Supporting Information

Probing the importance of π-stacking interactions in DNA-templated self-assembly of bisfunctionalized guanidinium compounds

Delphine Paolantoni, [⊥a] Jenifer Rubio-Magnieto, [⊥b] Sonia Cantel,[a] Jean Martinez,[a] Pascal Dumy,[a] Mathieu Surin,[b]* and Sébastien Ulrich[a]*

[a]Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, Ecole Nationale Supérieure de Chimie de Montpellier 8 Rue de l'Ecole Normale, 34296 Montpellier cedex 5, France; Fax: (+) 33467144353; E-mail: Sebastien.Ulrich@enscm.fr
[b]Laboratory for Chemistry of Novel Materials, University of Mons–UMONS–, 20, Place du Parc B-7000 Mons, Belgium; E-mail: mathieu.surin@umons.ac.be

[⊥]These authors contributed equally to this work

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General methods and materials

All reagents were purchased from commercial sources (Sigma-Aldrich, Alpha Aesar or Fisher Scientific) and used as received. Dry solvents were purchased in anhydrous quality from Sigma-Aldrich. For dichloromethane, amylene was the stabiliser.
TLC were performed on silica gel 60 F_{254} plates purchased from Merck. Flash column chromatography was performed on silica gel 60 (40 – 63 µm) purchased from Merck. NMR spectra were recorded on Bruker Avance 400 or 250 MHz instruments and were referenced with respect to the residual solvent peak for the deuterated solvent. Data are reported as follows: chemical shift (δ in ppm), multiplicity (s for singlet, d for doublet, m for multiplet), coupling constant (J in Hertz) and integration. High resolution mass spectrometry analyses were carried out at the Laboratoire de Mesures Physiques, IBMM – Université Montpellier 2, and were obtained on a Waters Micromass QTof mass spectrometer (positive mode). LC-MS analyses were performed on a Waters 2695 HPLC separation module equipped with a C18 column (Macherey-Nagel EC Nucleosil 300-5 125 mm x 3 mm), connected to Waters 996 photodiode array detector and Waters micromass ZQ mass spectrometer. Eluent A: H_2O/TFA 99.9%/0.1%; eluent B: CH_3CN/H_2O/TFA 90%/9.9%/0.1%. Linear gradient: 5% eluent B → 100% eluent B in 5 minutes. Flow: 1 mL/min. IR spectra were measured on a Perkin Elmer Spectrum 100 FT-IR spectrometer equipped with an universal ATR sampling accessory. Wavenumbers $\tilde{\nu}$ are indicated in cm^{-1} and band intensities are reported in brackets as weak (w), medium (m) or strong (s).
UV-Vis absorption, circular dichroism, and fluorescence measurements were recorded using a ChirascanPlus CD Spectrophotometer from Applied Photophysics (UK). The measurements were carried out using 2 mm Suprasil quartz cells from Hellma Analytics. The spectra were recorded between 240 and 500 nm, with a bandwidth of 1 nm and time per point 1 s. Fluorescence measurements were carried out at different temperatures by using a 10 mm quartz cells (1 mL) from Lightpath Optical. The excitation wavelength was set at 348 nm. The spectra were recorded between 355 and 650 nm, with a bandwidth of 10 nm and time per point 0.5 s.The buffered water solvent reference spectra were used as baselines and were automatically subtracted from the CD spectra of the samples.
The variable temperature spectroscopic experiments were performed using a TC125 Temperature Controller from Quantum Northwestern running on the ChirascanPlus Spectrophotometer. The temperatures were varied from -5 ºC to 80 ºC at rate of 0.5 ºC/min. The temperature within the quartz cells was determined using a temperature probe. The rate for decreasing the temperature from 80 ºC to -5 ºC was of 0.5 ºC/min in order to allow equilibration. For the heating/cooling cycles, the temperatures were varied from -5 ºC to 80 ºC using the following conditions:

- First Heating Cycle (H-C): From -5 ºC to 80 ºC at a rate of 10 ºC/min.
- 10 minutes of stabilization at 80 ºC.
- First Cooling Cycle (C-C): From 80 ºC to -5 ºC at a rate of 0.5 ºC/min.
- Second Heating Cycle (H-C): From -5 ºC to 80 ºC at a rate of 0.5 ºC/min.
- Second Cooling Cycle (C-C): From 80 ºC to -5 ºC at a rate of 0.5 ºC/min.
- After about 14 hours, the solution was stirred.
- Third Heating Cycle (H-C): From -5 ºC to 80 ºC at a rate of 0.5 ºC/min.
- Third Cooling Cycle (C-C): From 80 ºC to -5 ºC at a rate of 0.5 ºC/min.

MALDI-ToF mass spectrometry. *Sample preparation*: Stock solutions of guanidinium were prepared in DMSO at a concentration of 10 mM (**GuaBiPy**) or 100 mM (**GuaBiPhe** and **GuaBiNaph**). A dT_{10} (Eurogentec, RP-Cartridge purification) stock solution was prepared by dilution with MilliQ water to a concentration of 10 mM. Samples were then prepared by dilution of stock solutions to final concentrations of 1 mM in guanidinium and 0.1 mM in

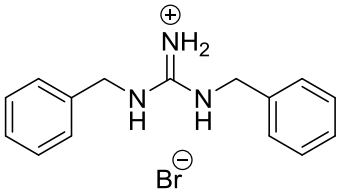
dT_{10} in a mixture of MilliQ water and DMSO (final proportion of DMSO: 1% with **GuaBiPhe**, 40% with **GuaBiNaph** and 80% with **GuaBiPy**). Samples were further diluted 4 times in MilliQ water prior to analyses. According to the dried droplet procedure, 0.5 µl of a solution of the 4-NA matrix in ethanol (0,1M) was deposited on the MALDI target (AnchorChip™, Bruker), then mixed with the sample in equal amount. Sample spots were dried at room temperature. *Mass spectrometric analysis*: MALDI mass spectra were performed on an Ultraflex III TOF/TOF instrument (Bruker Daltonics, Wissembourg, France) equiped with LIFT capability. A pulsed Nd:YAG laser at a wavelength of 355 nm was operated at a frequency of 100 Hz. MS analyses were conducted in positive reflectron ion mode with a pulse ion extraction delay of 30ns. An acceleration voltage of 25.0 kV (IS1) was applied for a final acceleration of 21.95 kV (IS2). Mass spectra were acquired from at least 150 laser shots, over a mass range from m/z 500 to 5000. A deflection at 2000 Da could be applied. The laser fluence was adjusted for each studied sample above the threshold for generation of molecular ions. Data were acquired with the Flex Control software and processed with the Flex Analysis software. External calibration was systematically performed with commercial peptide mixture (Calibration peptide standard II) in a linear correction calibration.

Synthesis and characterization of guanidinium compounds:

1. Bis-*N*,*N*-benzylguanidinium bromide (**GuaBiPhe**)

The synthetic procedure was adapted from a previous report.[1]

A solution of cyanogen bromide (103 mg, 0.96 mmol) in anhydrous acetonitrile (1 mL) was added dropwise to a solution of benzylamine (0.21 mL, 1.92 mmol) in anhydrous acetonitrile (2.5 mL) cooled at 0°C. The reaction mixture was then refluxed for 14 hours.

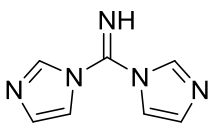


The suspension was then filtrated and the white solid was washed with acetonitrile and diethyl ether. The filtrate was concentrated *in vacuo* and the resulting oil was crystallized in dichloromethane at -20°C. The solid was filtered, washed with the minimum volume of dichloromethane and dried *in vacuo*, yielding **GuaBiPhe** as white crystals (165 mg, 54% yield). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.39-7.27 (m, 5H, C$H_{(aro)}$), 4.46 (s, 2H, CH_2NH); ^{13}C NMR (CD_3OD, 63 MHz): δ 157.4, 137.6, 129.8, 128.8, 127.7, 45.9; HR-ESI-MS: m/z calcd for $C_{15}H_{18}N_3^+$ ($[M+H]^+$): 240.1501, found: 240.1500. IR (ATR): $\tilde{\nu}$ = 3323 (w), 3157 (m), 3025 (w), 2876 (w), 1644 (m), 1623 (s), 1595 (m), 1496 (m), 1469 (m), 1441 (m), 1358 (m), 1325 (w), 1245 (w), 1206 (w), 1136 (w), 1103 (w), 1057 (w), 1029 (w), 989 (w), 1029 (w), 989 (w), 961 (w), 903 (w), 875 (w), 750 (m), 740 (m), 720 (m), 702 (s), 673 (m), 621 (w).

2. Di(1H-imidazol-1-yl)methanamine (**2**)

This compound was prepared as previously described and the characterization were conformed to the literature data.[2]

To a solution of imidazole (5.52 g, 84.9 mmol) in dry dichloromethane (408 mL) under argon was added a 3M solution of cyanogen bromide in dichloromethane (9.44 mL, 28.3 mmol). The reaction mixture was refluxed for 35 minutes and the formation of a white precipitate was observed. The suspension was then filtrated. The filtrate was concentrated *in vacuo* to 10% of its initial volume and crystallized at -20°C. The resulting suspension was filtrated, washed with cold dichloromethane and dried *in vacuo*, yielding compound **2** as white crystals (3.32 g, 73%



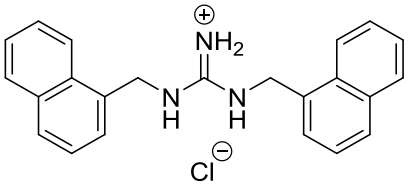
[1] Lovick, H. M.; Michael, F. E. *Tetrahedron Lett.* **2009**, *50*, 1016–1019.

yield). ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 10.19 (s, 1H, N*H*), 8.08 (d, J = 20.8 Hz, 2H, CNC*H*CHN), 7.56 (d, J = 29.3 Hz, 2H, CNCHC*H*N), 7.11 (s, 2H, NC*H*N); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 140.9, 137.4, 129.6, 118.9.

3. Bis-*N*,*N*-(naphtalen-1-ylmethyl)guanidinium chloride (**GuaBiNaph**)

The procedure was adapted from a previous report.[2]

A mixture of di(1*H*-imidazol-1-yl)methanamine (**2**) (203 mg, 1.26 mmol), 1-naphtylmethylamine (0.36 mL, 2.52 mmol) and TFA (74.5 µL, 0.98 mmol) in a screwed-cap tube was stirred at 105°C for 4 hours. Once cooled to room temperature, the obtained yellow-brownish oil was dissolved in dichloromethane (20 mL). The organic solution was washed with saturated ammonium chloride solution (20 mL). The aqueous phase was extracted with dichloromethane (3 x 10 mL). The organic phases were combined and washed with a saturated aqueous ammonium chloride solution, dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (dichloromethane/methanol: 100/0→90/10). Further recrystallization in chloroform/acetonitrile yielded the desired **GuaBiNaph** as a white solid (145 mg, 31% yield). LC-MS: t_R 4.30 minutes; extracted m/z calcd for $C_{23}H_{22}N_3^+$ ([M+H]$^+$): 340.18, found: 340.62; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.99-7.87 (m, 6H, C$H_{(aro)}$), 7.59-7.44 (m, 8H, C$H_{(aro)}$), 4.95 (s, 4H, CH_2NH); ^{13}C NMR (CD_3OD, 63 MHz): δ 157.7, 135.4, 132.7, 132.4, 130.0, 130.0, 127.8, 127.2, 126.5, 126.4, 123.9, 44.6; HR-ESI-MS: m/z calcd for $C_{23}H_{22}N_3^+$ ([M+H]$^+$): 340.1814, found: 340.1815. IR (ATR): $\tilde{\nu}$ = 3290 (m), 3146 (m), 3055 (m), 3014 (m), 1679 (m), 1660 (s), 1639 (s), 1623 (s), 1600 (s), 1513 (m), 1451 (w), 1399 (w), 1362 (w), 1324 (w), 1264 (w), 1216 (w), 1166 (w), 1110 (m), 1030 (w), 1005 (w), 883 (w), 853 (w), 786 (s), 765 (s), 731 (m), 645 (m).



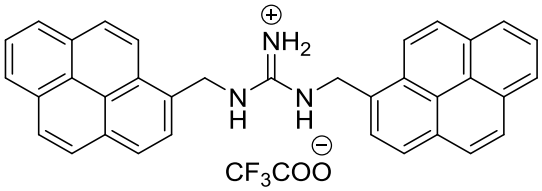
4. 1-pyrenemethylamine (**3**)

1-pyrenemethylamine was prepared from 1-pyrenemethylammonium chloride as follows:
1-pyrenemethylammonium chloride (500 mg, 1.86 mmol) was suspended in an aqueous 10% sodium hydroxide solution (10 mL). Extraction with dichloromethane (10 mL), drying over sodium sulfate and concentration *in vacuo* afforded the desired compound as a pale yellow solid (431 mg, 99% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): 8.12-8.08 (m, 3H, C$H_{(aro)}$), 8.00-7.92 (m, 5H, C$H_{(aro)}$), 7.90 (d, J = 7.8 Hz, 1H, C$H_{(aro)}$), 4.36 (s, 2H, CH_2NH).

5. Bis-*N*,*N*-(pyren-1-ylmethyl)guanidinium trifluoroacetate (**GuaBiPy**)

The procedure was adapted from a previous report.[2]

A mixture of di(1*H*-imidazol-1-yl)methanamine (**2**) (87.8 mg, 0.54 mmol), 1-pyrenemethylamine (**3**) (241.5 mg, 1.13 mmol) and TFA (32.7 µL, 0.42 mmol) in a screwed-cap tube was stirred at 105°C for 4 hours. Once cooled to room temperature, the solidified orange - brown oil was re-suspended in dichloromethane. The solid was filtrated, further washed with dichloromethane and diethyl ether, and dried *in vacuo* to obtain **GuaBiPy** as a powder (19.7 mg, 24% yield). LC-MS: t_R 5.34 minutes, 97.8% peak area at 254 nm; extracted m/z calcd for $C_{35}H_{26}N_3^+$ ([M+H]$^+$): 488.21, found: 488.67; ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 8.34-8.06 (m, 20H, C$H_{(aro)}$ and N*H*/NH_2^+), 7.90 (broad s, 2H, N*H*/NH_2^+), 5.25 (d, J = 4.7 Hz, 4H,



[2] Jadhav, V. D.; Schmidtchen, F. P. *J. Org. Chem.* **2007**, *73*, 1077–1087.

CH_2NH); ^{13}C NMR (DMSO-*d6*, 63 MHz): δ 156.1, 130.8, 130.5, 130.3, 130.2, 128.0, 127.9, 127.5, 127.4, 126.5, 125.6, 125.5 (2C), 124.8, 124.1, 123.8, 122.8, 43.0; HR-ESI-MS: m/z calcd for $C_{35}H_{26}N_3^+$ ($[M+H]^+$): 488.2127, found: 488.2130. IR (ATR): $\tilde{\nu}$ = 3310 (m), 3129 (m), 3041 (m), 1663 (s), 1632 (s), 1489 (w), 1459 (w), 1433 (w), 1416 (w), 1392 (w), 1370 (w), 1341 (w), 1298 (w), 1207 (s), 1172 (s), 1132 (s), 958 (w), 888 (w), 834 (s), 801 (m), 752 (w), 724 (m), 702 (m), 678 (w), 656 (w).

Preparation of the oligonucleotides (ODN).

The buffer was prepared by using tris(hydroxymethyl)aminomethane ($(HOCH_2)_3CNH_2$), EDTA (Aldrich, HPLC grade) in Milli-Q water. The oligonucleotides (ODN) were purchased from Eurogentec (Belgium) as HPLC-RP purification (Ultrapure Gold, >95% purity) in dried format, and the purity of the ODN sequences was checked with MALDI-ToF. The oligonucleotides were dissolved in a volume of Tris-EDTA (TE) buffer at a concentration of 100 μM. The solution obtained was centrifuged during 2 minutes at 2000 rpm. 20 μL of this solution were used in order to prepare different aliquots. A solution of 280 μL of MQ water was added to each aliquot in order to obtain a final volume of 300 μL (1.33 mM Tris buffer and 67 μM EDTA) and the final diluted solution was mixed using a vortex.

Preparation of the DNA–Guanidinium solutions.

The concentration of the aliquot of DNA in TE buffer solution (1.33 mM Tris buffer and 67 μM EDTA) was determined by UV-Vis at 25 ºC using the specific extinction coefficients (ε_{260}) of each DNA, which are 81600 L.mol^{-1}.cm^{-1}, 162600 L.mol^{-1}.cm^{-1}, and 324600 L.mol^{-1}.cm^{-1}, 412900 L.mol^{-1}.cm^{-1} and 675815 L.mol^{-1}.cm^{-1} for ssDNAd$(T)_{10}$, ssDNAd$(T)_{20}$, ssDNAd$(T)_{40}$, ssDNAd$(R)_{43}$ and dsDNAd$(R)_{43}$, respectively. The structure of these DNA ODNs is described in the Chart 1 below. The guanidinium compounds were dissolved in DMSO with a 10 mM concentration (stock solution). The stock solution of Guanidinium was added to the DNA solution and the molar ratio between guanidinium compounds and DNA was adjusted to the DNA concentration. Both compounds were stirred using the vortex at vigorous speed during 2 minutes.

dT$_{10}$: 5'-TTT TTT TTT T-3'

dT$_{20}$: 5'-TTT TTT TTT TTT TTT TTT TT-3'

dT$_{40}$: 5'-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT T-3'

dR$_{43}$: 5'-CGT CAC GTA AAT CGG TTA ACA AAT GGC TTT CGA AGC TAG CTT C-3'

dR$_{43}$·dR$_{rev43}$: 5'-CGT CAC GTA AAT CGG TTA ACA AAT GGC TTT CGA AGC TAG CTT C-3'
3'-GCA GTG CAT TTA GCC AAT TGT TTA CCG AAA GCT TCG ATC GAA G-5'

Chart S1. Oligonucleotide sequences.

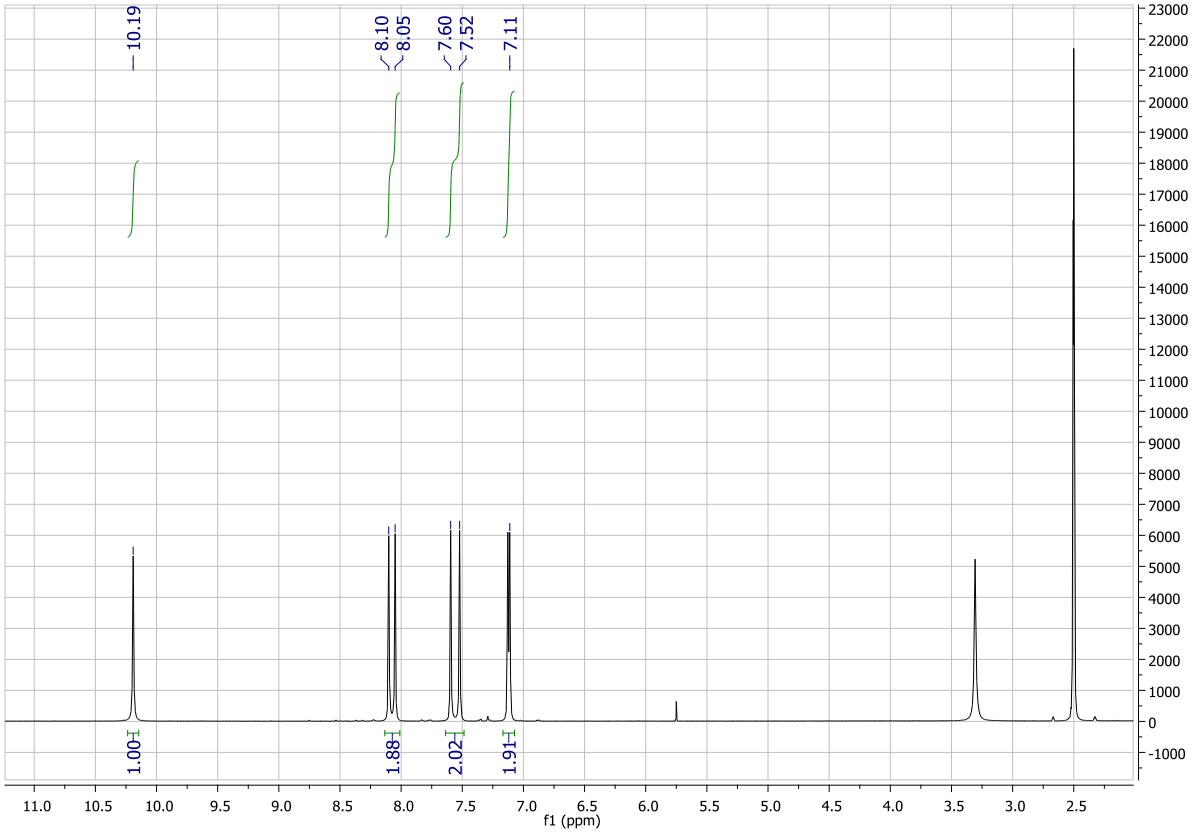


Fig. S1. ^{1}H NMR of **2**.

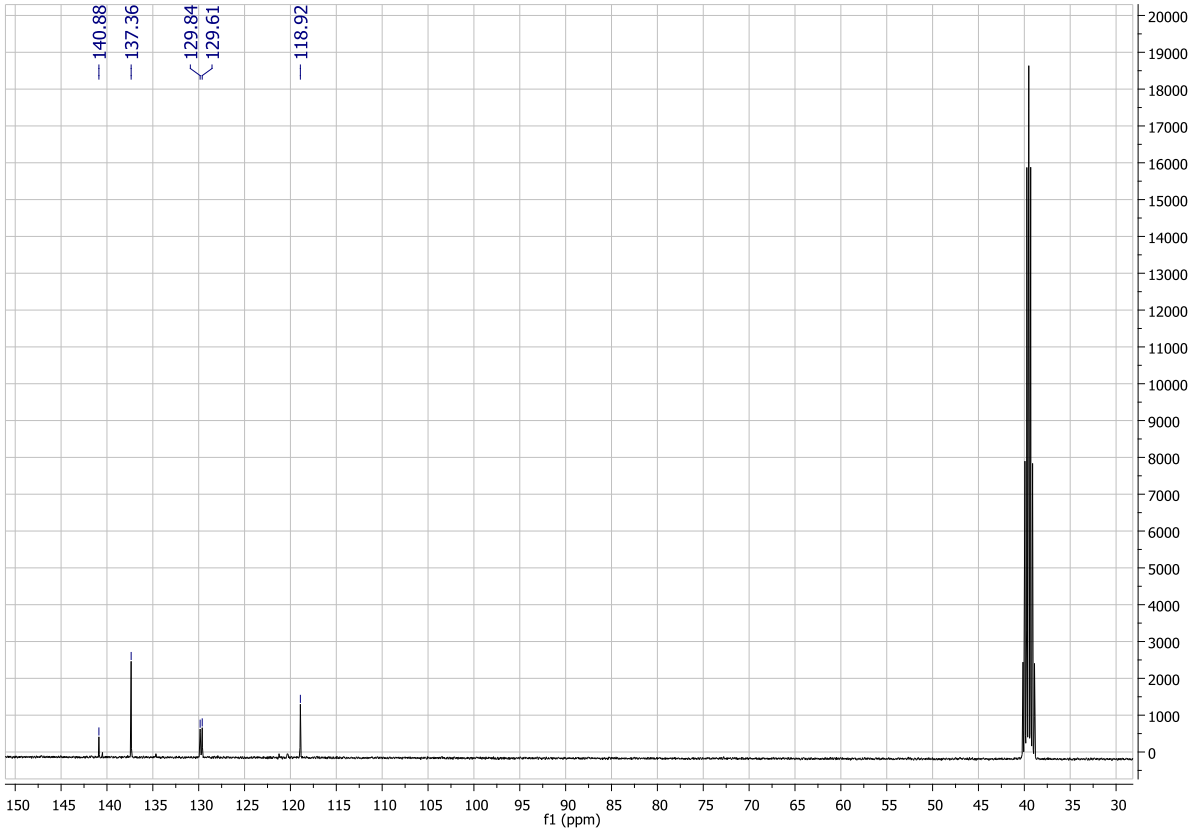


Fig. S2. ^{13}C NMR of **2**.

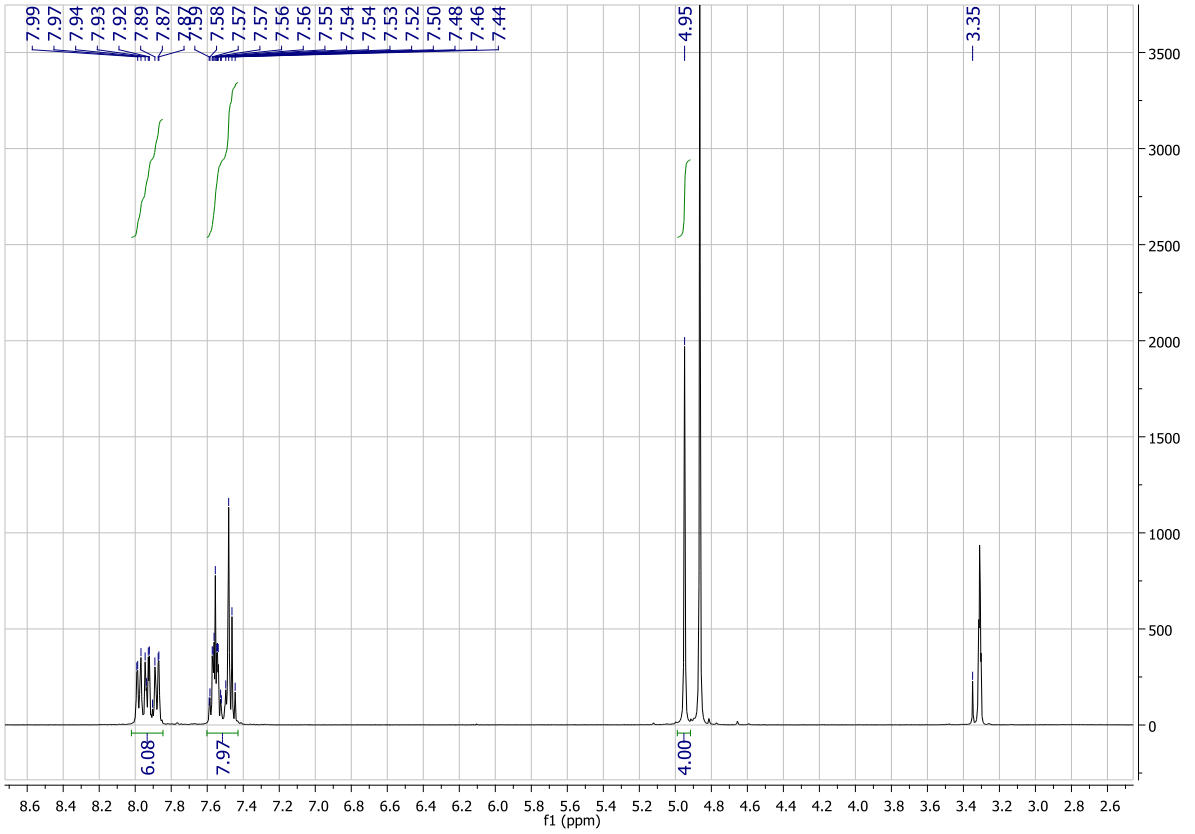


Fig. S3. ^{1}H NMR of **GuaBiNaph**.

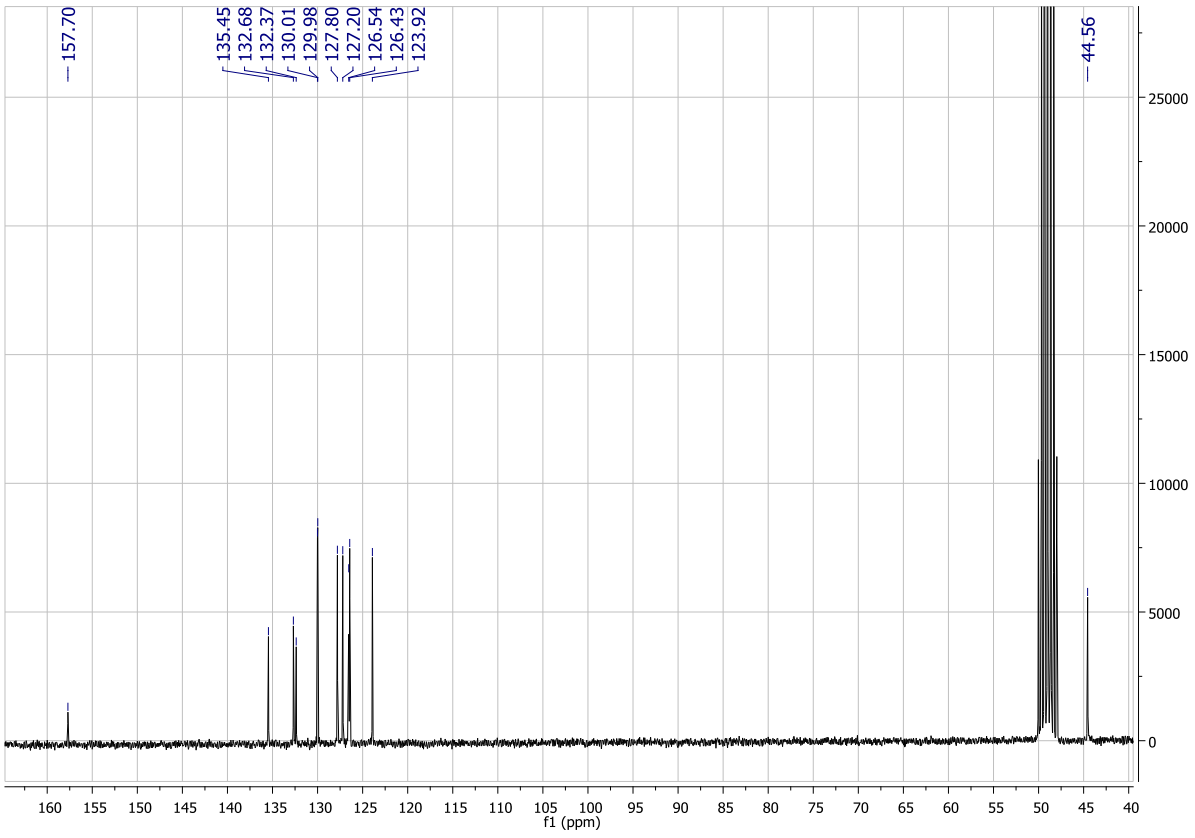


Fig. S4. ^{13}C NMR of **GuaBiNaph**.

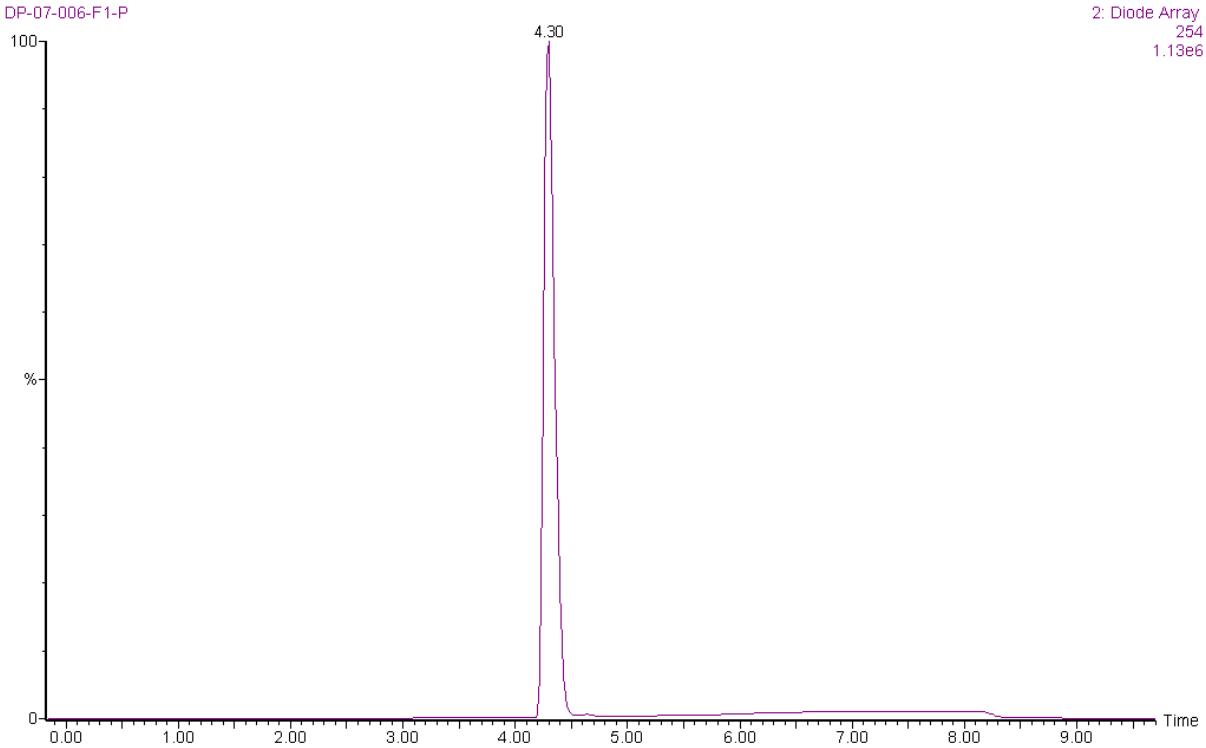


Fig. S5. HPLC chromatogram of **GuaBiNaph** (detection at 254 nm).

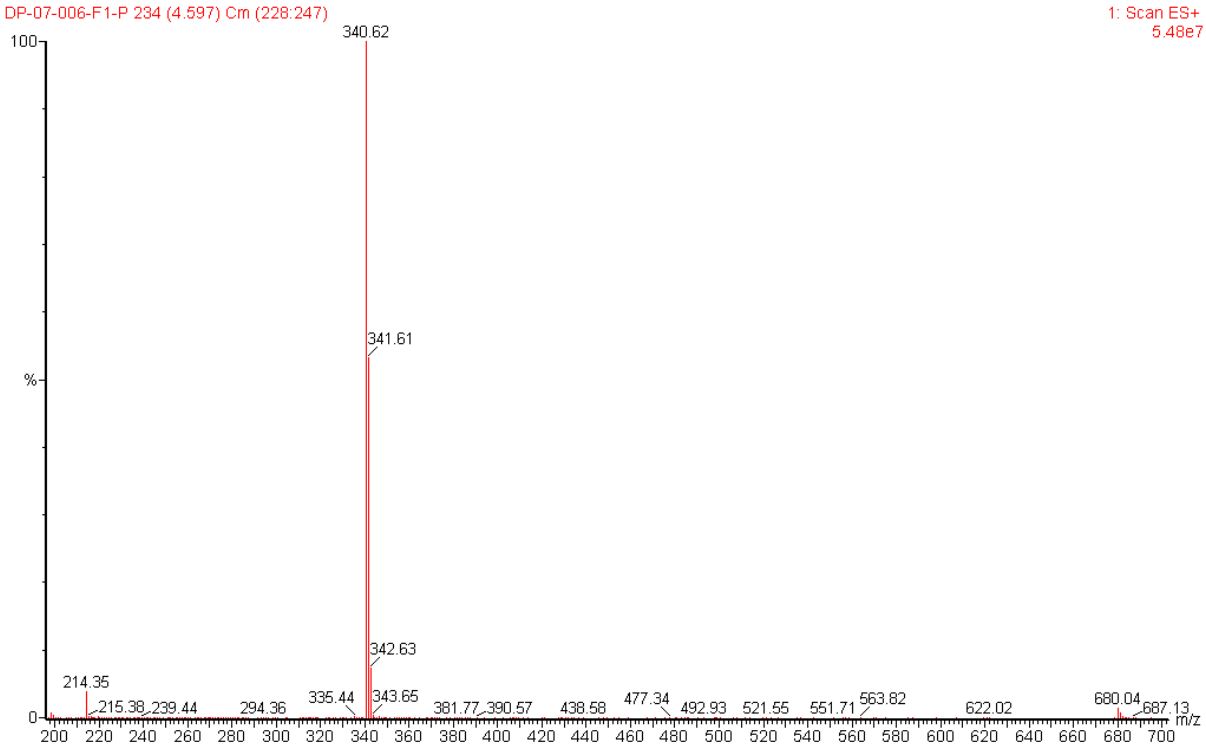


Fig. S6. Extracted mass spectrum of peak at 4.30 minutes from LC-MS.

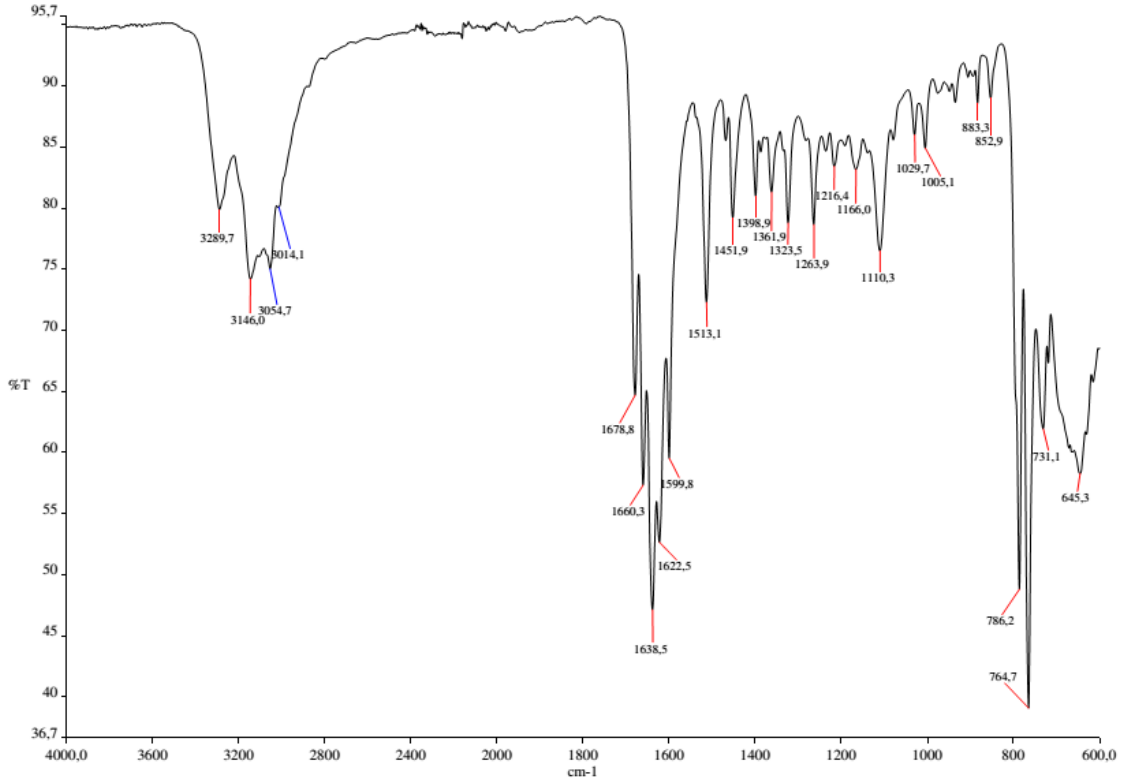


Fig. S7. IR spectra of **GuaBiNaph**.

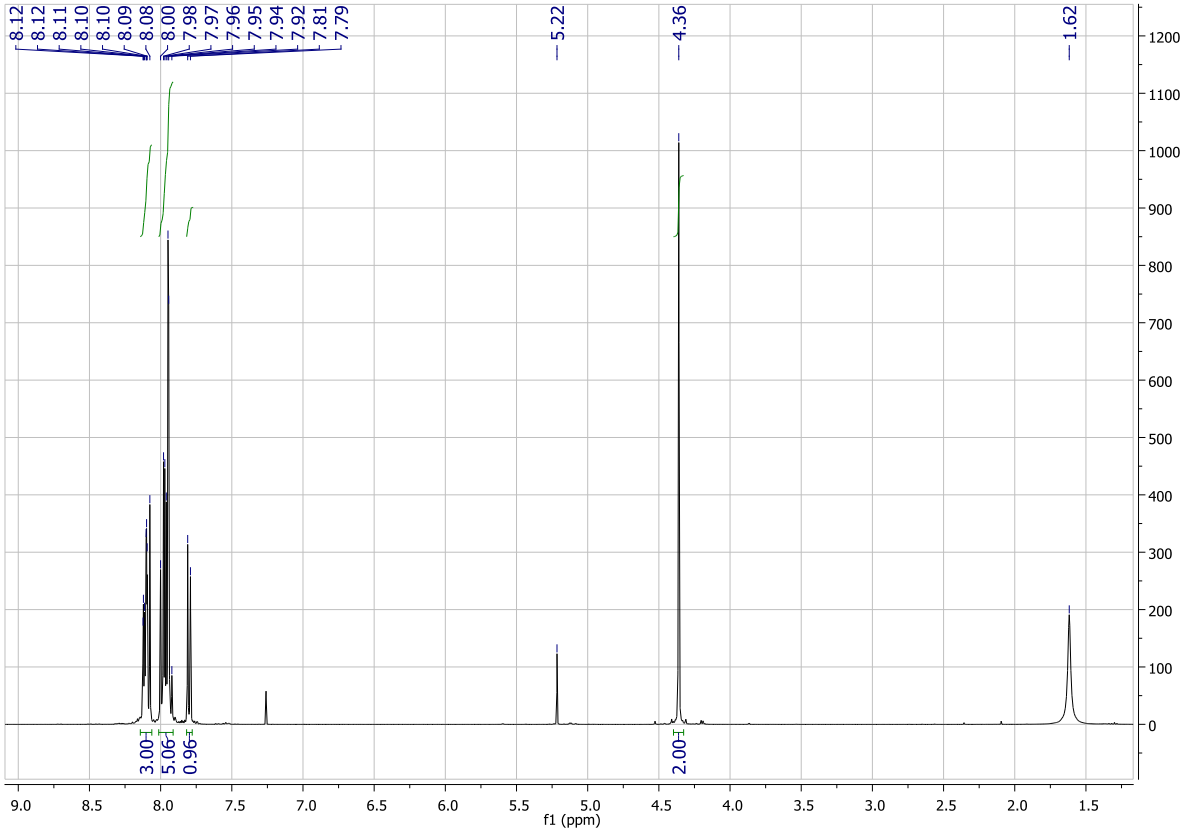


Fig. S8. ^{1}H NMR of **3**.

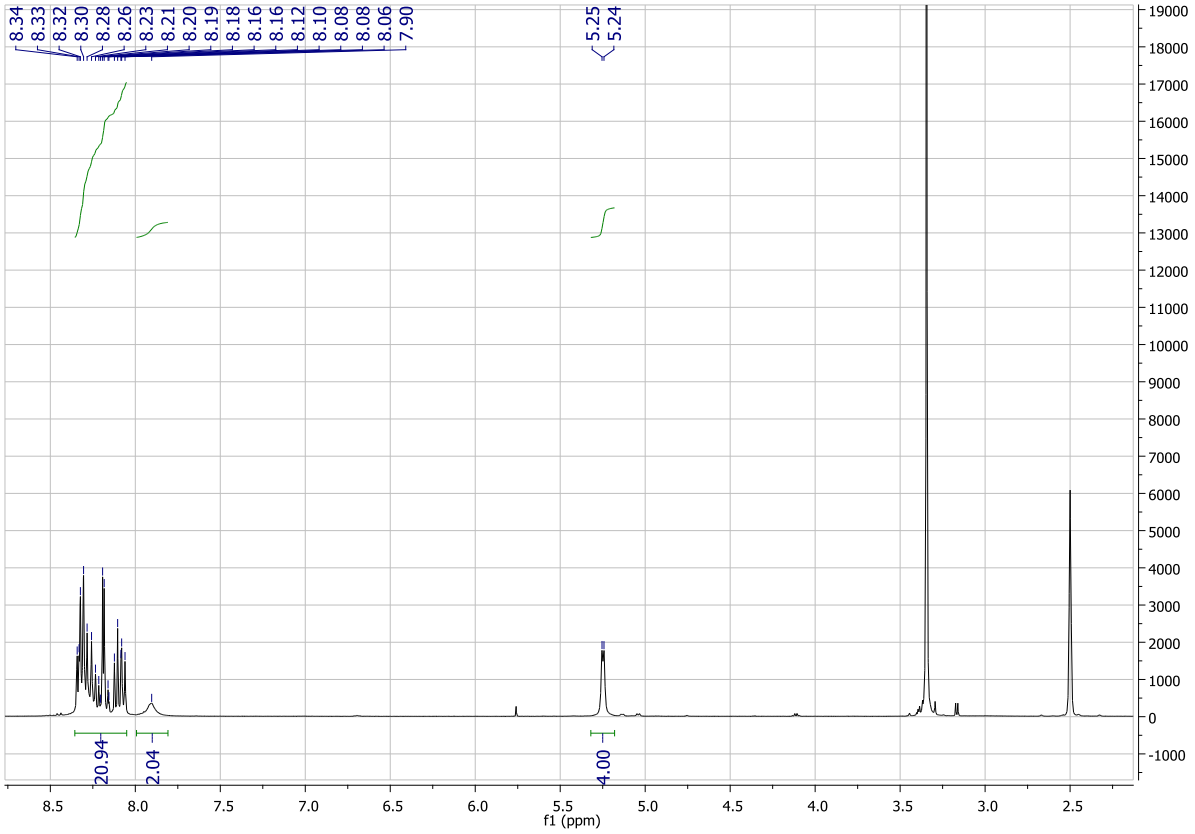


Fig. S9. ^{1}H NMR of **GuaBiPy**.

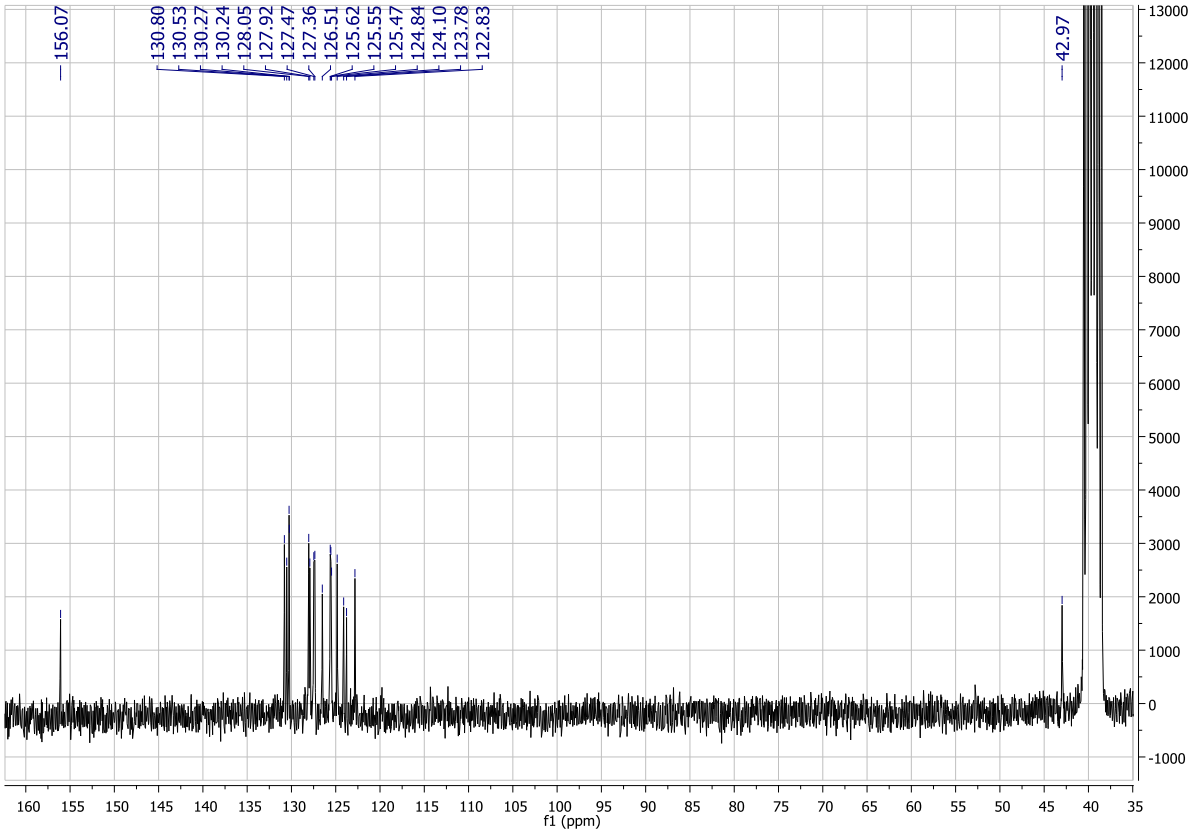


Fig. S10. ^{13}C NMR of **GuaBiPy**.

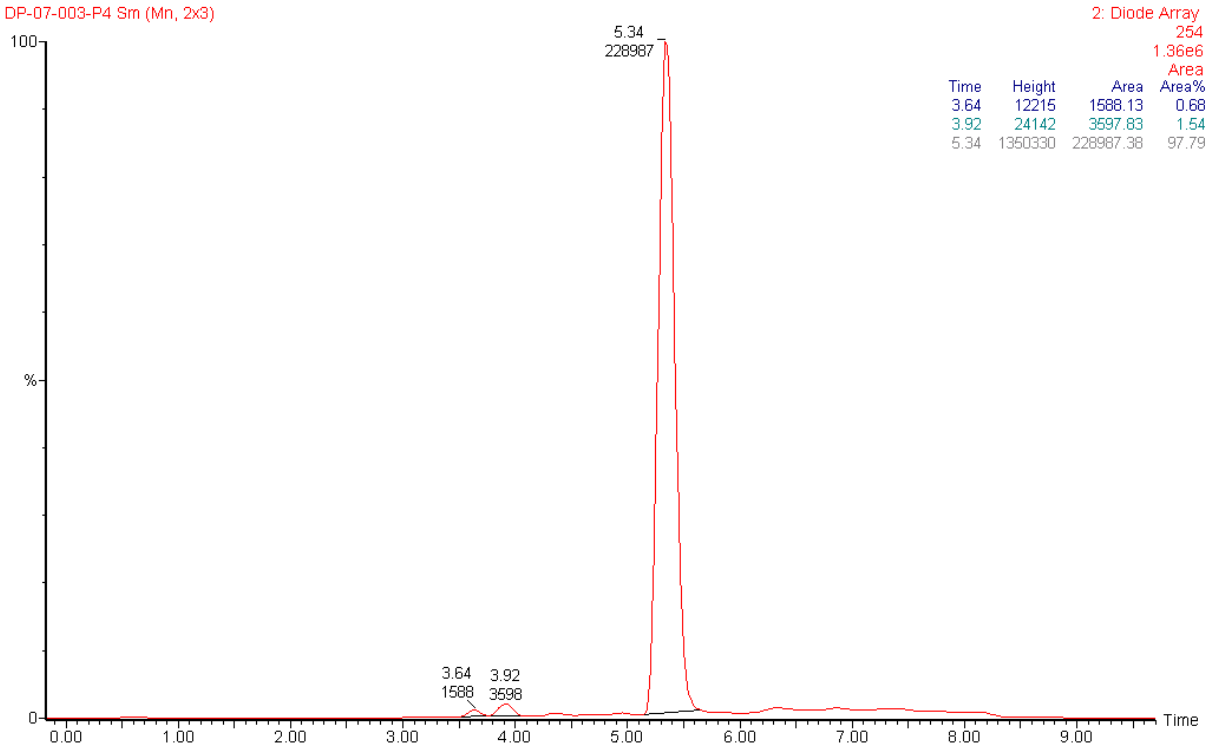


Fig. S11. HPLC chromatogram of **GuaBiPy** (detection at 254 nm).

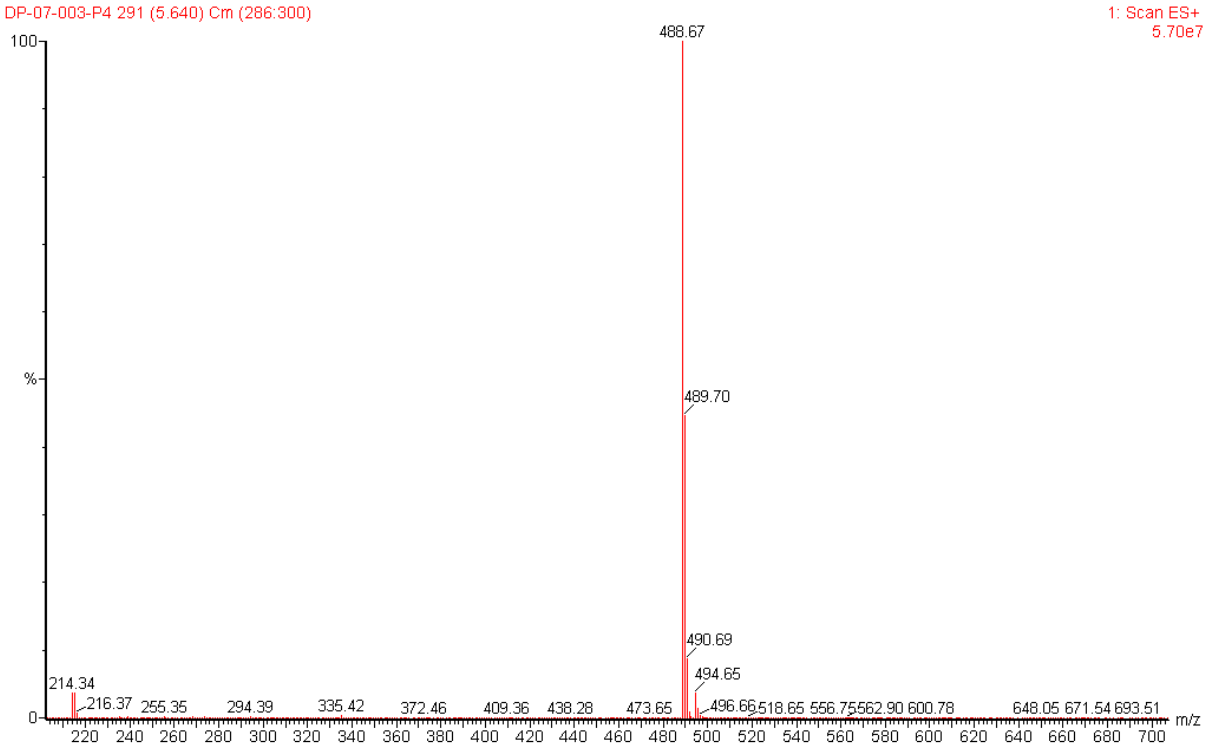


Fig. S12. Extracted mass spectrum of peak at 5.34 minutes from LC-MS.

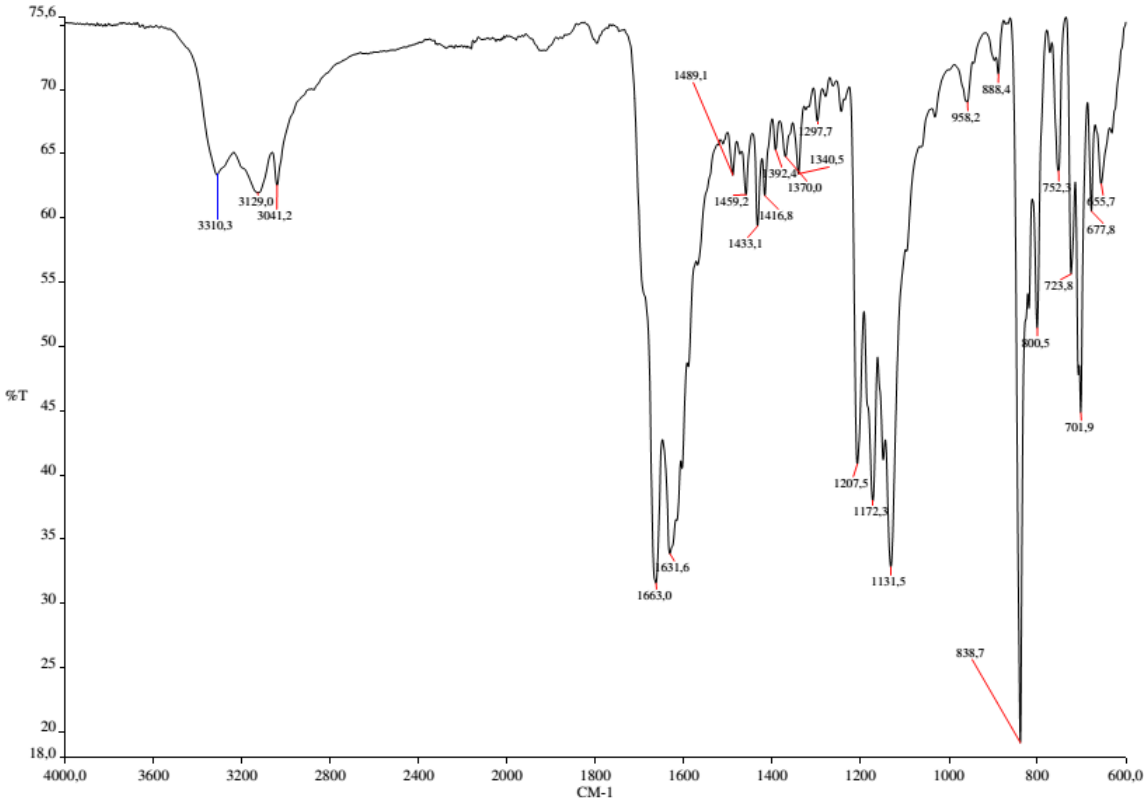


Fig. S13. IR spectra of **GuaBiPy**.

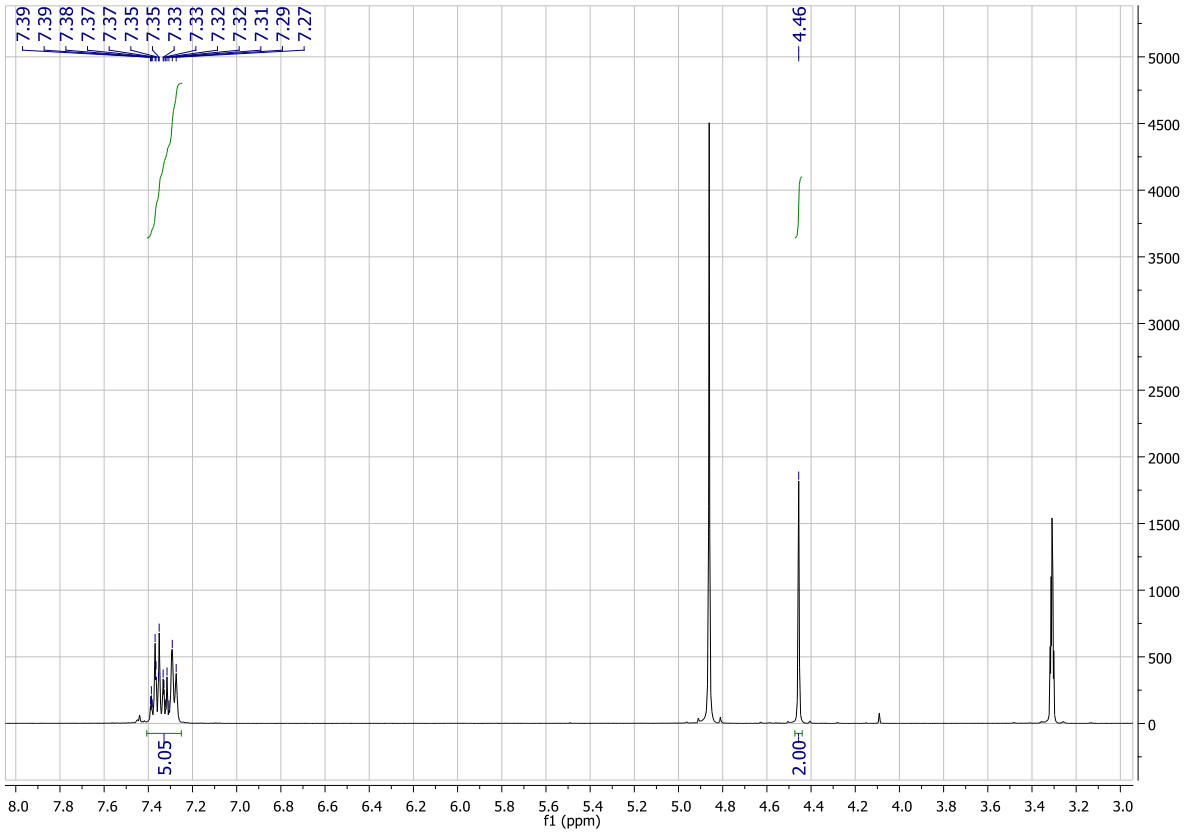


Fig. S14. ^{1}H NMR of **GuaBiPhe**.

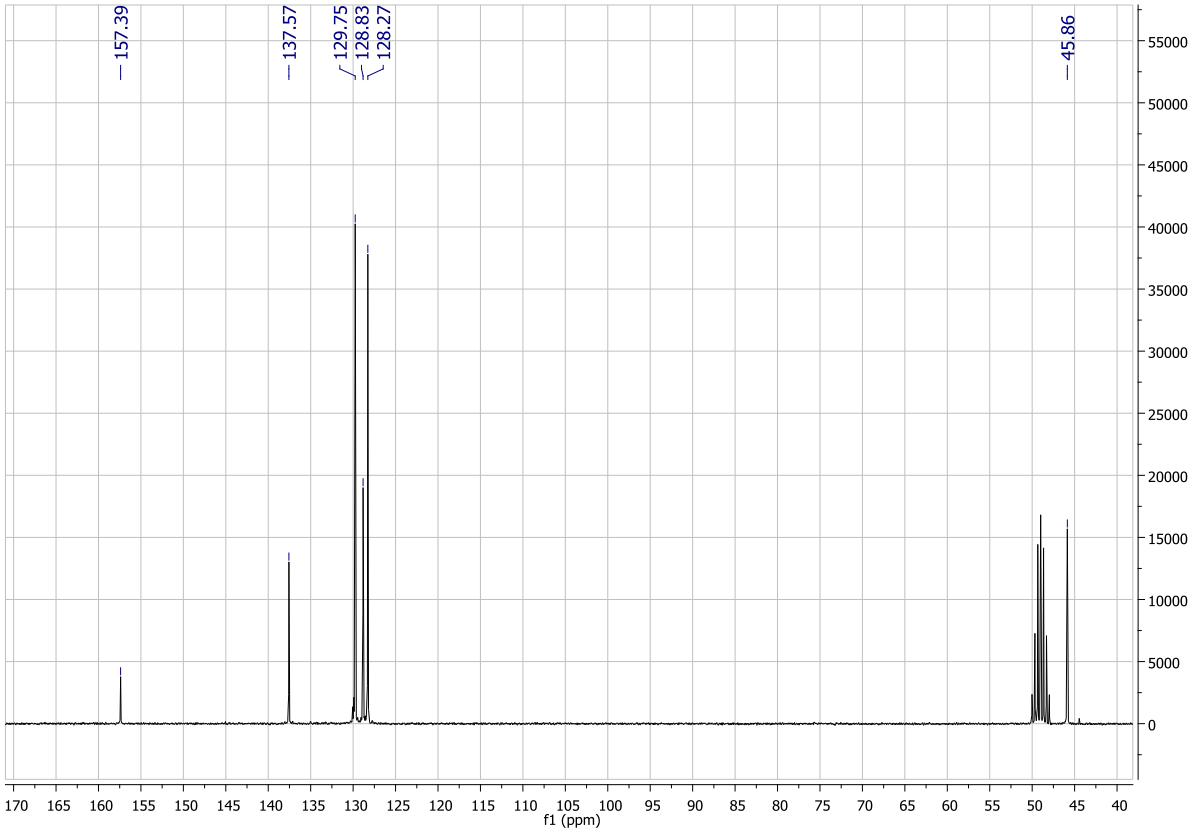


Fig. S15. ^{13}C NMR of **GuaBiPhe**.

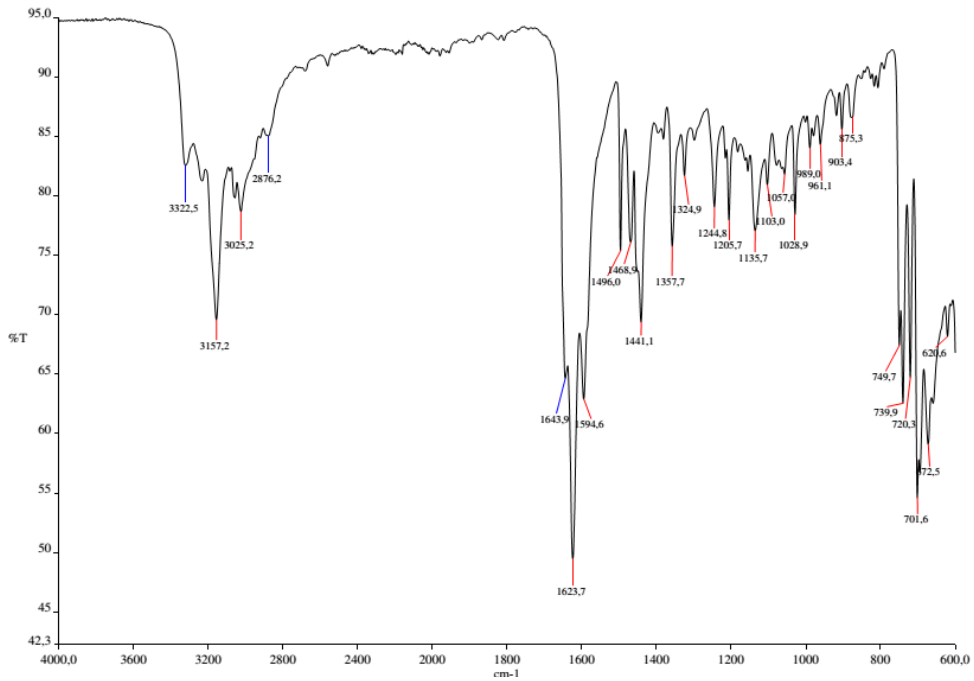


Fig. S16. IR spectra of **GuaBiPhe**.

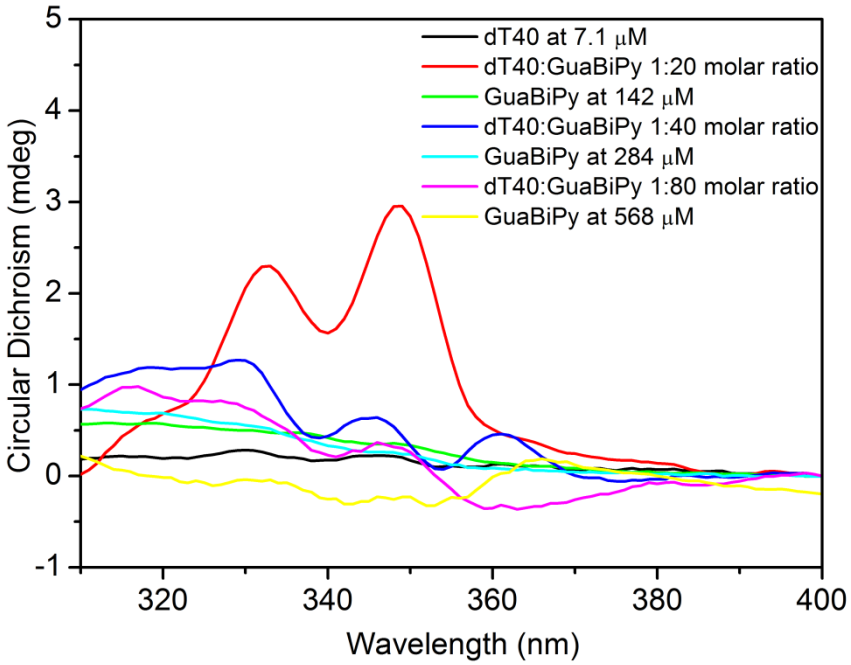


Fig. S17. Circular dichroism spectra of pure **GuaBiPy** and dT_{40}:**GuaBiPy** at different molar ratio.

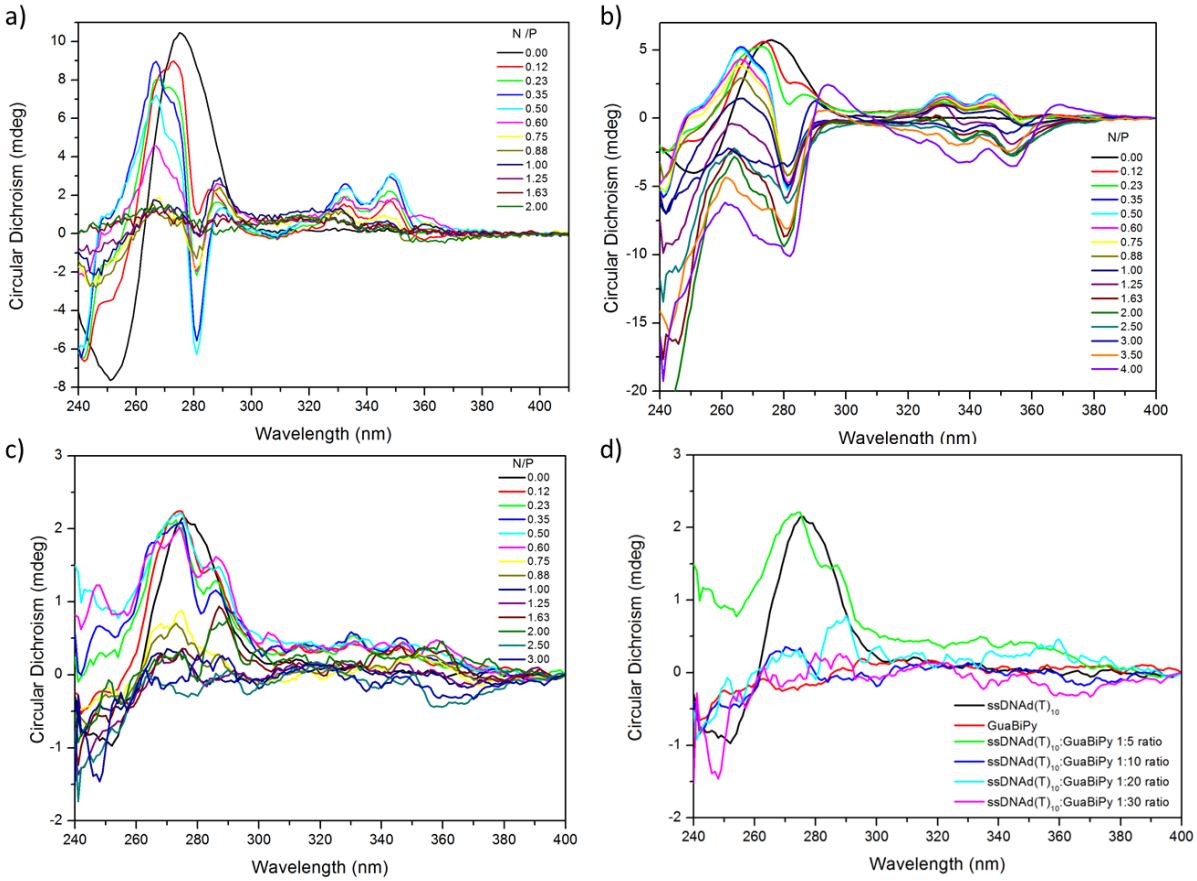


Fig. S18. Titration experiments of a) dT_{40}, b) dT_{20}, and c) and d) dT_{10} by **GuaBiPy** in TE buffer at -5 ºC, monitored by circular dichroism spectroscopy. N/P is the number **GuaBiPy** molecules per phosphate group (or nucleobase) in DNA. $[dT_n]$ ~ 5.7 μM.

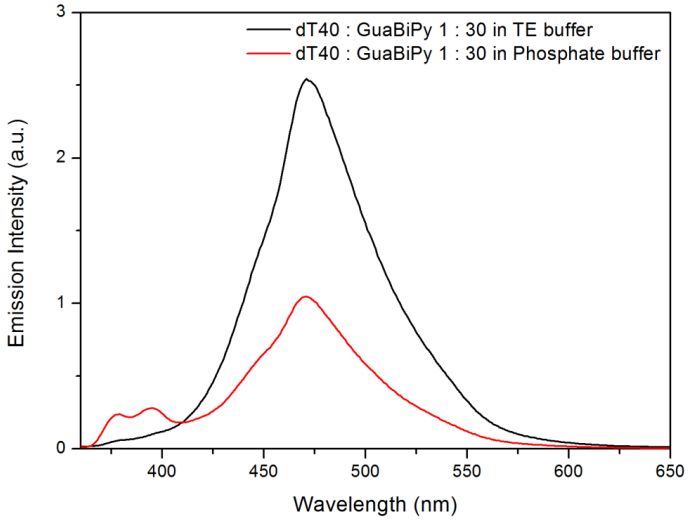


Fig. S19. Emission spectra (at λ_{exc} = 348 nm) of a mixture of dT_{40}:**GuaBiPy** 1:30 ratio in TE buffer (black line) and in phosphate buffer (red line) at -5 ºC in the second heating cycle (H-C). [dT_{40}] ~ 5.7 µM.

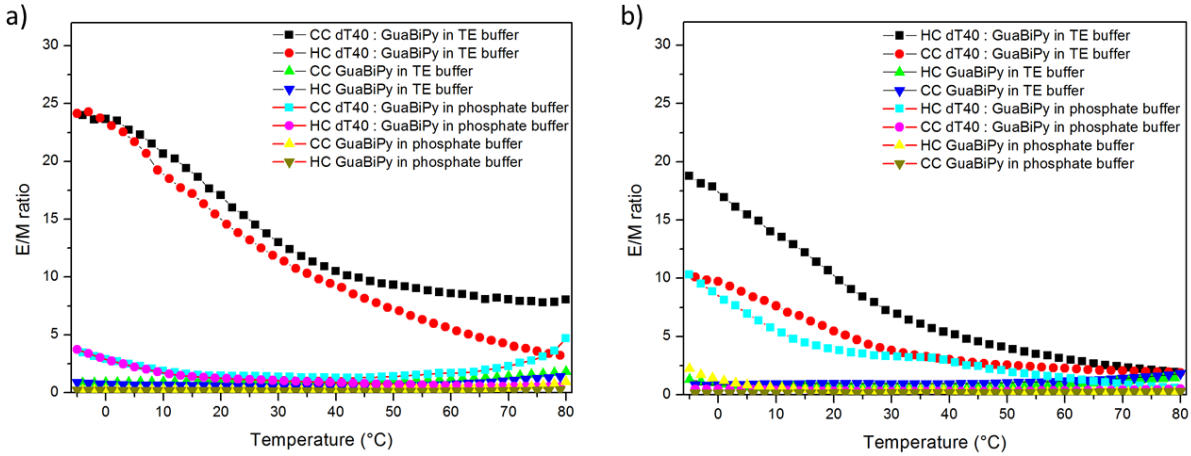


Fig. S20. Plots of the Excimer to Monomer (E/M) ratio of dT_{40}:**GuaBiPy** 1:30 and pure **GuaBiPy** in TE buffer and phosphate buffer. a) First cooling cycle (C-C) and second heating cycle (H-C); and b) third heating cycle (H-C) and third cooling cycle (C-C). [dT_{40}] ~ 5.7 µM.

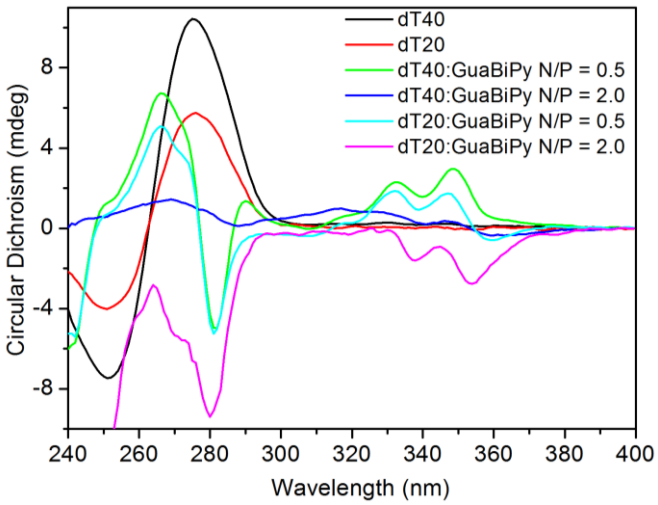


Fig. S21. Circular Dichroism (CD) spectra of dT_{40}:**GuaBiPy** and dT_{20}:**GuaBiPy** at different molar ratio N/P = 0.5 and 2.0 in TE buffer at -5 ºC. [dT_n] ~ 5.7 µM.

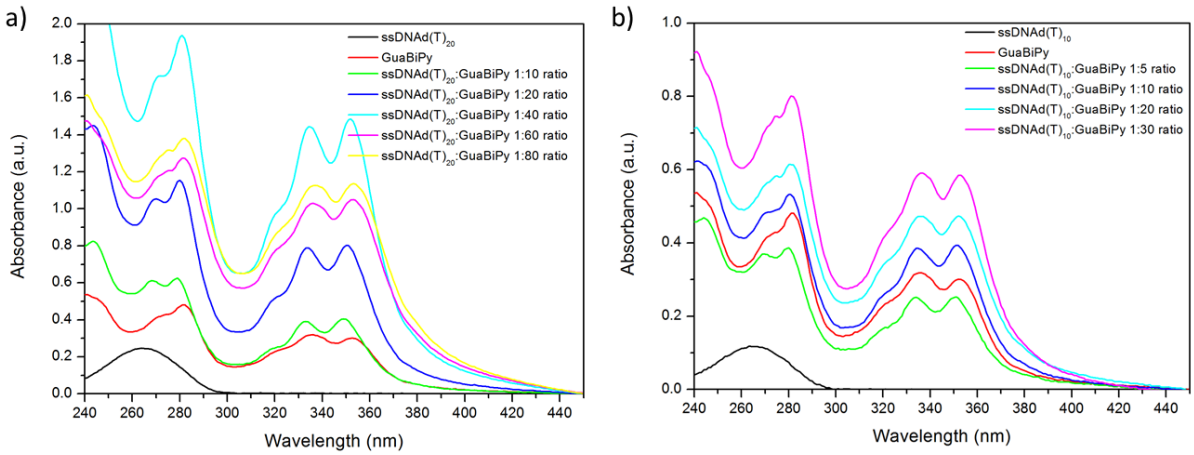


Fig. S22. UV-Vis spectra of a) dT_{20} and b) dT_{10} by **GuaBiPy** in TE buffer at -5 ºC. [dT_{20}] ~ 7.4 µM and [dT_{10}] ~ 7.5 µM.

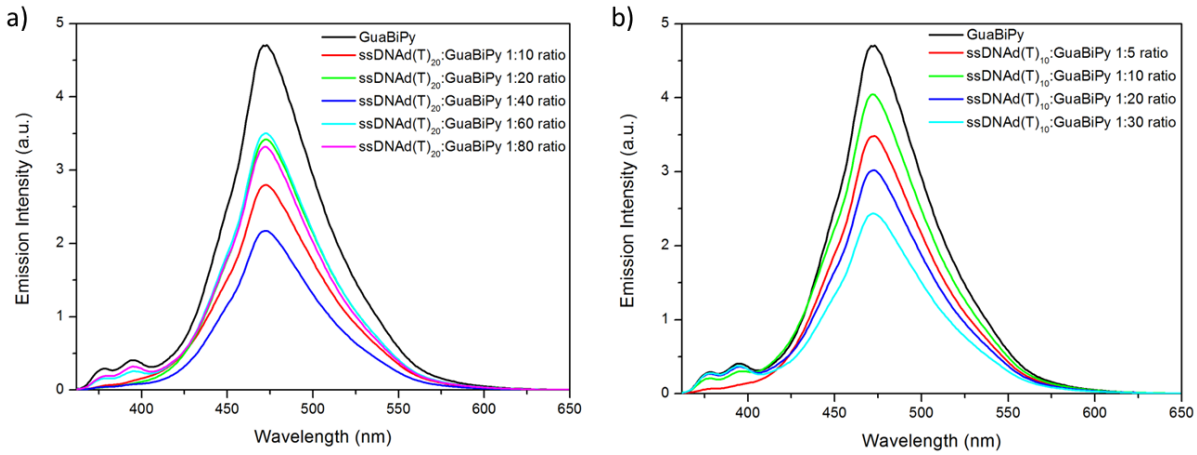


Fig. S23. Fluorescence spectra of mixtures of a) dT_{20}:**GuaBiPy** and b) dT_{10}:**GuaBiPy** at various molar ratio in TE buffer at -5 ºC. [dT_{20}] ~ 7.4 µM and [dT_{10}] ~ 7.5 µM. [**GuaBiPy**] ~ 111 µM.

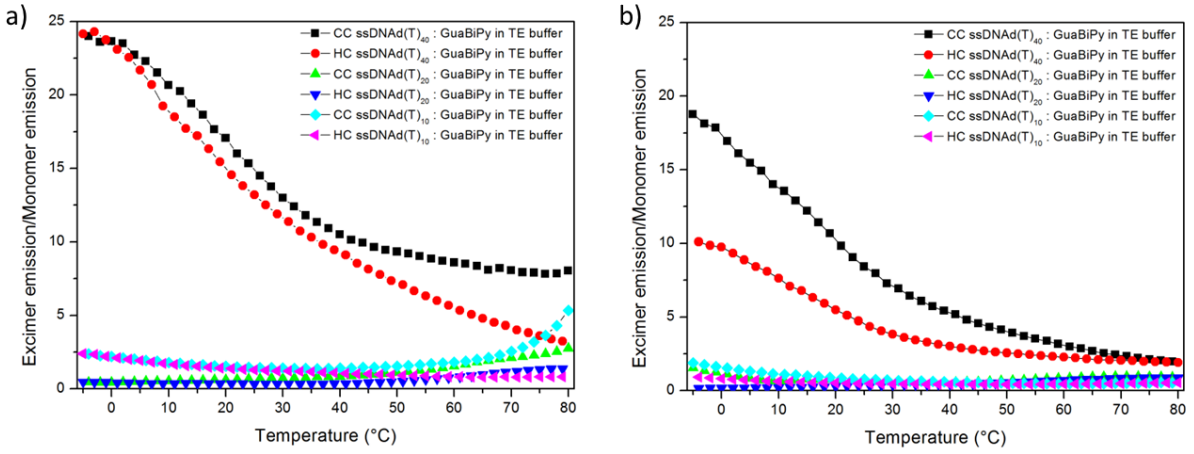


Fig. S24. Plots of the Excimer to Monomer ratio of dT_n:**GuaBiPy** 1:X in TE buffer. a) First cooling cycle (C-C) and second heating cycle (H-C); and b) third heating cycle (H-C) and third cooling cycle (C-C). [dT_n] ~ 4.6 µM.

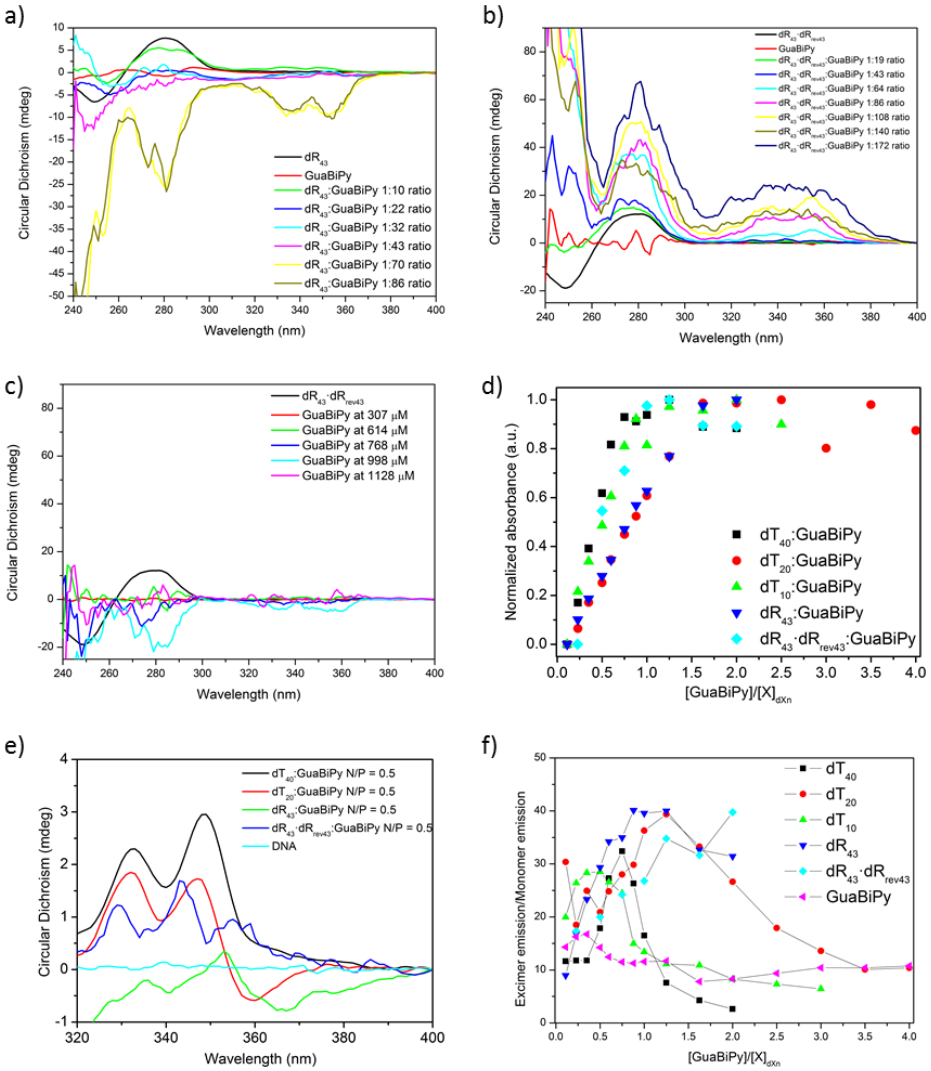


Fig. S25. a) Spectroscopic titration of dR_{43} by **GuaBiPy** in TE buffer at -5 ºC. [dR_{43}] ~ 7.1 µM. [**GuaBiPy**] ~ 612 µM; b) titration of $dR_{43}{\cdot}dR_{rev43}$ by **GuaBiPy** in TE buffer at -5 ºC. [$dR_{43}{\cdot}dR_{rev43}$] ~ 7.1 µM. [**GuaBiPy**] ~ 614 µM; c) titration of **GuaBiPy** in TE buffer at -5 ºC.; d) normalized UV-Vis titration monitored at 349 nm of $xDNAd(X)_n$ by **GuaBiPy**; e) circular dichroism (CD) spectra of dT_{40}, dT_{20}, dT_{10}, dR_{43} and $dR_{43}{\cdot}dR_{rev43}$ in the presence of **GuaBiPy** at N/P ratios of 0.5; f) plot of the excimer emission (472 nm)/monomer emission (396 nm) ratio of different $xDNAd(X)_n$ mixtures.

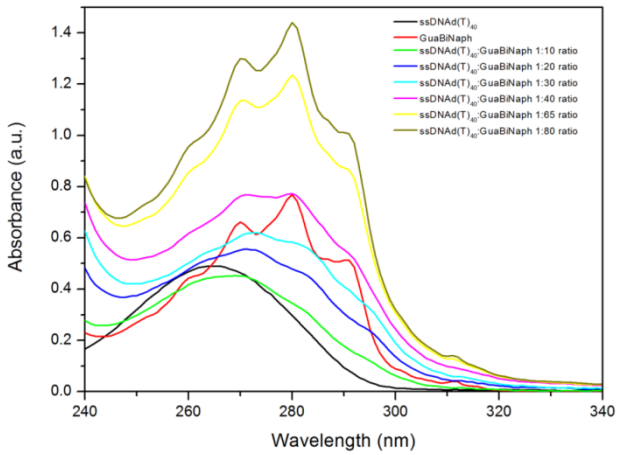


Fig. S26. UV-Vis spectra of dT_{40}:**GuaBiNaph** solutions in TE buffer at -5 ºC. [dT_{40}] ~ 7.5 µM.

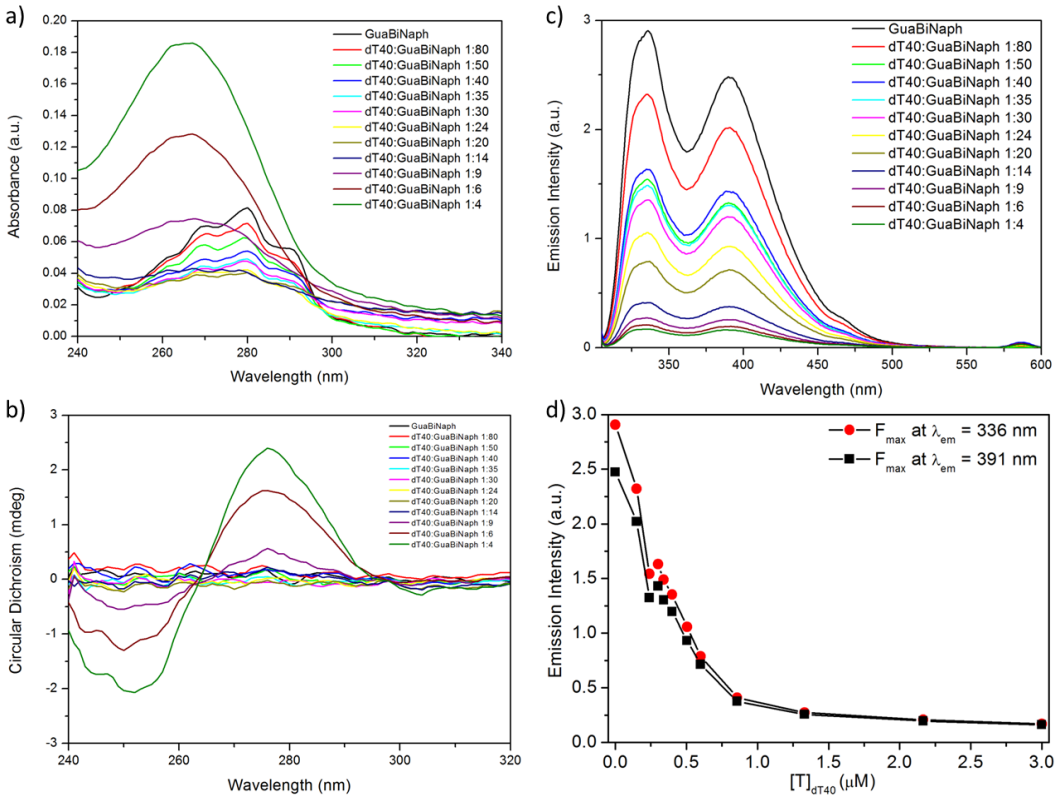


Fig. S27. a) UV-Vis spectra; b) Circular Dichrosim (CD) spectra; c) Fluorescence spectra of mixtures of dT_{40}:**GuaBiNaph**; and d) Emission spectra (at λ_{exc} = 292 nm) of dT_{40} into a solution of **GuaBiNaph** at various molar ratio in TE buffer at -5 ºC. [**GuaBiNaph**] = 12 μM. [**GuaBiNaph**] $_{stock}$= 10 mM in DMSO.

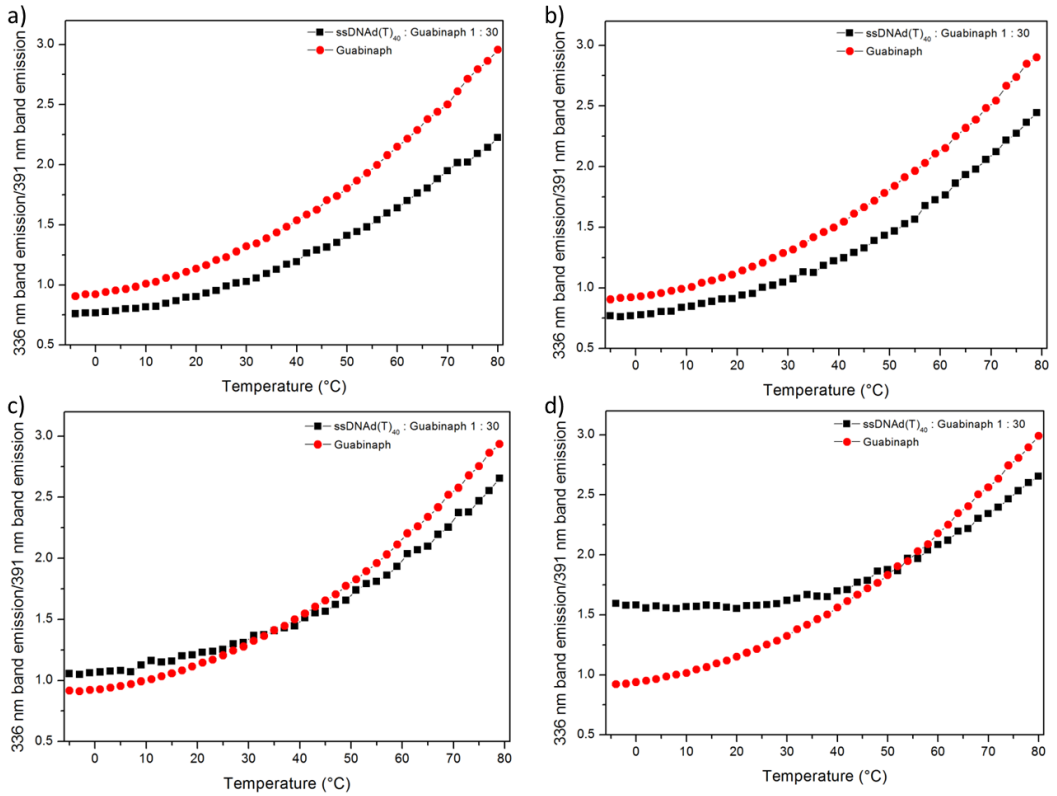


Fig. S28. Plots of intensity ratios in fluorescence spectra of dT_{40}:**GuaBiNaph** 1:30 and pure **GuaBiNaph** in TE buffer. a) First cooling cycle (C-C); b) Second heating cycle (H-C); c) Third heating cycle (H-C); and d) Third cooling cycle (C-C). [dT_{40}] ~ 4.6 μM.

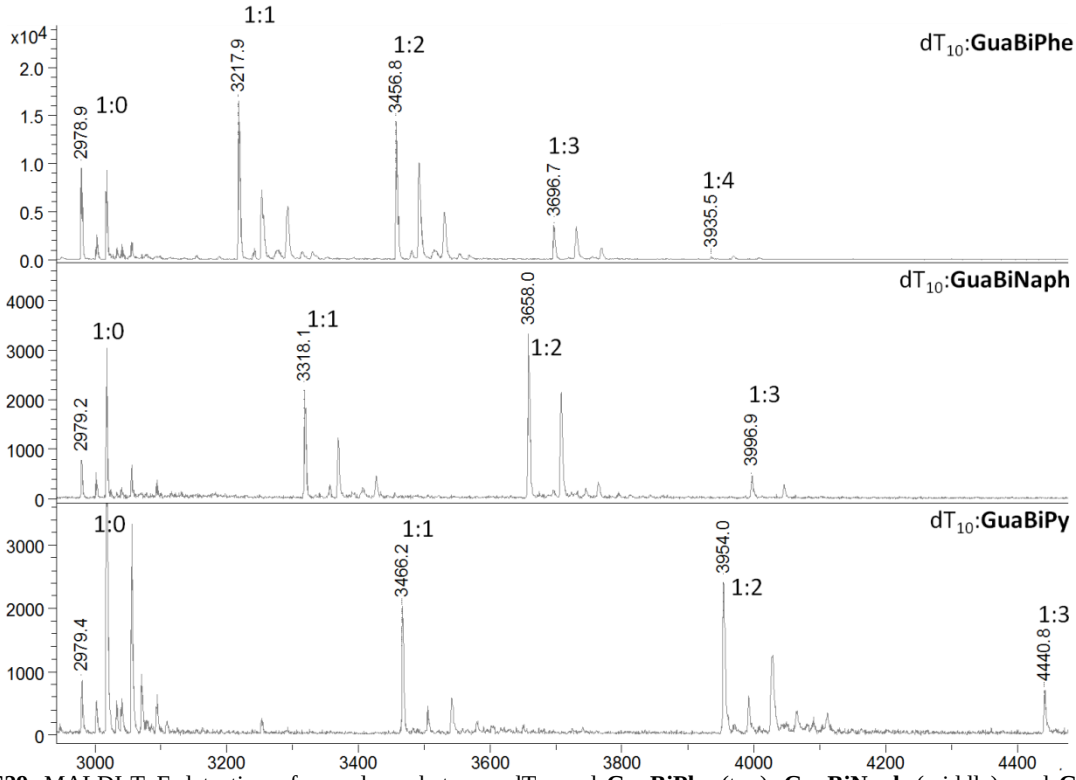


Fig. S29. MALDI-ToF detection of complexes between dT_{10} and **GuaBiPhe** (top), **GuaBiNaph** (middle), and **GuaBiPy** (bottom).

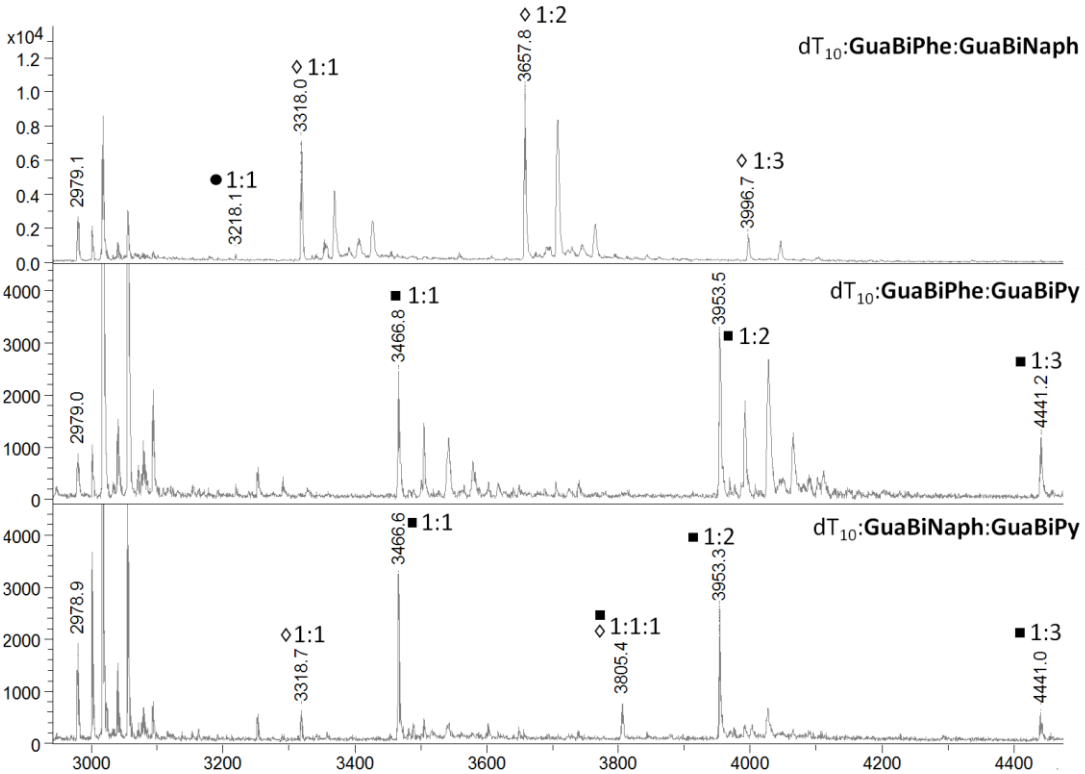


Fig. S30. Competition experiments analysed by MALDI-ToF MS. **GuaBiPhe** vs. **GuaBiNaph** (top), **GuaBiPhe** vs. **GuaBiPy** (middle), and **GuaBiNaph** vs. **GuaBiPy** (bottom). Complexes of dT_{10} with **GuaBiPhe** are marked with a circle (●), complexes of dT_{10} with **GuaBiNaph** are marked with a lozenge (◊) and complexes of dT_{10} with **GuaBiPy** are marked with a square (▪).