Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY INFORMATION

Alternative activators in oxathiaphospholane (OTP) method to the solid phase synthesis of P-stereodefined phosphorothioate analogs

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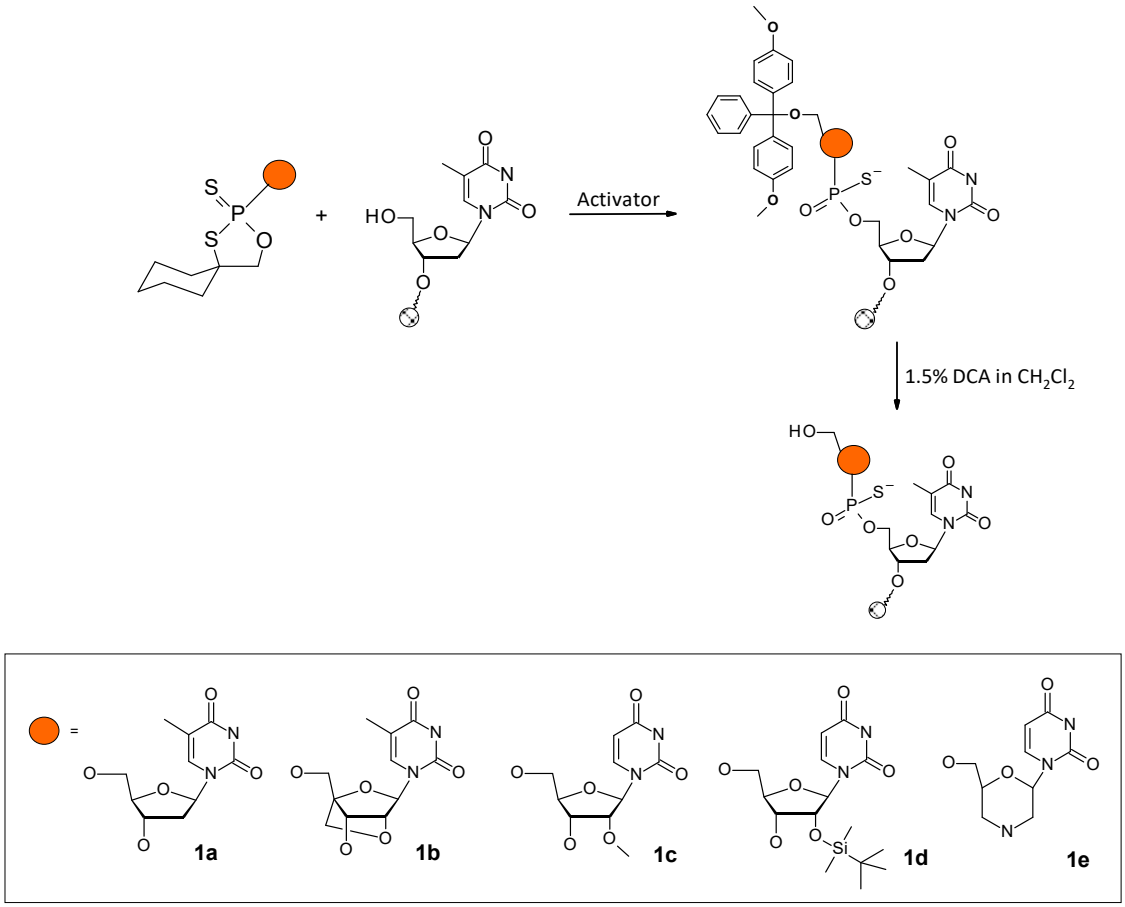
Table of Contents

Experimental information

Supporting data

1. General 'solid phase' synthesis of dinucleotides

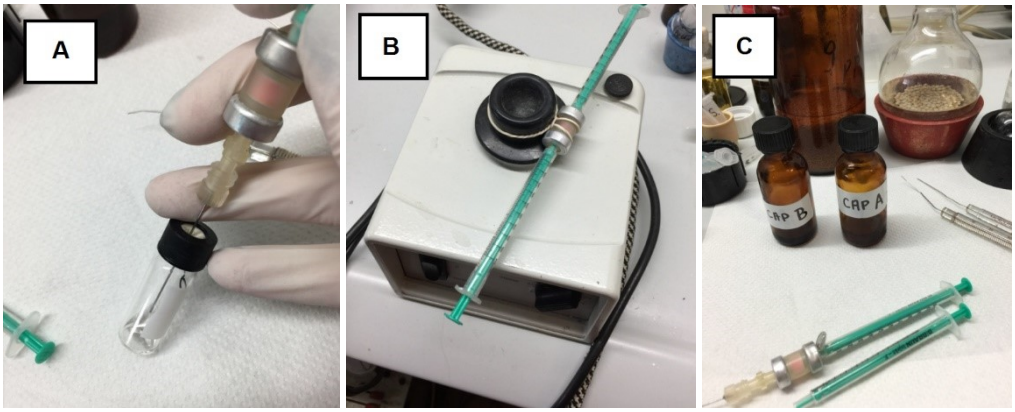


In the column 5'-O-DMT nucleoside unit (1 mmol load) anchored to the LCA CPG support was detritylated with a 3% solution of DCA (dichloroacetic acid) in methylene chloride, washed with 5 mL of dry acetonitrile followed by 5 mL of dry methylene chloride and dried under high vacuum. For the coupling step, a solution of a suitable oxathiaphospholane monomer **1** (20 µmol) and activator (50 µmol) in dry acetonitrile (150 µL) was prepared and instantly introduced into the column (**Picture A**). After 15 minutes of intensive swirling the column (**Picture B**) was washed with dry acetonitrile (5 mL) and dry methylene chloride (5 mL). Unreacted 5'-hydroxyl groups were capped using the standard DMAP–Ac_2O–pyridine solution in THF (**Picture C**), washed with 5 mL of dry acetonitrile followed by 5 mL of dry methylene chloride and dried under high vacuum. Then the column containing the dimer was detritylated with a 3% solution of DCA in methylene chloride, washed with 5 mL of dry acetonitrile followed by 5 mL of dry methylene chloride and dried under high vacuum. The coupling efficiency is controlled by measuring the absorbance at 504 nm coming from the released DMT^+ cation (**Picture D**). In the case of synthesis longer oligomer, a solution of the next oxathiaphospholane monomer 1 (20 µmol) and activator (50 µmol) in dry acetonitrile (150 µL) was prepared and introduced into the column. When the synthesis was complete, the dimer was cleaved from the support under standard conditions (25% NH_4OH, 2h). The sample was concentrated under reduced pressure in a Speed-Vac concentrator.

Table 1. Detailed procedure of condensation

Detailed procedure			
Solution (stock)	1 M DBU	3 M TBD	2M Verkade
	40µL DBU + 210 µL CH_3CN	104mg TBD + 250 µL CH_3CN	108mg Verkade + 250 µL CH_3CN
One condensation: 20mg of monomer were dissolved in:	50µL + 100µL CH_3CN (50µmols stock of DBU were used)	50µL + 100µL CH_3CN (150µmols stock of TBD were used)	50µL + 100µL CH_3CN (100µmols stock of Verkade were used)

Loading of the support with the respective nucleoside as determined by trityl assay: CPG-sarcosinyl-dT: 35.0 µmol/g. The coupling reactions were performed at the 1 µmol scale (in all experiments thymidine was attached to the support) using ~25-fold molar excess of each OTP monomer and 50-, 150- and 100-fold excess of DBU (1M), TBD (3M) and Verkade (2M), respectively.



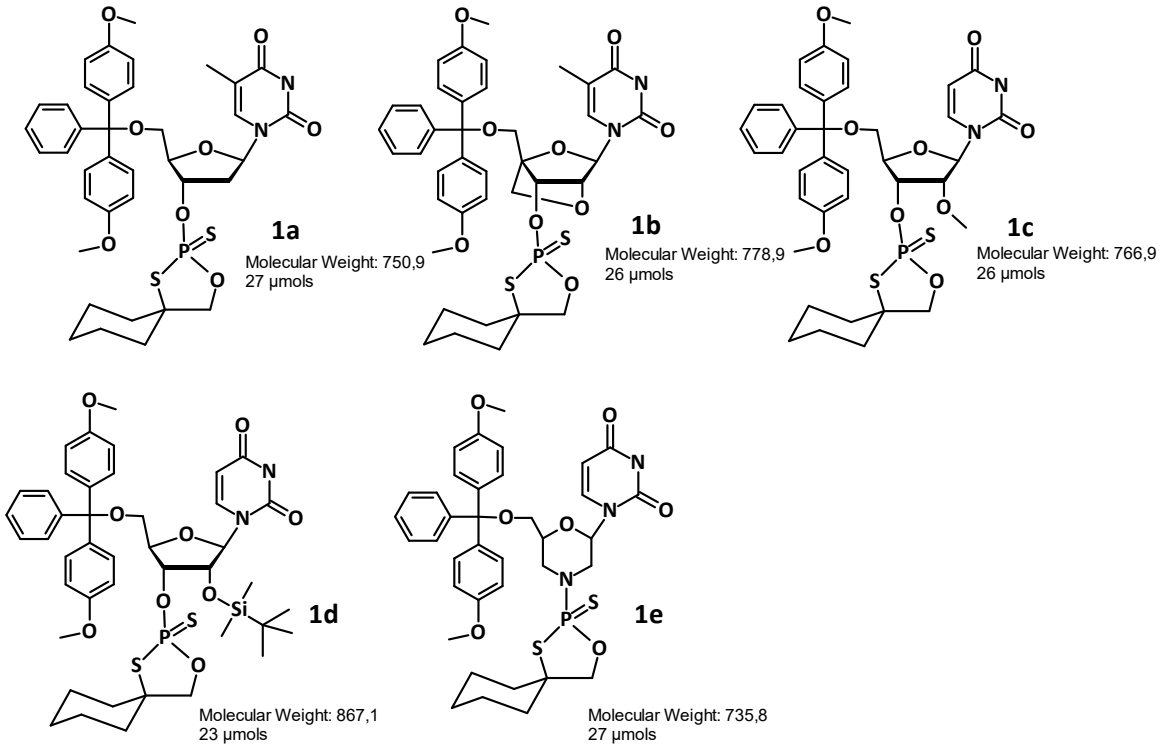


Figure S1. Structures of the studied compounds **1a-1e**, their molecular weight and numbers of µmols

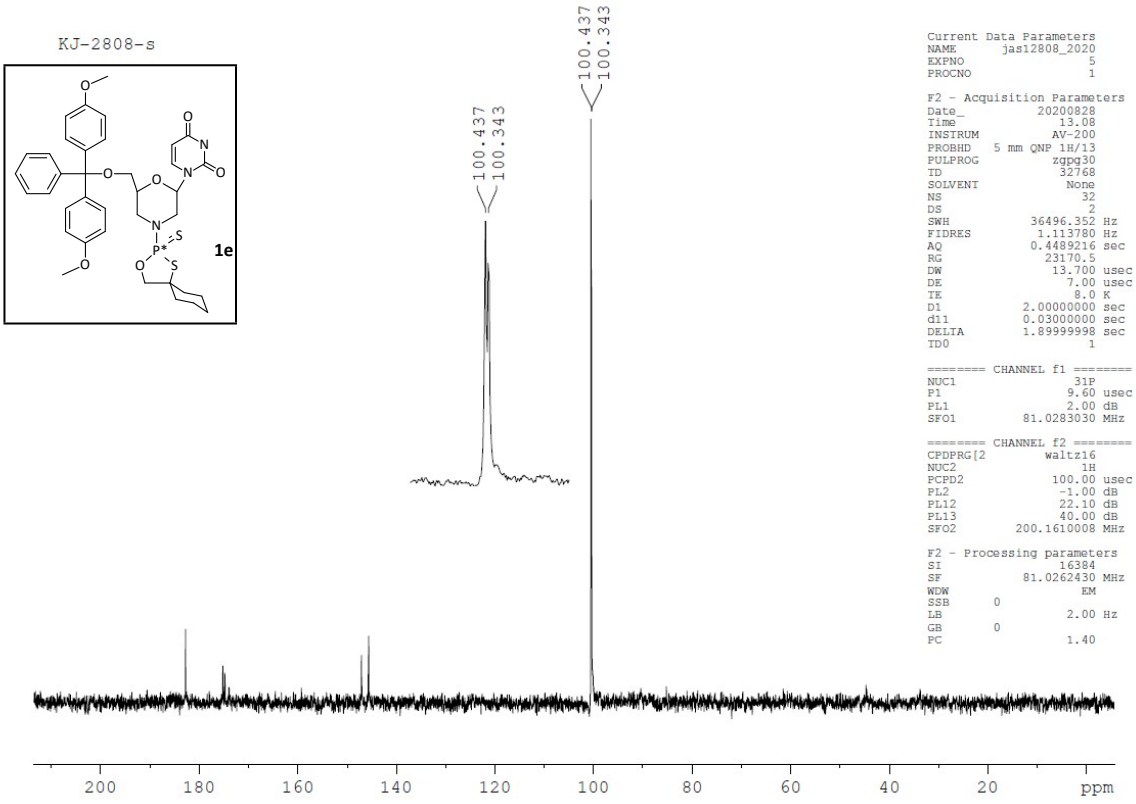


Figure S2. ^{31}P NMR spectrum of crude reaction mixture of *m*U-OTP formation (**1e**) (analysis performed in non-deuterium solvent).

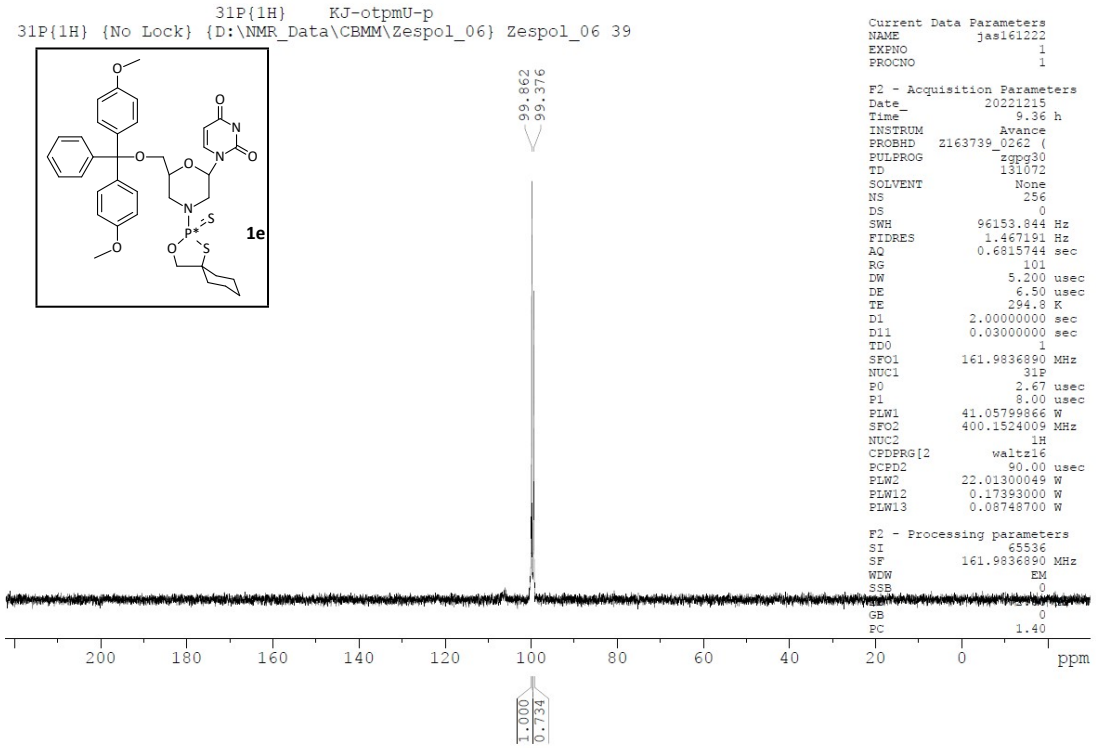
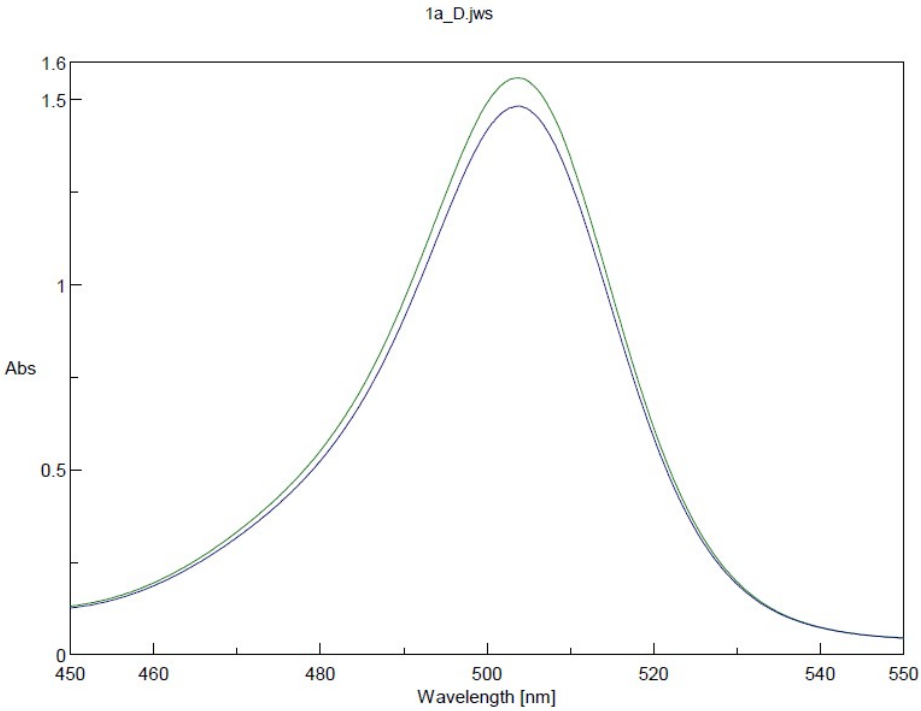


Figure S3. ^{31}P NMR spectrum of purified *m*U-OTP (**1e**) (analysis performed in non-deuterium solvent).



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User
Division
Company CBMiM PAN

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Figure S4. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1a** in the presence of 1M DBU (at 1 µmole scale)

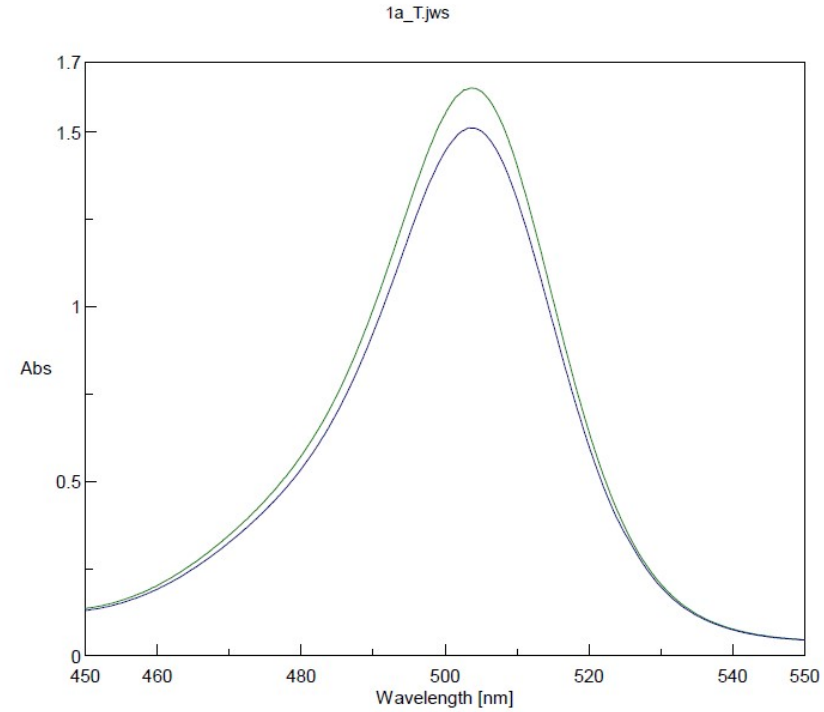

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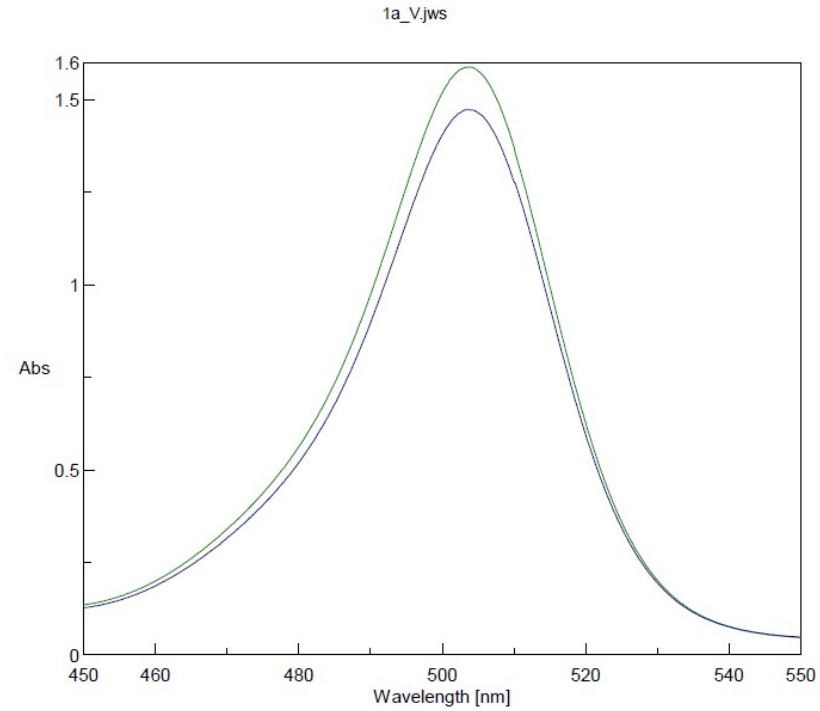
Figure S5. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1a** in the presence of 3M TBD (at 1 µmole scale).



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Company CBMiM PAN

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1a_V.jws

[Detailed Information]
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Measurement date 08.12.2023 13:46

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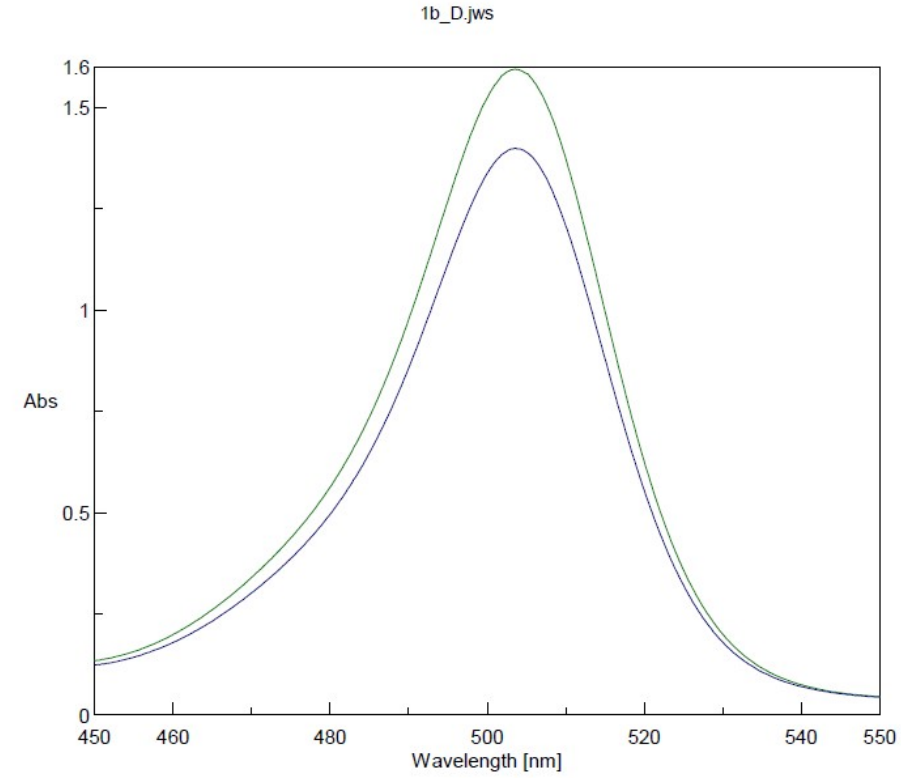
5	1a_V.jws			2023/12/08 13:46	503.8	1.47227

Figure S6. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1a** in the presence of 2M Verkade base (at 1 µmole scale)



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User
Division
Company CBMiM PAN

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Creation date 09.12.2023 09:00

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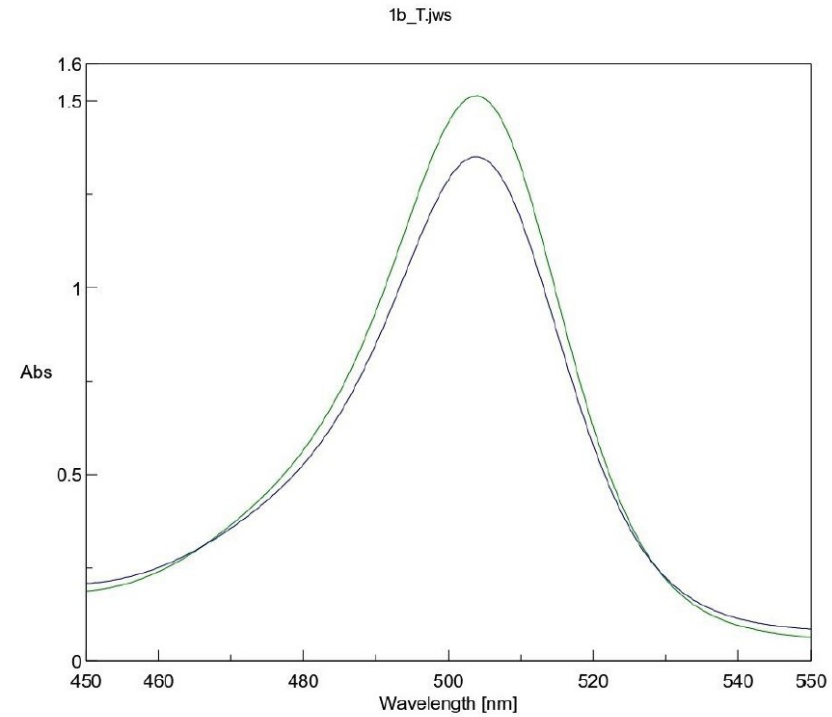
Figure S7. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1b** in the presence of 1M DBU (at 1 µmole scale)



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Company CBMiM PAN

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Figure S8. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1b** in the presence of 3M TBD (at 1 µmole scale).

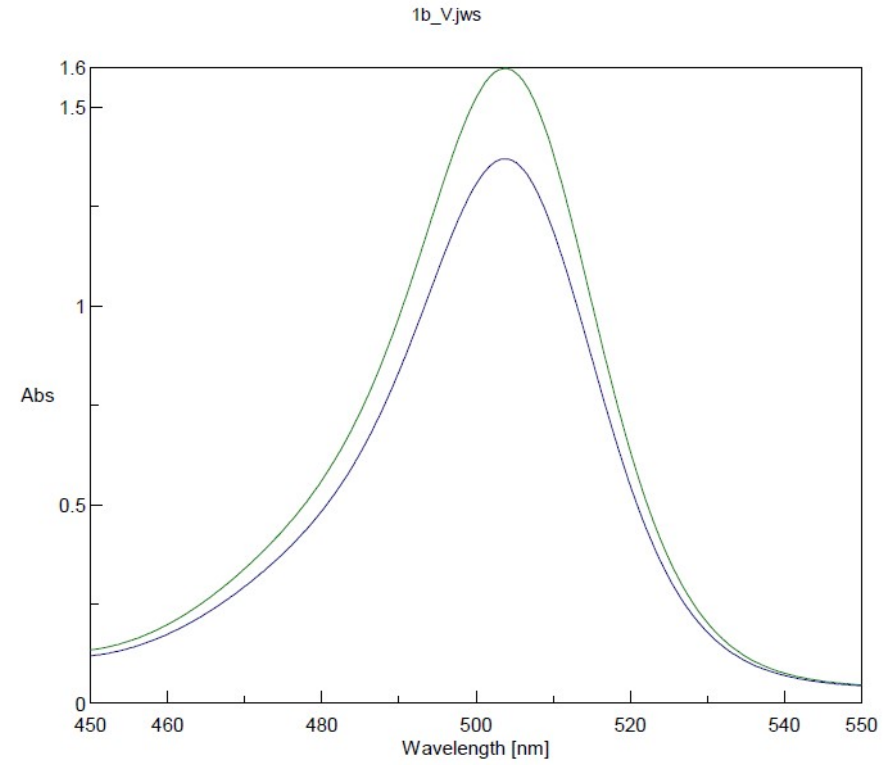

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Figure S9. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1b** in the presence of 2M Verkade base (at 1 µmole scale).

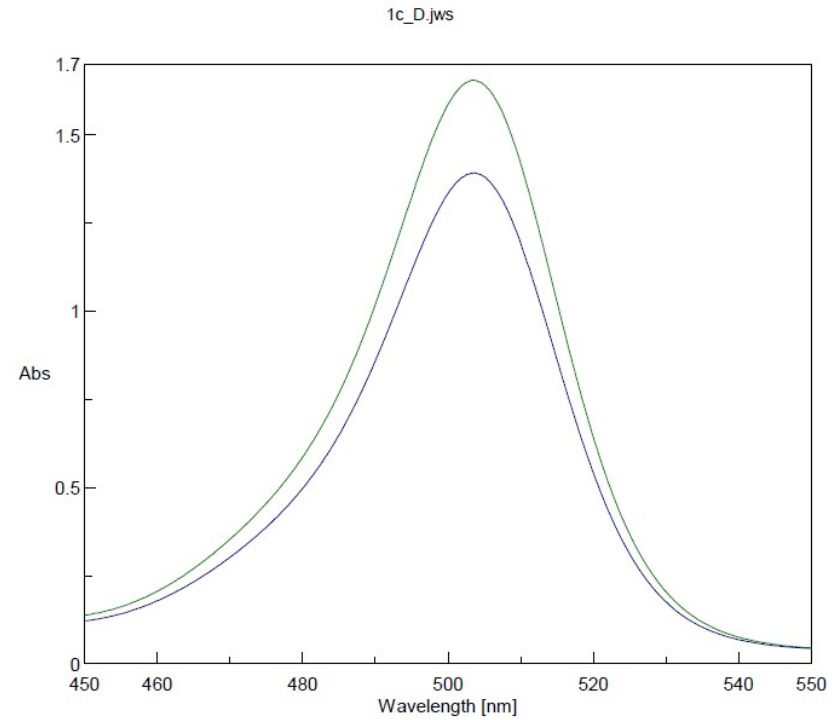

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Figure S10. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1c** in the presence of 1M DBU (at 1 µmole scale)

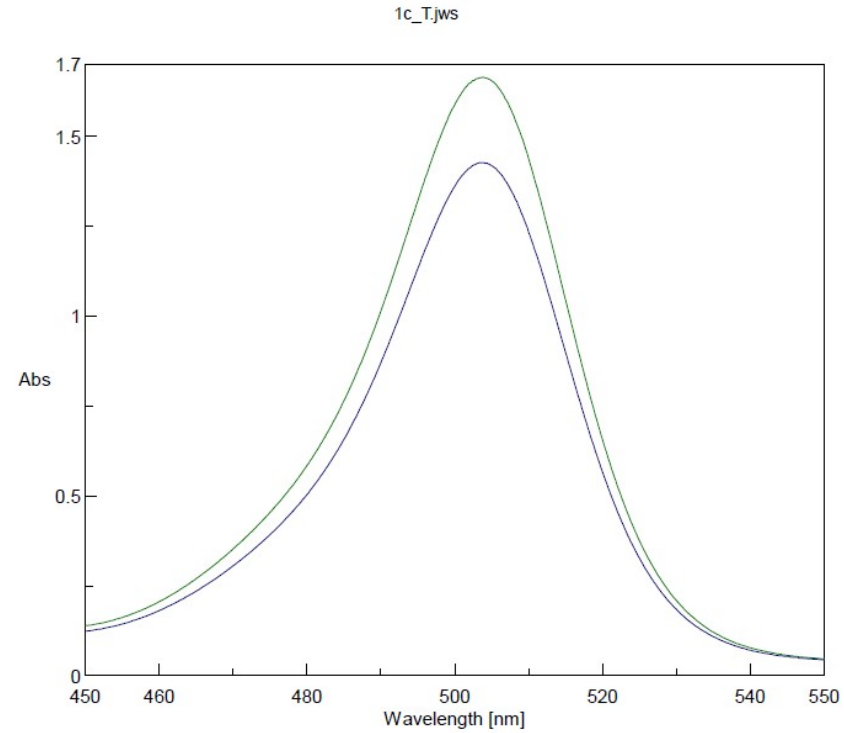

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Figure S11. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1c** in the presence of 3M TBD (at 1 µmole scale).

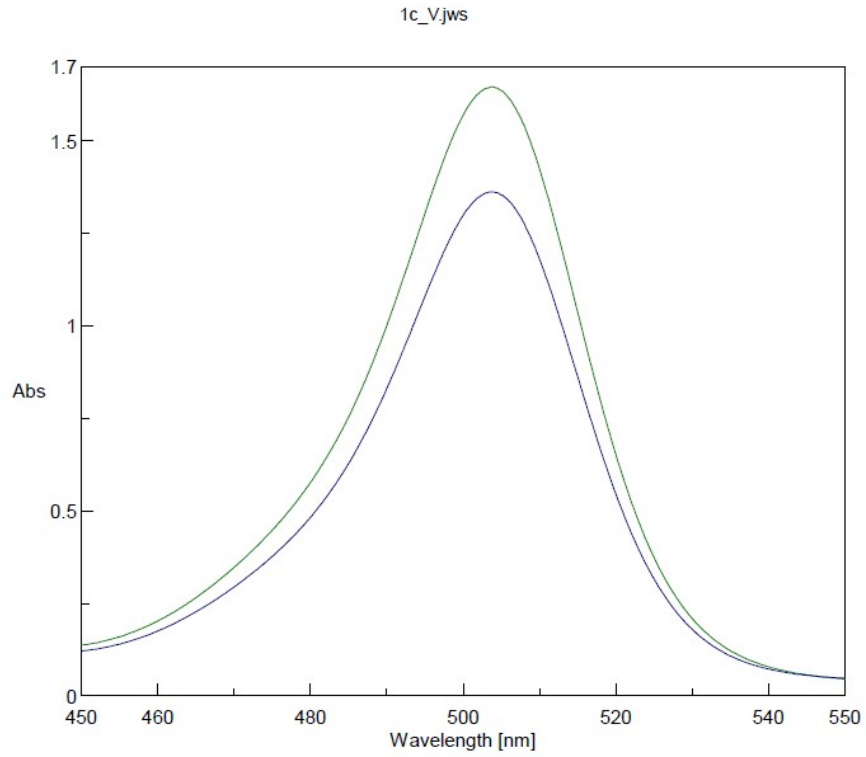

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Figure S12. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1c** in the presence of 2M Verkade base (at 1 µmole scale).

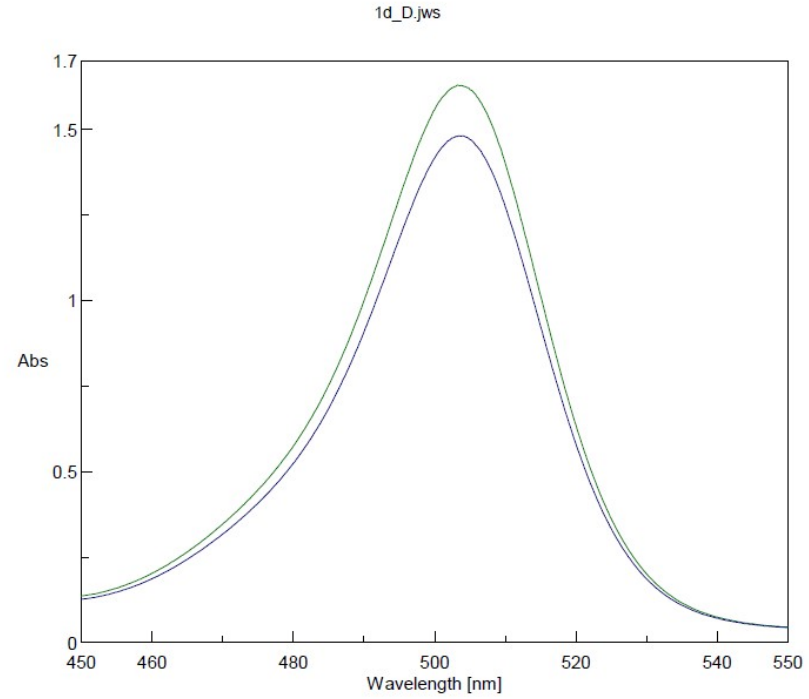

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Figure S13. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1d** in the presence of 1M DBU (at 1 µmole scale)

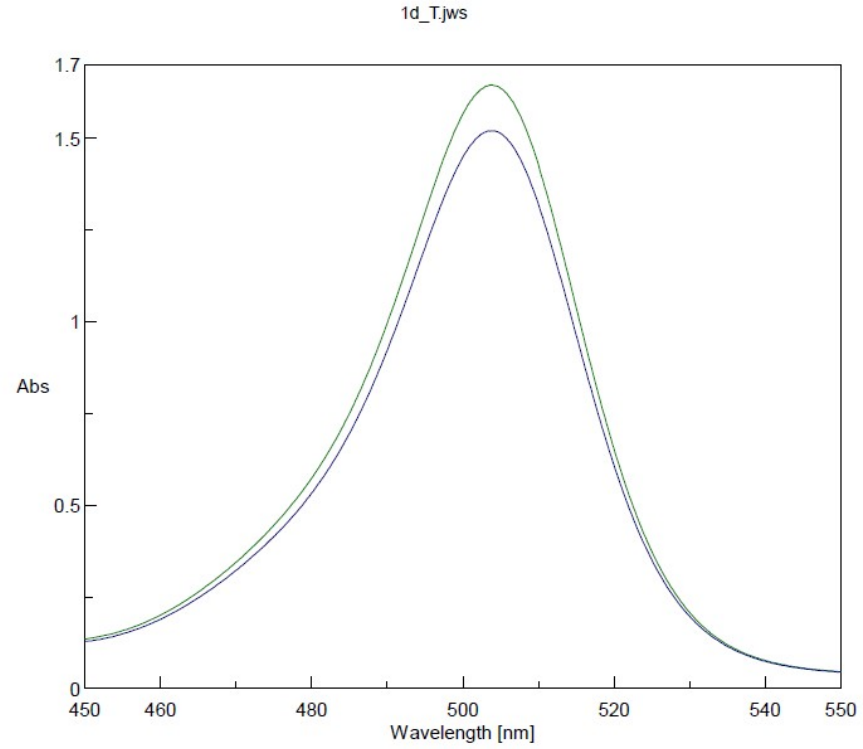

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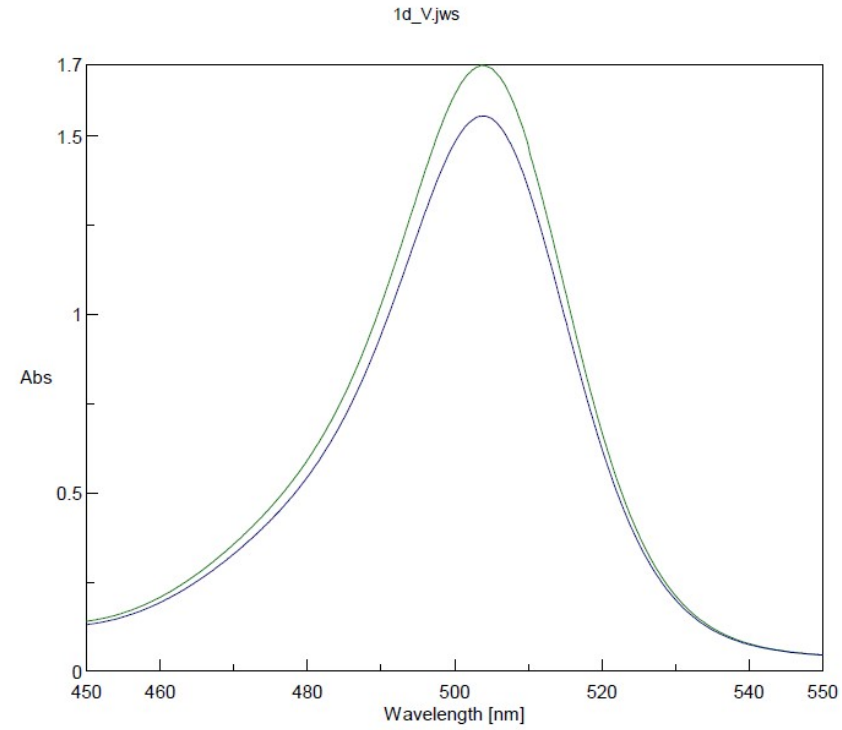
Figure S14. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1d** in the presence of 3M TBD (at 1 µmole scale).



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1d_V.jws

[Detailed Information]
Creation date 08.12.2023 14:12

Data array type Linear data array
Horizontal axis Wavelength [nm]
Vertical axis Abs
Start 550 nm
End 450 nm
Data interval 0.2 nm
Data points 501

[Measurement Information]
Instrument name V-770
Model name V-770
Serial No. D070961801

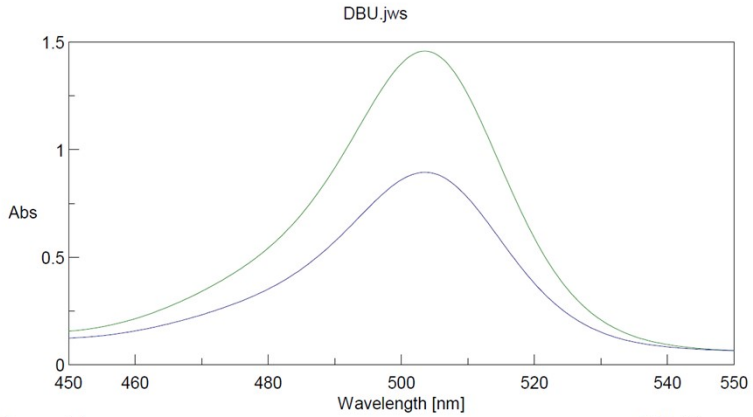
Accessory PAC-743R
Accessory S/N C014361221
Temperature 43254.43 C
Control sensor Holder
Monitor sensor Holder
Start mode Keep +/-0.10 C of the target temperature for 5 seconds

Measurement date 08.12.2023 14:12

Parameter file C:\Users\CBMiM PAN\Desktop\KJastrzebska\trityls\trityl.uvsp
Photometric mode Abs
Measurement range 550 - 450 nm
Data interval 0.2 nm
UV/Vis bandwidth 0.5 nm
NIR bandwidth 2.0 nm
UV/Vis response 0.06 sec
NIR response 0.06 sec
Scan mode Continuous

No.	File Name	Sample Name	Cc	Date	Peak 1	Peak Vakue 1
1	1d_0v.jws			2023/12/08 14:09	503.6	1.69721
2	1d_V.jws			2023/12/08 14:12	503.8	1.55512

Figure S15. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1d** in the presence of 2M Verkade base (at 1 µmole scale).



[Comments]
Sample name
Comment
User
Division
Company CBMiM PAN

[Detailed Information]
Creation date 11.01.2023 14:27
Date modified 01.08.2023 12:09

Data array type Linear data array
Horizontal axis Wavelength [nm]
Vertical axis Abs
Start 550 nm
End 450 nm
Data interval 0.5 nm
Data points 201

[Measurement Information]
Instrument name V-770
Model name V-770
Serial No. D070961801

Accessory PAC-743R
Accessory S/N C014361221
Temperature 43254.43 C
Control sensor Holder
Monitor sensor Holder

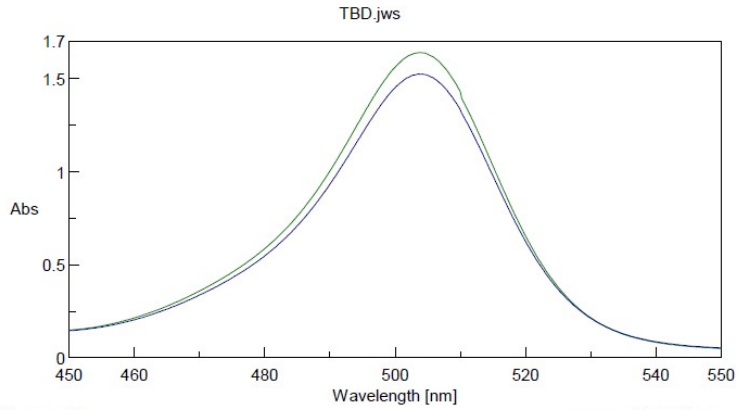
Measurement date 11.01.2023 14:27

Photometric mode Abs
Measurement range 550 - 450 nm
Data interval 0.5 nm
UV/Vis bandwidth 2.0 nm
NIR bandwidth 8.0 nm
UV/Vis response 0.06 sec
NIR response 0.06 sec
Scan mode Continuous

No.	File Name	Sample Name	Comment	Date	Peak 1
1	trityl_2.jws			2023/01/11 14:2	503.5
2	DBU.jws			2023/01/11 14:2	503.5

No.	Peak Vakue 1
1	1.45763
2	0.89423

Figure S16. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1e** in the presence of 1M DBU (at 1 µmole scale)



[Comments]
Sample name
Comment
User
Division
Company CBMiM PAN

[Detailed Information]
Creation date 01.08.2023 11:58
Date modified 01.08.2023 12:19

Data array type Linear data array
Horizontal axis Wavelength [nm]
Vertical axis Abs
Start 550 nm
End 450 nm
Data interval 0.2 nm
Data points 501

[Measurement Information]
Instrument name V-770
Model name V-770
Serial No. D070961801

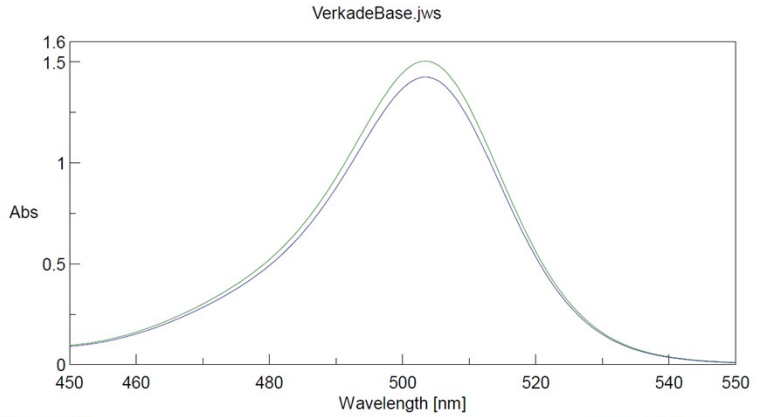
Accessory PAC-743R
Accessory S/N C014361221
Temperature 43254.43 C
Control sensor Holder
Monitor sensor Holder
Start mode Keep +/-0.10 C of the target temperature for 5 seconds

Measurement date 01.08.2023 11:58

Parameter file C:\Users\CBMiM PAN\Desktop\KJastrzebska\trityls\trityl.uvsp
Photometric mode Abs
Measurement range 550 - 450 nm
Data interval 0.2 nm
UV/Vis bandwidth 0.5 nm
NIR bandwidth 2.0 nm
UV/Vis response 0.06 sec

No.	File Name	Sample Name	C	Date	Peak 1	Peak Vakue 1
1	trityl_1.jws			2023/08/01 11:5	503.8	1.63857
2	TBD.jws			2023/08/01 11:5	504.2	1.52392

Figure S17. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1e** in the presence of 3M TBD (at 1 µmole scale).



[Comments]
Sample name
Comment
User
Division
Company CBMiM PAN

[Detailed Information]
Creation date 11.01.2023 14:02
Date modified 01.08.2023 12:11

Data array type Linear data array
Horizontal axis Wavelength [nm]
Vertical axis Abs
Start 550 nm
End 450 nm
Data interval 0.5 nm
Data points 201

[Measurement Information]
Instrument name V-770
Model name V-770
Serial No. D070961801

Accessory PAC-743R
Accessory S/N C014361221
Temperature 66192.56 C
Control sensor Holder
Monitor sensor Holder

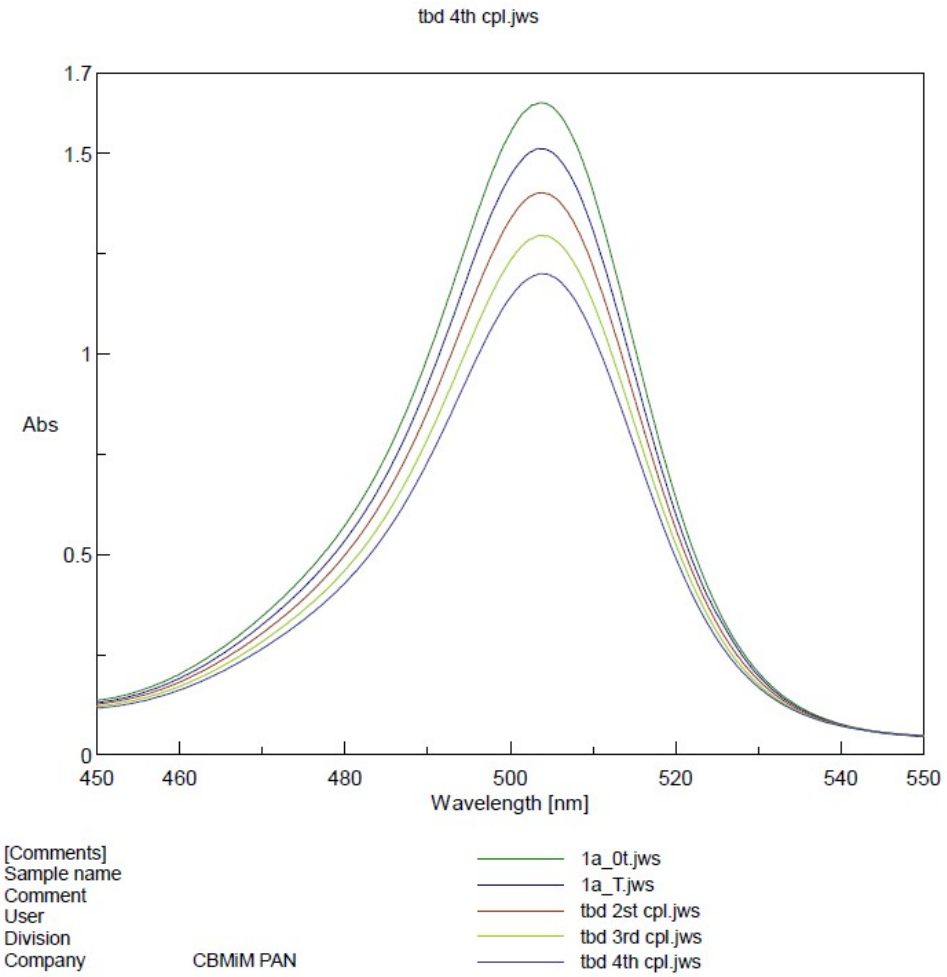
Measurement date 11.01.2023 14:02

Photometric mode Abs
Measurement range 550 - 450 nm
Data interval 0.5 nm
UV/Vis bandwidth 2.0 nm
NIR bandwidth 8.0 nm
UV/Vis response 0.06 sec
NIR response 0.06 sec
Scan mode Continuous

No.	File Name	Sample Name	Comment	Date	Peak 1
1	trityl.jws			2023/01/1	503.5
2	VerkadeBase.jw			2023/01/1	503.5

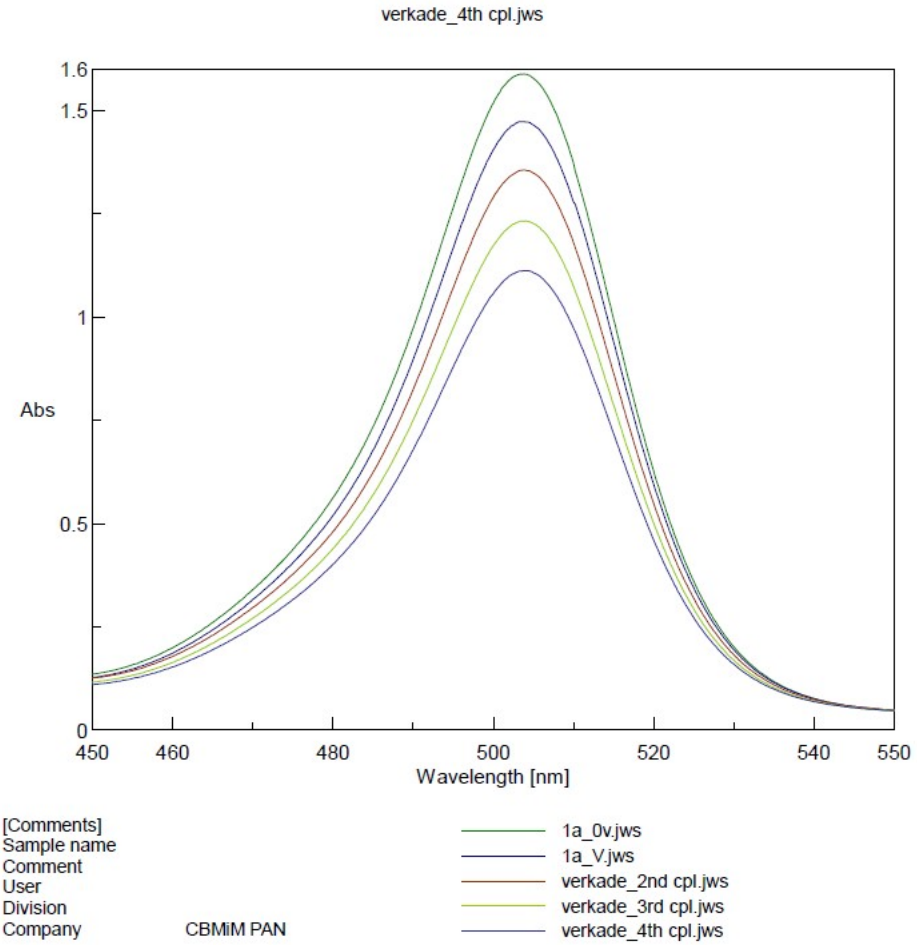
No.	Peak Vakue 1
1	1.50311
2	1.42448

Figure S18. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1e** in the presence of 2M Verkade base (at 1 µmole scale).



No.	File Name	Sample	C	Date	Peak 1	Peak Vakue 1
1	1a_0t.jws			2023/11	503.6	1.62487
2	1a_T.jws			2023/11	503.4	1.51065
3	tbd 2st cpl.jws			2023/11	503.8	1.40092
4	tbd 3rd cpl.jws			2023/11	503.8	1.29472
5	tbd 4th cpl.jws			2023/11	503.4	1.19887

Figure S19. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the coupling of **1a** in the presence of 3M TBD base (at 1 µmole scale).



No.	File Name	Sample I	C	Date	Peak 1	Peak Vakue 1
1	1a_0v.jws			2023/11	503.8	1.58746
2	1a_V.jws			2023/11	503.8	1.47227
3	verkade_2nd cpl.jws			2023/11	504	1.35457
4	verkade_3rd cpl.jws			2023/11	504	1.23209
5	verkade_4th cpl.jws			2023/11	504	1.11158

Figure S20. Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λmax=504 nm) after consecutive detritylation steps during the couplings of **1a** in the presence of 2M Verkade base (at 1 µmole scale).

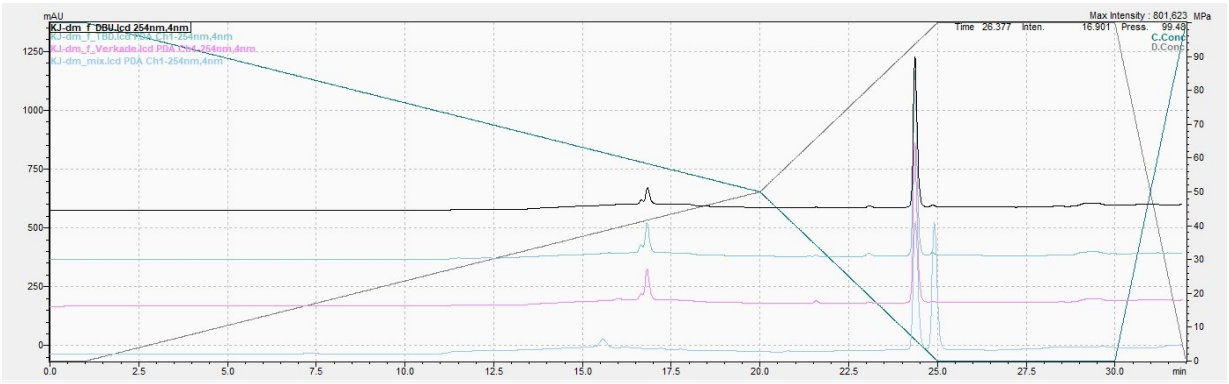


Figure S21. HPLC profiles recorded for P-stereodefined dinucleotides formed from OTP **1a** (*fast*-eluting P-diastereomer) in the presence of DBU (black line), TBD (green line) and Verkade base (pink line) and dinucleotide formed from OTP **1a** (mixture of P-diastereomers) in the presence of DBU (blue line).

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: KJ-dm_f_DBU		
Sample ID	:		
Data Filename	: KJ-dm_f_DBU.lcd		
Method Filename	: KJ-dimer_OFF.lcm		
Batch Filename	:		
Vial #	: -1	Sample Type	: Unknown
Injection Volume	: 100 uL		
Date Acquired	: 2/1/2024 11:18:11 AM	Acquired by	: Sterownik
Date Processed	: 2/1/2024 11:50:12 AM	Processed by	: Sterownik

<Chromatogram>

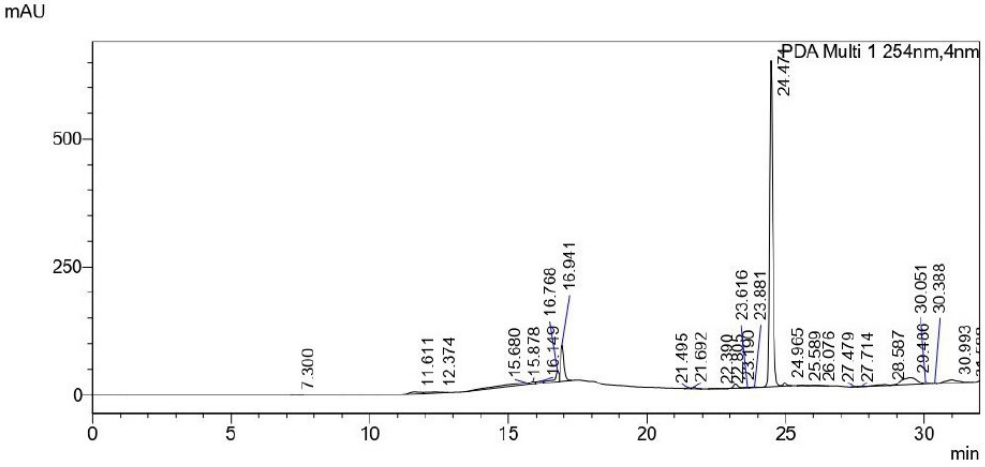


Figure S22. HPLC profile recorded for P-stereodefined dinucleotide formed from OTP **1a** (*fast*-eluting P-diastereomer) in the presence of DBU

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: KJ-dm_f_TBD		
Sample ID	:		
Data Filename	: KJ-dm_f_TBD.lcd		
Method Filename	: KJ-dimer_OFF.lcm		
Batch Filename	:		
Vial #	: -1	Sample Type	: Unknown
Injection Volume	: 100 uL		
Date Acquired	: 2/1/2024 12:59:03 PM	Acquired by	: Sterownik
Date Processed	: 2/1/2024 1:31:03 PM	Processed by	: Sterownik

<Chromatogram>

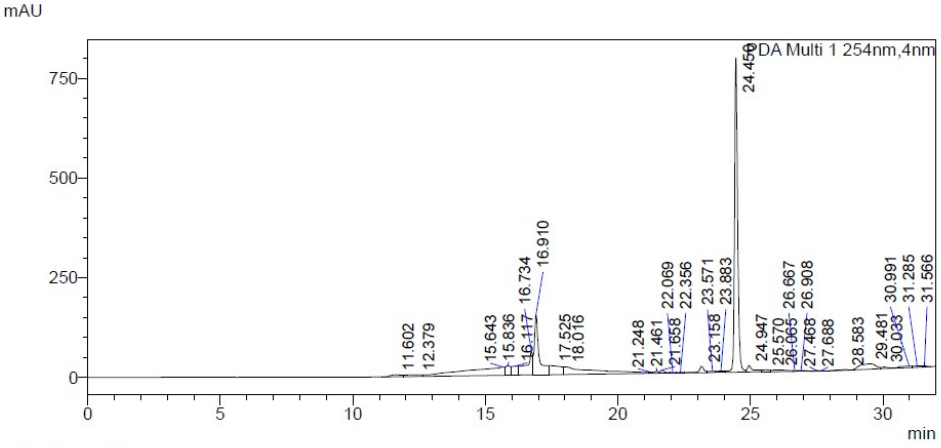


Figure S23. HPLC profile recorded for P-stereodefined dinucleotide formed from OTP **1a** (*fast*-eluting P-diastereomer) in the presence of TBD

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: KJ-dm_f_Verkade		
Sample ID	:		
Data Filename	: KJ-dm_f_Verkade.lcd		
Method Filename	: KJ-dimer_OFF.lcm		
Batch Filename	:		
Vial #	: -1	Sample Type	: Unknown
Injection Volume	: 100 uL		
Date Acquired	: 2/1/2024 1:45:35 PM	Acquired by	: Sterownik
Date Processed	: 2/1/2024 2:17:35 PM	Processed by	: Sterownik

<Chromatogram>

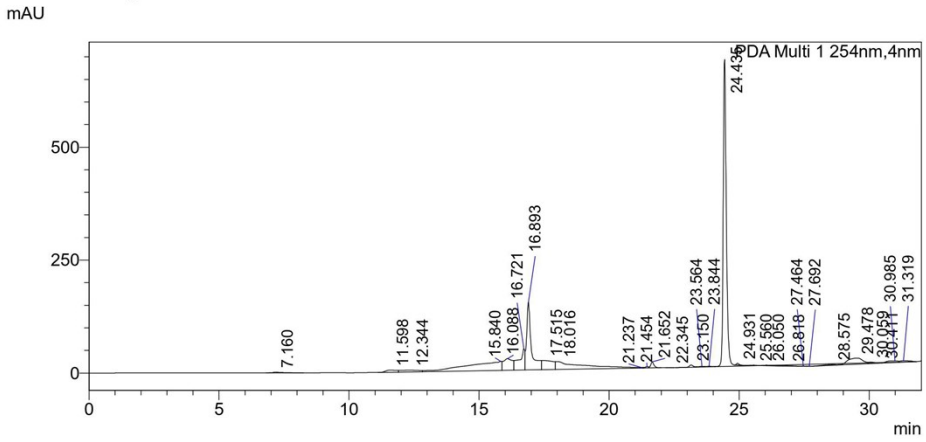


Figure S24. HPLC profile recorded for P-stereodefined dinucleotide formed from OTP **1a** (*fast*-eluting P-diastereomer) in the presence of Verkade base

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: KJ-dm_mix		
Sample ID	:		
Data Filename	: KJ-dm_mix.lcd		
Method Filename	: KJ-dimer_OFF.lcm		
Batch Filename	:		
Vial #	: -1	Sample Type	: Unknown
Injection Volume	: 100 uL		
Date Acquired	: 2/2/2024 9:27:58 AM	Acquired by	: Sterownik
Date Processed	: 2/2/2024 9:59:59 AM	Processed by	: Sterownik

<Chromatogram>

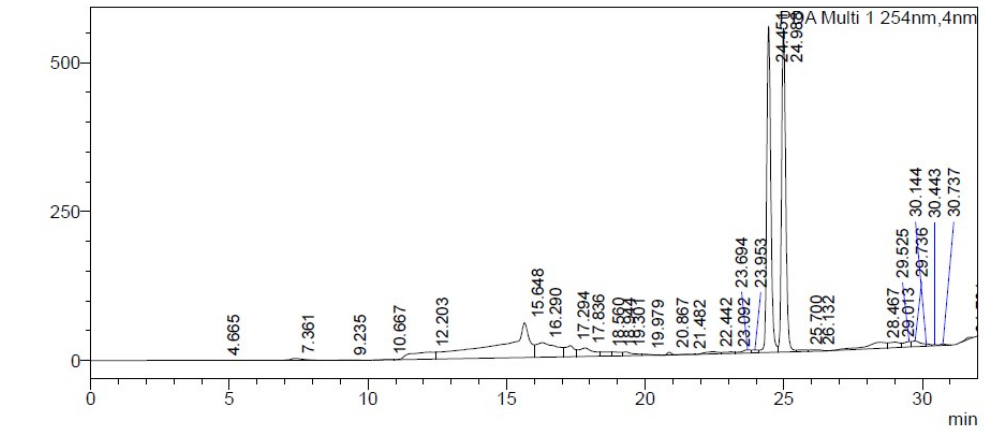


Figure S25. HPLC profile recorded for dinucleotide formed from OTP **1a** (mixture of P-diastereomers) in the presence of DBU