82$ -0.86 (m, 6H), 1.20-1.23 (m, 4H), 1.68-1.75 (m, 1H), 1.78 (s, 3H), 1.81 (s, 3H), 2.06-2.16 (m, 6H), 2.43-2.47 (m, 1H), 2.52-2.58 (m, 1H), 2.74-2.79 (m, 1H), 2.97-3.01 (m, 1H), 3.52-3.69 (m, 4H), 4.03-4.08 (m, 1H), 4.13-4.16 (m, 1H), 4.25-4.31 (m, 1H), 4.38 (s, 1H), 4.43-4.48 (m, 2H), 4.57-4.62 (m, 1H), 5.75 (bs, 1H), 6.32-6.35 (m, 1H), 7.00 (s, 1H), 7.11 (s, 1H), 7.16-7.25 (m, 5H), 7.37 (s, 1H), 7.47 (s, 1H), 7.92-8.10 (m, 7H), 11.4 (s, 1H) ppm. ^{13}C NMR (125.7 MHz, DMSO): $\delta = 12.5, 18.0, 18.3, 19.3,$

22.4, 27.5, 30.5, 31.7, 33.3, 36.9, 37.5, 38.1, 41.9, 48.2 49.8, 51.4, 52.1, 53.6, 57.6, 73.6, 84.7, 85.8, 109.7, 126.3, 128.0, 129.3, 136.4, 137.7, 150.7, 163.7, 168.9, 169.6, 170.6, 170.9, 171.0, 171.8, 172.0, 172.2, 173.2 ppm. HRMS (ESI) m/z : $[M+H]^+$ Calcd for $C_{42}H_{61}N_{12}O_{16}S$: 1021.4049; found 1021.4057.

BASIC PROTOCOL 3

SYNTHESIS OF 3'-AZIDO-4'-(CARBOXAMIDO)ETHANE-SULFONYL AZIDE-3'-DEOXYTHYMIDINE

The protocol of this section describes in details the synthesis of the bis-azido thymidine **11**. The presented reaction strategy started with commercially available azidothymidine **9** which underwent oxidation at the 5'-OH with TEMPO/BAIB in a mixture of MeCN and water to obtain compound **10** (Fig. 4). Then, the coupling of carboxylic acid **10** to 2-aminoethane-sulfonyl azide using BOP reagent in DMF led to the desired bis-azido thymidine **11** (Fig. 4).

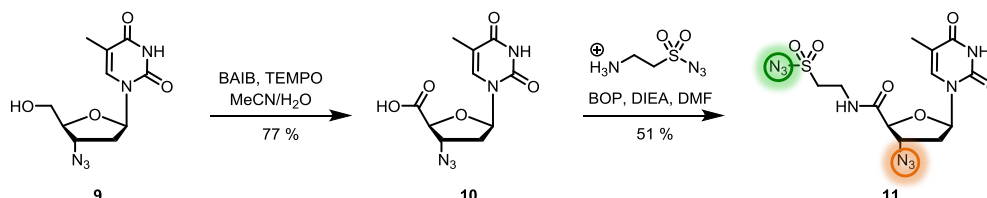


Figure 4 Synthesis of 3'-azido-4'-(carboxamido)ethane-sulfonyl azide-3'-deoxythymidine **11**.

Materials:

3'-Azido-3'-deoxythymidine (AZT, Sigma-Aldrich, CAS Number 30516-87-1)
2-aminoethane-sulfonyl azide hydrobromide (Brouwer, Merck, Dabrowska, Rijkers, & Liskamp, 2006)
(Diacetoxyiodo)benzene (BAIB, Sigma-Aldrich, CAS Number 3240-34-4)
2,2,6,6-Tetramethylpiperidine 1-oxyl (TEMPO, Sigma-Aldrich, CAS Number 2564-83-2)
(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, Sigma-Aldrich, CAS Number 56602-33-6)
N,N-Diisopropylethylamine, dry (DIEA, Sigma-Aldrich, CAS Number 7087-68-5)
Acetonitrile, analytical grade (MeCN, Sigma-Aldrich, CAS Number 75-05-8)
Dimethyl formamide, dry (DMF, Sigma-Aldrich, CAS Number 68-12-2)
Dichloromethane, analytical grade (DCM, Sigma-Aldrich, CAS Number 75-09-2)
Methanol, analytical grade (MeOH, Sigma-Aldrich, CAS Number 67-56-1)
Ethyl acetate, analytical grade (AcOEt, Sigma-Aldrich, CAS Number 141-78-6)
Toluene, analytical grade (Sigma-Aldrich, CAS Number 108-88-3)
Sodium sulfate (Na₂SO₄, Sigma-Aldrich, CAS Number 7757-82-6), anhydrous
Deionized water
Brine (saturated aqueous NaCl solution)
10 w% aqueous citric acid solution
Saturated aqueous NaHCO₃ solution

20- and 10-mL round-bottom flasks with ground-glass stoppers
Rubber septa
Magnetic stirrer
Magnetic stir bars
Rotary evaporator
Vacuum source (water aspirator/membrane pump; vacuum oil pump)
UV lamp, 254 nm
Thin-layer chromatography (TLC) plate (Silica gel 60 F254 aluminum sheets, Merck)

Flash chromatography system [e.g., Biotage Isolera 1[®] system]
Pre-packed silica column [e.g., Büchi FlashPure[®]]
Stainless steel spatula
Cotton
Argon gas

Prepare 4'-carboxylic acid-3'-azido-3'-deoxythymidine 10

1. Add 115 mg (430 μ mol) AZT **9** to a 20-mL round-bottomed flask equipped with a magnetic stir bar.
2. Add 5 mL MeCN and stir until complete dissolution.
3. Add 5 mL deionized water.
4. In one portion, add 277 mg (860 μ mol, 2 equivalents) BAIB.
5. In one portion, add 13 mg (86 μ mol, 0.2 equivalents) TEMPO.
6. Stir reaction mixture 18 hr at room temperature with an open flask.
7. Monitor reaction by TLC (9:1 DCM/MeOH); TLC analysis indicates the end of the reaction by disappearance of nucleoside **9** from the reaction mixture.

*If the reaction is not complete and does not seem to progress any more, add BAIB and TEMPO again in the same proportions as many times as necessary every 6 hr until complete disappearance of AZT **9**.*

8. Evaporate solvents under reduced pressure using a vacuum pump.
9. Add 10 mL toluene and co-evaporate using rotary evaporator (three times).
10. Purify product on a silica gel column. Eluent: DCM/MeOH; 24-g flash column, linear gradient 0->20% in 40 minutes. Pool fractions containing product and evaporate using rotary evaporator.
11. Dry the resulting residue 2 hr in vacuo.
13. Characterize product **10** by TLC, ¹H NMR, ¹³C NMR, and HRMS.

Yield of white powder 93 mg (77%). R_f (9:1 DCM/MeOH) = 0.03. ¹H NMR (400 MHz, MeOD) δ = 1.89 (s, 3H), 2.26-2.45 (m, 1H), 4.52 (d, J = 2,8 Hz, 1H), 4.62-4.65 (m, 1H), 6.26-6.29 (m, 1H), 8.08 (s, 1H) ppm. ¹³C NMR (100.6 MHz, MeOD) δ = 12.6, 37.3, 65.5, 83.0, 87.8, 111.7, 138.3, 152.3, 166.3, 173.2 ppm. HRMS (ESI) m/z: [M+H]⁺ Calcd for C₁₀H₁₂N₅O₅: 282.0833; found 282.0851.

Prepare 3'-azido-4'-(carboxamido)ethane-sulfonyl azide-3'-deoxythymidine **11**

1. Add 60 mg (214 μmol) 4'-carboxylic acid-3'-azido-3'-deoxythymidine **10** to a 10-mL round-bottomed flask equipped with a magnetic stir bar.
2. Add 1 mL dry DMF and stir until complete dissolution.
3. In one portion, add 74 mg (321 μmol , 1.5 equivalents) 2-aminoethane-sulfonyl azide hydrobromide.
4. In one portion, add 142 mg (321 μmol , 1.5 equivalents) BOP reagent.
5. In one portion, add 75 μL (428 μmol , 2 equivalents) dry DIEA.
6. Stir reaction mixture 2 hr at room temperature under an argon atmosphere.
7. Monitor reaction by TLC (9:1 DCM/MeOH) and (5:5 DCM/AcOEt); TLC (9:1 DCM/MeOH) analysis indicates the end of the reaction by disappearance of 4'-carboxylic acid-3'-azido-3'-deoxythymidine **10** from the reaction mixture. TLC (5:5 DCM/AcOEt) analysis indicates the formation of 3'-azido-4'-(carboxamido)ethane-sulfonyl azide-3'-deoxythymidine **11**.
8. Add 50 mL AcOEt to the crude and wash with 50 mL aq. 10% citric acid, then 50 mL aq. sat. NaHCO_3 and 50 mL brine. Repeat wash step with brine three times to ensure the elimination of DMF of the organic layer.
9. Dry the organic layer over anhydrous Na_2SO_4 and filter off the drying agent on cotton.
10. Purify product on a silica gel column. Eluent: DCM/AcOEt; 12-g flash column, linear gradient 0-40% in 40 minutes. Pool fractions containing product and evaporate using rotary evaporator.
11. Dry the resulting residue 2 hr in vacuo.
12. Characterize product **11** by TLC, ^1H NMR, ^{13}C NMR, and HRMS.

Yield of white powder 45 mg (51%). R_f (9:1 DCM/AcOEt) = 0.15. ^1H NMR (400 MHz, CDCl_3): δ = 1.91 (s, 3H), 2.21-2.27 (m, 1H), 2.59-2.66 (m, 1H), 3.56-4.04 (m, 4H), 4.36 (d, J = 3.6 Hz, 1H), 4.56 (quin., J = 3.7 Hz), 6.03 (t, J = 6.8 Hz, 1H), 7.19 (s, 1H), 7.68 (t, J = 5.8 Hz, 1H), 9.56 (s, 1H) ppm. ^{13}C NMR (100.6 MHz, CDCl_3): δ = 12.4, 34.0, 35.2, 54.3, 63.6, 83.4, 88.9, 112.2, 137.6, 150.8, 163.9, 170.0 ppm. HRMS (ESI) m/z : $[M+H]^+$ Calcd for $\text{C}_{12}\text{H}_{16}\text{N}_9\text{O}_6\text{S}$: 414.0939; found 414.0956.

BASIC PROTOCOL 4

DETAILED SYNTHETIC PROCEDURE FOR ONE-POT DOUBLE CLICK CONJUGATIONS

The 3'-azido-4'-(carboxamido)ethane-sulfonyl azide-3'-deoxythymidine **11** (basic protocol 4) is subjected to a biocompatible one-pot double-labelling procedure (Fig. 5). The sulfonyl azide moiety is first engaged in a sulfo-click reaction with 4'-tetrabutylammonium thioacetate-uridine **4d** (basic protocol 1) in a buffered aqueous media without the addition of any other reactant. Once the reaction is completed, the biotin bearing a terminal alkyne is used to perform a CuAAC reaction leading to the desired bis-conjugate **13**.

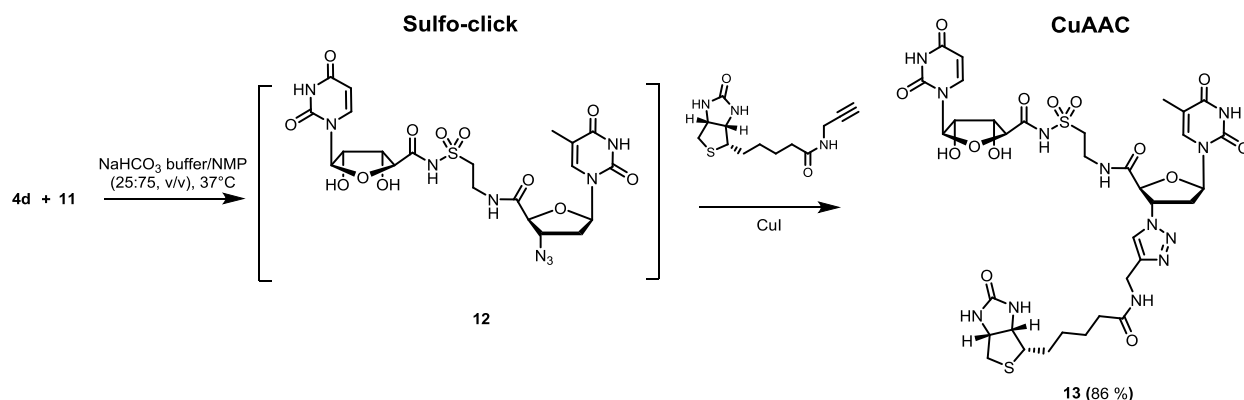


Figure 5 Synthesis of the deoxythymidine bis-conjugate **13**.

Materials:

- 4'-tetrabutylammonium thioacetate-uridine **4d** (basic protocol 1)
 - 3'-azido-4'-(carboxamido)ethane-sulfonyl azide-3'-deoxythymidine **11** (basic protocol 4)
 - 1-Methyl-2-pyrrolidinone, anhydrous (Sigma-Aldrich, CAS Number 872-50-4)
 - Acetonitrile, analytical grade (MeCN, Sigma-Aldrich, CAS Number 75-05-8)
 - Milli-Q water 18.2 M Ω .cm (e.g., from Millipore filtration system)
 - 100 mM NaHCO_3 buffer, pH 8.5
 - 0.1% aqueous trifluoroacetic acid buffer (aq. TFA, 0.1%, v/v, pH 2.0)
 - N,N*-Diisopropylethylamine, dry (DIEA, Sigma-Aldrich, CAS Number 7087-68-5)
 - Copper(I) iodide (CuI, Sigma-Aldrich, CAS Number 7681-65-4)
 - Biotin alkyne (Lumiprobe, CAS Number 773888-45-2)
-
- 1.5-mL Eppendorf Safe-Lock Tubes
 - Vortex (e.g., Vortex-Genie[®] 2T mixer)
 - Microcentrifuge (Eppendorf)
 - Micropipet [e.g., Gilson[™] PIPETMAN Classic[™]]
 - HPLC system equipped with analytical RP-C18 column (e.g., RP-HPLC Accucore[™] C18 aQ column, 2.6 μm , 4.6 \times 50 mm)
 - Lyophilizer (freeze-drier) [e.g., FreeZone[®] bench top freeze dryer]
 - Vacuum source (water aspirator/membrane pump; vacuum oil pump)
 - Flash chromatography system [e.g., Biotage Isolera 1[®] system]
 - Pre-packed RP-silica column [e.g., Büchi FlashPure[®]]

Stainless steel spatula

Perform the sulfo-click reaction

1. Add 5 mg (12 μmol) 3'-azido-4'-(carboxamido)ethane-sulfonyl azide-3'-deoxythymidine **11** to a 1.5-mL Eppendorf Safe-Lock Tube.
2. Add 200 μL dry NMP and vortex until complete dissolution. Centrifuge the microtube.
3. Add 9.3 mg (18 μmol , 1.5 equivalent) 4'-tetrabutylammonium thioacetate-uridine **4d** to a 1.5-mL Eppendorf Safe-Lock Tube.
4. Add 200 μL dry NMP and vortex until complete dissolution. Centrifuge the microtube.
5. Mix the two solutions using a micropipet and immediately add 100 μL of 0.1 M aq. NaHCO_3 buffer.
6. Vortex for 10 sec and place the microtube at 37°C in a microtube heater without agitation for 10 min.

At this point, you can visually check that the reaction occurs after five minutes by observing nitrogen bubbles along with elemental Sulphur precipitates within the mixture.

7. Monitor the reaction by RP-HPLC analysis using the following gradient: (Accucore™ C18 aQ column, 2.6 μm , 4.6×50 mm) with CH_3CN and 0.1% aqueous trifluoroacetic acid (aq. TFA, 0.1%, v/v, pH 2.0) as the eluents [0% CH_3CN (2 min), followed by linear gradient from 0 to 100% (25 min) of CH_3CN] at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. Triple UV detection was achieved at 210, 260 and 650 nm (Fig. 6).

Perform the CuAAC reaction

1. Add 4.6 mg (24 μmol , 2 equivalent) CuI to a 1.5-mL Eppendorf Safe-Lock Tube.
2. Add 100 μL dry NMP and vortex.

The CuI is not entirely soluble resulting in a suspension.

3. In one portion, add 21 μL (120 μmol , 10 equivalents) dry DIEA to the CuI suspension and vortex for 10 s.
4. In one portion, add 6.5 mg (24 μmol , 2 equivalents) biotin alkyne to the CuI suspension and vortex for 10 s.
5. Transfer the sulfo-click crude to the CuI suspension using a micropipet, vortex for 10 sec and place the microtube at 37°C in a microtube heater without agitation for 30 min.

At this stage, the CuI catalyst is entirely soluble in the solvent.

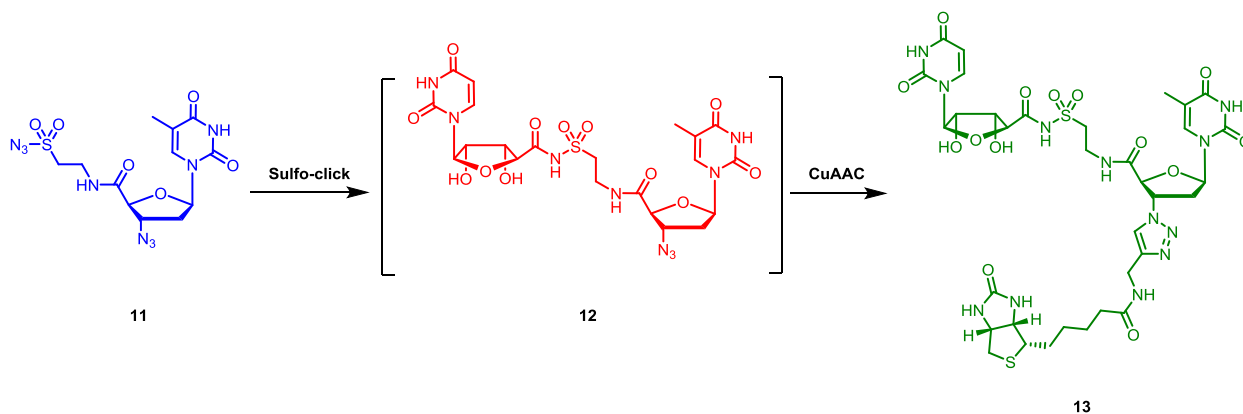
6. Monitor the reaction by RP-HPLC analysis using the following gradient: (Accucore™ C18 aQ column, 2.6 μm , 4.6 \times 50 mm) with CH_3CN and 0.1% aqueous trifluoroacetic acid (aq. TFA, 0.1%, v/v, pH 2.0) as the eluents [0% CH_3CN (2 min), followed by linear gradient from 0 to 100% (25 min) of CH_3CN] at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. Triple UV detection was achieved at 210, 260 and 650 nm (Fig. 6).

7. Take up the crude in a 10 mL syringe; dilute it by withdrawing 9 mL of aq. TFA, 0.1% and purify product on a RP-silica gel column. Eluent: Aq. TFA, 0.1% / MeCN; 12-g flash column, linear gradient 0 \rightarrow 30% in 40 minutes. Pool fractions containing product and lyophilize.

Alternatively, depending on the synthesis scale envisaged and the material available, the product can be purified via semi-preparative RP-HPLC using the same gradient and eluents.

8. Characterize product **13** by RP-HPLC, ^1H NMR, ^{13}C NMR, and HRMS.

Yield of white amorphous powder 9.5 mg (86%). $T_R = 8.7$ min. ^1H NMR (400 MHz, DMSO) $\delta = 1.27$ -1.62 (m, 6H), 1.80 (s, 3H), 2.07 (s, 1H), 2.11 (t, $J = 7.4$ Hz, 2H), 2.57 (d, $J = 12.3$ Hz, 1H), 2.65-2.78 (m, 2H), 2.79-2.84 (m, 1H), 3.07-3.12 (m, 1H), 3.50-3.64 (m, 4H), 4.08-4.14 (m, 3H), 4.29-4.31 (m, 3H), 4.43 (d, $J = 2.8$ Hz, 1H), 4.65 (d, $J = 3.6$ Hz, 1H), 5.55-5.58 (m, 1H), 5.75 (dd, $J_1 = 8.2$, $J_2 = 2.2$ Hz, 1H), 5.92 (d, $J = 6.4$ Hz, 1H), 6.36 (bs, 1H), 6.41 (bs, 1H), 6.51 (t, $J = 6.4$ Hz, 1H), 7.86 (s, 1H), 8.08 (t, $J = 3.5$ Hz, 2H), 8.32 (t, $J = 5.6$ Hz, 1H), 8.46 (t, $J = 5.6$ Hz, 1H), 11.40 (s, 2H) ppm. ^{13}C NMR (100.6 MHz, DMSO) $\delta = 12.3$, 25.2, 28.0, 28.2, 33.2, 34.1, 35.0, 36.0, 39.9, 51.3, 55.4, 59.2, 61.0, 62.0, 72.8, 73.0, 82.3, 85.9, 88.0, 102.3, 109.8, 122.9, 137.0, 140.6, 145.4, 150.7, 150.9, 162.7, 163.1, 163.8, 169.1, 170.9, 172.1 ppm. HRMS (ESI) m/z : $[M+H]^+$ Calcd for $\text{C}_{34}\text{H}_{45}\text{N}_{12}\text{O}_{14}\text{S}_2$: 909.2620; found 909.2623.



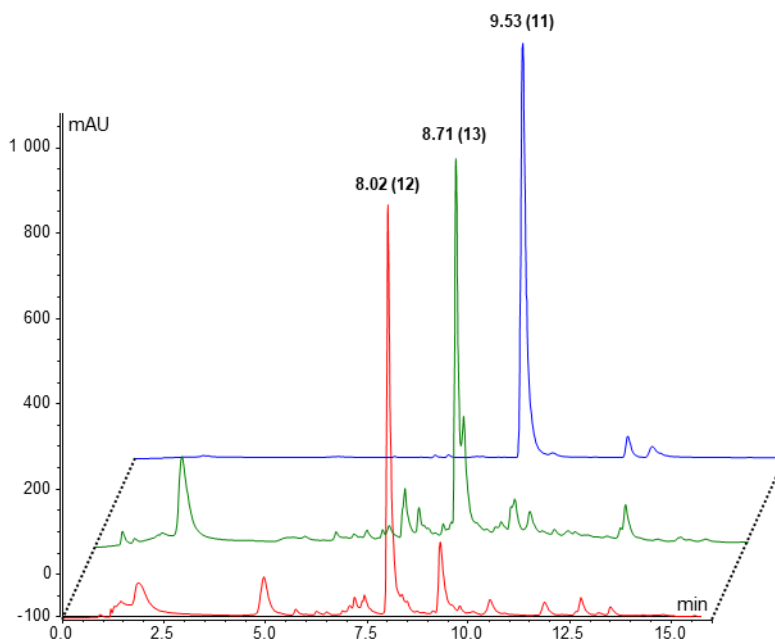


Figure 6 RP-HPLC analyses (260 nm) of the one-pot double-click conjugations. Analysis of purified compound **11** (in blue); analysis of crude sulfo-click reaction (in red); analysis of crude CuAAC reaction (in green).

COMMENTARY

BACKGROUND INFORMATION:

The selective 5' and/or 3' functionalization of ribo- and deoxyribonucleotides is of primary importance for anticancer and antiviral drug development (Jordheim, Durantel, Zoulim, & Dumontet, 2013). Such modifications are used to enhance the physico-chemical properties of the corresponding nucleosides. Thus, many methodologies have been developed over the years to functionalize a nucleoside core with a specific moiety or another biomolecule derivative (e. g. an amino acid, a nucleoside, a carbohydrate, a phospholipid or an alkaloid). Most of the time, reactions that do not meet the criteria of click chemistry are implemented. As illustration, a series of modified nucleosides incorporating zinc-binding pharmacophores as inhibitors of the DNA repair nuclease SNM1A which is responsible for the resistance of certain cancer against traditional DNA crosslinking agents have been synthesized using amide forming coupling reactions and Peterson olefination (Doherty et al., 2019). In another example, dinucleoside analogues were synthesized through a one-pot tandem Staudinger/aza-Wittig/cyclization under microwave irradiation. The dinucleosides obtained containing (*R*)- or (*S*)-thiazolidin-4-one linkages are active to inhibit HIV reverse transcriptase (Shen et al., 2011). Moreover, in the literature various dinucleoside analogues bearing non-natural linkages have been demonstrated as possible therapeutic agents (Arenas-Jal, Sune-Negre, & Garcia-Montoya, 2020; Chen et al., 2008). The reactions described above are not compatible with fragile biomolecules and may lack efficiency. Therefore biocompatible click reactions represent a valuable alternative. For instance the CuAAC (Rostovtsev, Green, Fokin, & Sharpless, 2002; Tornøe, Christensen, & Meldal, 2002) has been used by several groups to enhance the discovery of new nucleoside analogues with interesting properties (Amblard, Cho, & Schinazi, 2009; Baraniak, Kacprzak, & Celewicz, 2011; Baraniak, Ruzskowski, Baranowski, Framski, & Boryski, 2019; Roy et al., 2011; Sirivolu et al., 2013). The sulfo-click reaction belongs to the class of Thioacid/Azide based Ligation (TAL) reactions. The TAL was originally described as a side reaction in 1980 (Hakimelahi & Just, 1980) but its potential as an alternative amidation reaction was highlighted more than 20 years later by Williams and co-workers (Kolakowski, Shangguan, Sauers, & Williams, 2006; Shangguan, Katukojvala, Greenberg, & Williams, 2003). In particular, they demonstrated that electron-deficient azides readily react with thioacids at room temperature while aliphatic azides require heating at 60 °C for several hours. In 2010, Liskamp and co-workers denominated "sulfo-click reaction" the specific use of electron-deficient sulfonyl azides in their study devoted to the site specific conjugation of peptides. This designation was subsequently employed in the field of bioconjugation of various classes of molecules (e. g. sugars, peptides, fluorophores or chelators and one example of protein). For instance 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelator (Yim et al., 2010) was conjugated to vector

biomolecules for imaging or treatment of cancer by bounding with radionuclides. An application in the context of peptides coupling was published in 2012 (Raz & Rademann, 2012), thus illustrating the significance of the reaction for the formation of an amide linkage between the two partners. The sulfo-click reaction was also applied to carbohydrates- and fluorophores-peptides conjugates (Rohmer et al., 2015). A recent example was published concerning its use in the context of radiolabeling by ^{18}F for positron emission tomography imaging (Urkow, Bergman, & Wuest, 2019). Noteworthy, perfluoroaryl azides can be an interesting alternative to sulfonyl azides to perform highly efficient conjugation with thioacid derivatives (Xie, Fukumoto, Ramström, & Yan, 2015). The reaction is equally fast and compatible with aqueous media but the introduction of the perfluoroaryl azide moiety on molecules is tricky and adds a significant steric hindrance. All these examples demonstrate the potential of the sulfo-click as a surrogate click reaction. Indeed, complete conversions are reached within 10 minutes at room temperature in organic or aqueous media using mild conditions. Moreover, the sulfo-click reaction involves unobtrusive polar moieties compared to other click reactions (e.g., cyclooctyne, norbornene, tetrazine, etc.). Despite its significance, the sulfo-click reaction was never applied to nucleic acids. In this context, the protocols presented in this unit describe a straightforward sequence to synthesize 4'-thioacid deoxyribo- and ribonucleosides allowing the implementation of the sulfo-click reaction to nucleoside derivatives in biocompatible aqueous conditions. Moreover, we have developed an efficient procedure allowing the chemoselective one-pot dual functionalization of a bis-azide nucleoside derivative via the sulfo-click and the CuAAC reactions (Clavé et al., 2020). This method has the potential to be applied to any kind of molecules for the development of highly functionalized nucleoside conjugates for various applications.

CRITICAL PARAMETERS AND TROUBLESHOOTING:

As it is mentioned within the detailed protocols, it may be difficult to reach quantitative conversion during the oxidation of the 5'-OH of nucleosides **1a-d** and **10** using TEMPO/BAIB as oxidant. It is very important to keep the flask opened to ensure oxygen renewal in the solvent. However, complete conversion is always reachable by successive addition of oxidants. The coupling procedures to obtain **3a-d** and **11** in DMF are efficient thanks to the high concentration of the reactants. It is important to perform the five aqueous washings of the AcOEt organic phase to ensure the total elimination of DMF. The TBAF deprotection of **3a-d** requires fresh solutions. Indeed, side reactions leading to poor yields have been observed with old solutions. The tetrabutylammonium-thioacetate nucleoside **4** must be stored at -20°C to avoid degradations observed at room temperature.

UNDERSTANDING RESULTS:

The protocols described in this article allow the preparation of 4'-thioacid-nucleoside derivatives **4a-d** with a straightforward three-step sequence (basic protocol 1). The implementation of the sulfo-click reaction use versatile conditions applicable to numerous polar compounds in aqueous conditions (basic protocol 2) leading to quantitative conversion within ten minutes and high isolated yields (>75%). The preparation in two steps of the 3'-azido-4'-(carboxamido)ethane-sulfonyl azide-3'-deoxythymidine **11** (basic protocol 3) allowed the synthesis of the deoxythymidine bis-conjugate **13** (basic protocol 4) using 4'-tetrabutylammonium thioacetate-uridine **4d** and the biotin alkyne. The yields of all the reactions in these protocols are good to excellent if correctly executed. The double-click procedure is performed with an excess of one of the partner at each step to ensure quantitative conversions. As indicated Fig. 6, both click reactions are chemoselective and should lead to high isolated yields. Any reduction in yield must be attributed to the purification and freeze-drying stages.

TIME CONSIDERATIONS:

The synthesis of 4'-tetrabutylammonium thioacetate-nucleoside derivatives **4a-d** starting from **1a-d** can be accomplished in one week for each derivative. Each implementation of the sulfo-click reaction can be performed in one working day. Lyophilization of the products **6-8** can be achieved overnight. 3'-azido-4'-(carboxamido)ethane-sulfonyl azide-3'-deoxythymidine **11** can be prepared in 3-4 days starting from commercially available 3'-azido-3'-deoxythymidine **9**. The double-labelling of 3'-azido-4'-(carboxamido)ethane-sulfonyl azide-3'-deoxythymidine **11** via the sulfo-click and the CuAAC reactions is achievable within a day. Lyophilization of the purified final conjugate **13** can be achieved overnight.

ACKNOWLEDGEMENTS:

The authors thank the Agence Nationale de la Recherche (ANR "TALAN"-ANR-19-CE07-0004-01) for financial support.

LITERATURE CITED:

- Amblard, F., Cho, J. H., & Schinazi, R. F. (2009). Cu(I)-Catalyzed Huisgen Azide–Alkyne 1,3-Dipolar Cycloaddition Reaction in Nucleoside, Nucleotide, and Oligonucleotide Chemistry. *Chem. Rev.*, *109*, 4207-4220. doi: 10.1021/cr9001462
- Arenas-Jal, M., Sune-Negre, J. M., & Garcia-Montoya, E. (2020). Therapeutic potential of nicotinamide adenine dinucleotide (NAD). [Review]. *Eur. J. Pharmacol.*, *879*, 173158. doi: 10.1016/j.ejphar.2020.173158
- Baraniak, D., Kacprzak, K., & Celewicz, L. (2011). Synthesis of 3'-azido-3'-deoxythymidine (AZT)--Cinchona alkaloid conjugates via click chemistry: Toward novel fluorescent markers and cytostatic agents. *Bioorg. Med. Chem. Lett.*, *21*, 723-726. doi: 10.1016/j.bmcl.2010.11.127
- Baraniak, D., Ruskowski, P., Baranowski, D., Framski, G., & Boryski, J. (2019). Nucleoside dimers analogs containing floxuridine and thymidine with unnatural linker groups: synthesis and cancer line studies. Part III. *Nucleosides Nucleotides Nucleic Acids*, *38*, 980-1005. doi: 10.1080/15257770.2019.1641206
- Brouwer, A. J., Merkx, R., Dabrowska, K., Rijkers, D. T., & Liskamp, R. M. (2006). Synthesis and applications of β -aminoethanesulfonyl azides. *Synthesis*, *2006*, 455-460. doi: 10.1055/s-2006-926273
- Chen, L., Petrelli, R., Olesiak, M., Wilson, D. J., Labello, N. P., & Pankiewicz, K. W. (2008). Bis(sulfonamide) isosters of mycophenolic adenine dinucleotide analogues: inhibition of inosine monophosphate dehydrogenase. *Bioorg. Med. Chem.*, *16*, 7462-7469. doi: 10.1016/j.bmc.2008.06.003
- Clavé, G., Dursun, E., Vasseur, J. J., & Smietana, M. (2020). An Entry of the Chemoselective Sulfo-Click Reaction into the Sphere of Nucleic Acids. *Org. Lett.*, *22*, 1914-1918. doi: 10.1021/acs.orglett.0c00265
- Crich, D., Sana, K., & Guo, S. (2007). Amino Acid and Peptide Synthesis and Functionalization by the Reaction of Thioacids with 2,4-Dinitrobenzenesulfonamides. *Org. Lett.*, *9*, 4423-4426. doi: 10.1021/ol701583t
- Doherty, W., Dürr, E.-M., Baddock, H. T., Lee, S. Y., McHugh, P. J., Brown, T., . . . McGouran, J. F. (2019). A hydroxamic-acid-containing nucleoside inhibits DNA repair nuclease SNM1A. *Org. Biomol. Chem.*, *17*, 8094-8105. doi: 10.1039/c9ob01133a
- Epp, J. B., & Widlanski, T. S. (1999). Facile preparation of nucleoside-5'-carboxylic acids. *J. Org. Chem.*, *64*, 293-295. doi: 10.1021/jo981316g
- Hakimelahi, G. H., & Just, G. (1980). A simple synthesis of 2, 2-disubstituted tetrahydrothiophenes. *Tetrahedron Lett.*, *21*, 2119-2122. doi: 10.1016/S0040-4039(00)78973-8
- Jordheim, L. P., Durantel, D., Zoulim, F., & Dumontet, C. (2013). Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat. Rev. Drug. Discov.*, *12*, 447-464. doi: 10.1038/nrd4010
- Kolakowski, R. V., Shangguan, N., Sauers, R. R., & Williams, L. J. (2006). Mechanism of Thio Acid/Azide Amidation. *J. Am. Chem. Soc.*, *128*, 5695-5702. doi: 10.1021/ja057533y
- Merkx, R., Brouwer, A. J., Rijkers, D. T. S., & Liskamp, R. M. J. (2005). Highly Efficient Coupling of β -Substituted Aminoethane Sulfonyl Azides with Thio Acids, toward a New Chemical Ligation Reaction. *Org. Lett.*, *7*, 1125-1128. doi: 10.1021/ol0501119
- Meyer, A., Spinelli, N., Dumy, P., Vasseur, J.-J., Morvan, F., & Defrancq, E. (2010). Oligonucleotide Sequential Bis-Conjugation via Click–Oxime and Click–Huisgen Procedures. *J. Org. Chem.*, *75*, 3927-3930. doi: 10.1021/jo100599m
- Patil, S. V., Mane, R. B., & Salunkhe, M. M. (1994). A Facile Method for Detritylation of 5'-O-Dimethoxytrityl-3'-O-tert-butylidimethylsilyl-2'-deoxynucleosides. *Synth. Commun.*, *24*, 2423-2428. doi: 10.1080/00397919408010549
- Raz, R., & Rademann, J. (2012). Fmoc-Based Synthesis of Peptide Thioacids for Azide Ligations via 2-Cyanoethyl Thioesters. *Org. Lett.*, *14*, 5038-5041. doi: 10.1021/ol302247h

- Reisacher, U., Ploschik, D., Rönicke, F., Cserép, G. B., Kele, P., & Wagenknecht, H.-A. (2019). Copper-free dual labeling of DNA by triazines and cyclopropenes as minimal orthogonal and bioorthogonal functions. *Chem. Sci.*, *10*, 4032-4037. doi: 10.1039/C8SC05588B
- Rohmer, K., Mannuthodikayil, J., & Wittmann, V. (2015). Application of the Thioacid-Azide Ligation (TAL) for the Preparation of Glycosylated and Fluorescently Labeled Amino Acids. *Isr. J. Chem.*, *55*, 437-446. doi: 10.1002/ijch.201500001
- Rostovtsev, V. V., Green, L. G., Fokin, V. V., & Sharpless, K. B. (2002). A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes. *Angew. Chem., Int. Ed.*, *41*, 2596-2599. doi: 10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4
- Roy, V., Obikhod, A., Zhang, H. W., Coats, S. J., Herman, B. D., Sluis-Cremer, N., . . . Schinazi, R. F. (2011). Synthesis and anti-HIV evaluation of 3'-triazolo nucleosides. *Nucleosides Nucleotides Nucleic Acids*, *30*, 264-270. doi: 10.1080/15257770.2011.580291
- Schoch, J., Staudt, M., Samanta, A., Wiessler, M., & Jäschke, A. (2012). Site-specific one-pot dual labeling of DNA by orthogonal cycloaddition chemistry. *Bioconjugate Chem.*, *23*, 1382-1386. doi: 10.1021/bc300181n
- Shangguan, N., Katukojvala, S., Greenberg, R., & Williams, L. J. (2003). The Reaction of Thio Acids with Azides: A New Mechanism and New Synthetic Applications. *J. Am. Chem. Soc.*, *125*, 7754-7755. doi: 10.1021/ja0294919
- Shen, F., Li, X., Zhang, X., Yin, Q., Qin, Z., Chen, H., . . . Ma, Z. (2011). Microwave-assisted synthesis of dinucleoside analogues containing a thiazolidin-4-one linkage via one-pot tandem Staudinger/aza-Wittig/cyclization. *Org. Biomol. Chem.*, *9*, 5766-5772. doi: 10.1039/c1ob05675a
- Sirivolu, V. R., Vernekar, S. K., Ilna, T., Myshakina, N. S., Parniak, M. A., & Wang, Z. (2013). Clicking 3'-azidothymidine into novel potent inhibitors of human immunodeficiency virus. *J. Med. Chem.*, *56*, 8765-8780. doi: 10.1021/jm401232v
- Tornøe, C. W., Christensen, C., & Meldal, M. (2002). Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. *J. Org. Chem.*, *67*, 3057-3064. doi: 10.1021/jo011148j
- Urkow, J., Bergman, C., & Wuest, F. (2019). Sulfo-click chemistry with 18F-labeled thio acids. *Chem. Commun.*, *55*, 1310-1313. doi: 10.1039/c8cc08717b
- Winz, M.-L., Linder, E. C., Becker, J., & Jäschke, A. (2018). Site-specific one-pot triple click labeling for DNA and RNA. *Chem. Commun.*, *54*, 11781-11784. doi: 10.1039/c8cc04520h
- Xie, S., Fukumoto, R., Ramström, O., & Yan, M. (2015). Anilide Formation from Thioacids and Perfluoroaryl Azides. *J. Org. Chem.*, *80*, 4392-4397. doi: 10.1021/acs.joc.5b00240
- Yim, C.-B., Dijkgraaf, I., Merckx, R., Versluis, C., Eek, A., Mulder, G. E., . . . Liskamp, R. M. J. (2010). Synthesis of DOTA-Conjugated Multimeric [Tyr3]Octreotide Peptides via a Combination of Cu(I)-Catalyzed "Click" Cycloaddition and Thio Acid/Sulfonyl Azide "Sulfo-Click" Amidation and Their in Vivo Evaluation. *J. Med. Chem.*, *53*, 3944-3953. doi: 10.1021/jm100246m
- Zhu, X.-F., Williams, H. J., & Scott, A. I. (2000). Facile and highly selective 5'-desilylation of multisilylated nucleosides. *J. Chem. Soc., Perkin Trans. 1*, 2305-2306. doi: 10.1039/b003562i