# Revisiting the assembly of amino ester-based benzene-1,3,5-tricarboxamides: chiral rods in solution

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## SUPPORTING INFORMATION

## General Procedures.

All amino acids were purchased from Sigma-Aldrich or Alfa Aesar (99%) and used as received. Benzene-1,3,5-tricarbonyl chloride was purchased from Alfa Aesar, 1-dodecanol and *p*-TsOH.$H_2O$ were acquired from Sigma Aldrich, and were used directly. **BTA C8*** and **BTA C8** were prepared following a published procedure.[1]

Unless otherwise noted, chromatography-grade solvents were used as received. Dried solvents were obtained from an SPS solvent purification system (IT-Inc) and stored on 4Å molecular sieves. Triethylamine was dried by distillation over $CaH_2$ and stored over 4Å molecular sieves. All inert atmosphere reactions were carried out under an argon atmosphere with standard Schlenk-line techniques.

NMR spectra were recorder on a Bruker Advance 300 spectrometer and calibrated to the residual solvent peak: DMSO-$d^6$ ($^1$H: 2.50 ppm; $^{13}$C: 39.52 ppm); acetone-$d^6$ ($^1$H: 2.05 ppm; $^{13}$C: 29.84 ppm). Peaks are reported with their corresponding multiplicity (s: singlet; d: doublet, t: triplet; q: quartet; p: pentuplet; hept: heptuplet; dt: doublet of triplets; td: triplet of doublets) and integration, and respective J coupling constants are given in Hertz. Exact mass measurements (HRMS) were obtained on TQ R30-10 HRMS spectrometer by ESI+ ionization and are reported in m/z for the major signal. **BTA (*R*,*R*,*R*)-Met**, **BTA (*rac*,*rac*,*rac*)-Met, BTA (*rac*),(*rac*),(*rac*)-Phe**, **BTA (*R*,*R*,*R*)-Nle** and **BTA (*rac*,*rac*,*rac*)-Nle** were prepared in the purpose of determining the optical purity of **BTA (*S*,*S*,*S*)-Met**, **BTA (*S*,*S*,*S*)-Phe** and **BTA (*S*,*S*,*S*)-Nle** thanks to chiral stationary phase HPLC (see pages S19-S21 for analytical details).

Preparation of BTA solutions for spectroscopic analyses: the desired BTA was weighed into a ø11.6 mm HPLC vial or a ø20 mm glass vial, the volume was adjusted to the desired end concentration with an adequate glass microsyringe, and verified by weighing the sample. Vials were sealed with PTFE-coated caps to avoid contamination from leaching plasticizers, heated to ~b.p. of the solvent (and sonicated for 1-5 min. when necessary) before letting it cool to r.t. on a shaking table (250 rpm) for 1h. After this, when required, low-concentration samples were prepared by dilution of mother liquors obtained as above, were re-heated and cooled again over 1h while shaking, prior to use.

Preparation of BTA solutions for "sergeants-and-soldiers" experiments: 2.0 mM cyclohexane solutions of ester BTAs and **BTA C8** were prepared as mentioned above and then mixed to the desired ratio. The mixtures were heated to ~b.p. of the solvent before letting them cool to r.t. on a shaking table (250 rpm) for 1h.

FT-IR measurements were performed on a Nicolet iS10 spectrometer in ATR (diamond probe) or in transmission between KBr disks (solid-state measurements). Solution spectra were measured in KBr or $CaF_2$ cells of 0.5 mm or 1.0 mm pathlength and are corrected for air, solvent and cell absorption.

Circular dichroism (CD) measurements were performed on a Jasco J-1500 spectrometer equipped with a Peltier thermostatted cell holder and Xe laser, in 0.5 mm (for 0.125 mM solutions) or in 0.1 mm (for 2 mM solutions) quartz cells. Data was recorded at 20°C, 20 $nm.min^{-1}$ sweep rate, and 0.05 nm data pitch, between 350 and 200 nm, and spectra were corrected for solvent and cell contribution. Molar ellipticities and reported in $L.mol^{-1}.cm^{-1}$ and are expressed as follows: $\Delta\varepsilon = \theta/(32980 \times l \times c)$ where $\theta$ is the measured ellipticity (mdeg), $l$ is the optical path length in cm and $c$ is the concentration in $mol.L^{-1}$. For all samples, linear dichroism (LD) contribution was negligible ($\Delta LD < 0.005$ dOD).

UV spectra were extracted to CD on each of the above samples. UV analyses were also performed on a Cary 300 UV-vis spectrometer between 350 and 190 nm with the same samples.

Isothermal titrating calorimetry (ITC) data were recorded on a Microcal VP-ITC apparatus at 20°C, injecting 5 mM cyclohexane solution of the sample into neat cyclohexane. Injections of 5 µL over 10 seconds were performed every 300 seconds at a stirring rate of 260 rpm.

Solution-phase differential scanning calorimetry (nanoDSC) measurements were performed on a TA Instruments nDSC III system in cyclohexane at 1 or 10 mM, between 15 and 75°C, using 3 full heating/cooling cycles, at $1°C.min^{-1}$.

Viscosimetry measurements were performed on an Anton Paar AMVn falling-ball microviscosimeter with a Ø 0.16 mm capillary, at 25°C at the various concentrations in cyclohexane, with 3 measurements at an angle of +20° and -20°. Results are reported as an average of those 6 measurements.

Small-angle neutron scattering measurements were made at the LLB (Saclay, France) on the Pace instrument, at two distance-wavelength combinations to cover the $4 \times 10^{-3}$ to $0.24 Å^{-1}$ q-range, where the scattering vector q is defined as usual, assuming elastic scattering ($q=(4\pi/\lambda)\sin(\theta/2)$, where $\theta$ is the angle between incident and scattered beam). Data were corrected for the empty cell signal and the solute and solvent incoherent background. A light water standard was used to normalize the scattered intensities to $cm^{-1}$ units.

**Supplementary figures.**

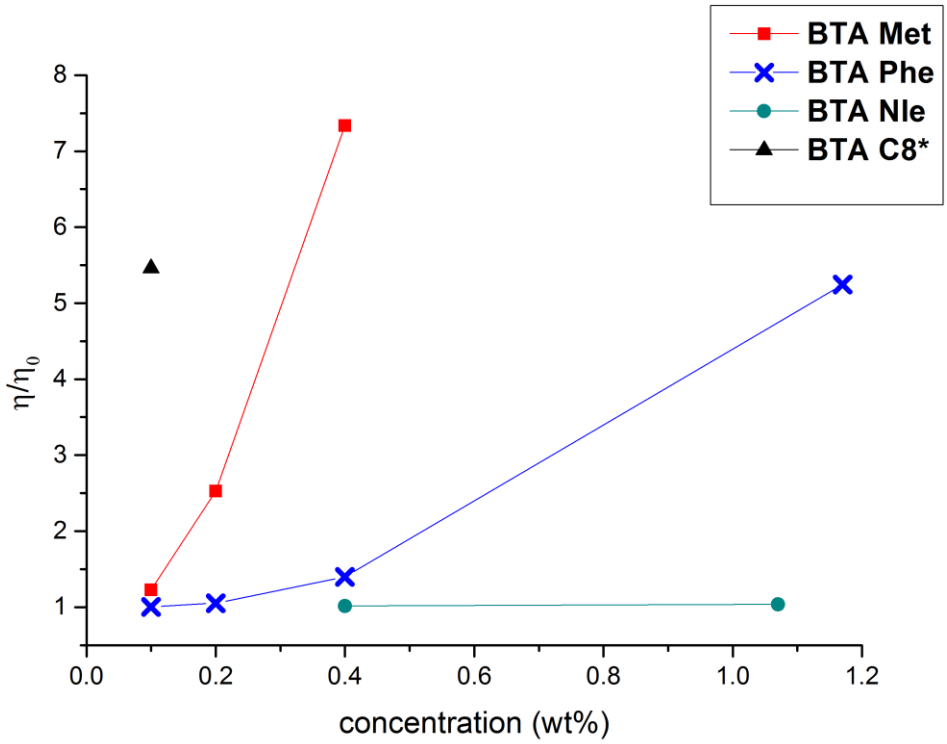



**Figure S1.** Relative viscosity of ester BTAs and **BTA C8*** in cyclohexane *vs* concentration (25°C).

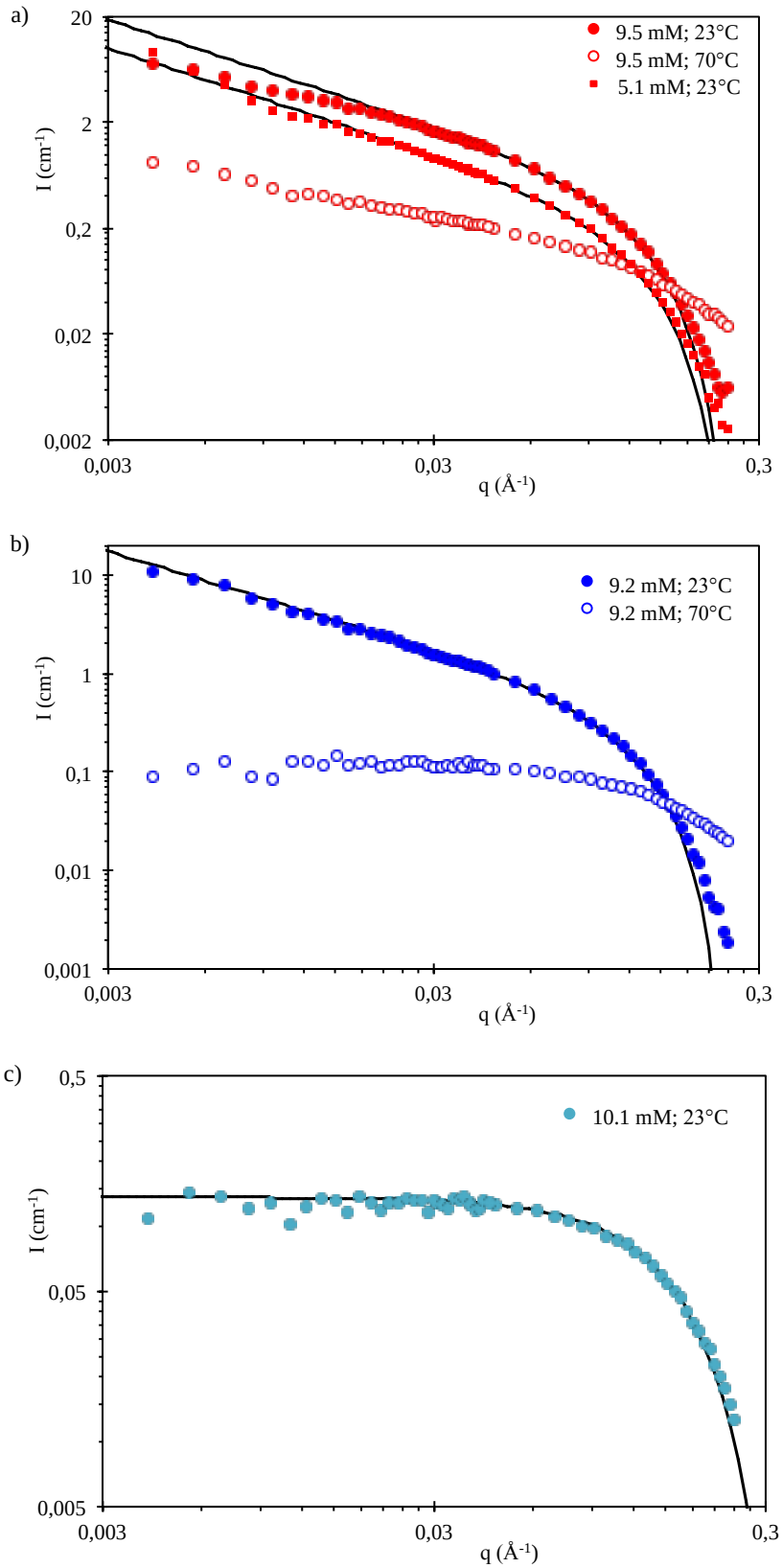



**Figure S2.** SANS analyses of **BTA Met** (23°C and 70°C) (a), **BTA Phe** (23°C and 70°C) (b) and **BTA Nle** (23°C) (c) in deuterated cyclohexane. The curves are fits according to the form factor for rigid rods with a circular cross section and a uniform scattering length density (a and b),[2] or for spheres with a uniform scattering length density (c),[3] respectively. The low intensity measured at low scattering vector for **BTA Met** at 9.5 mM is due to interactions between rods (contribution from the structure factor).

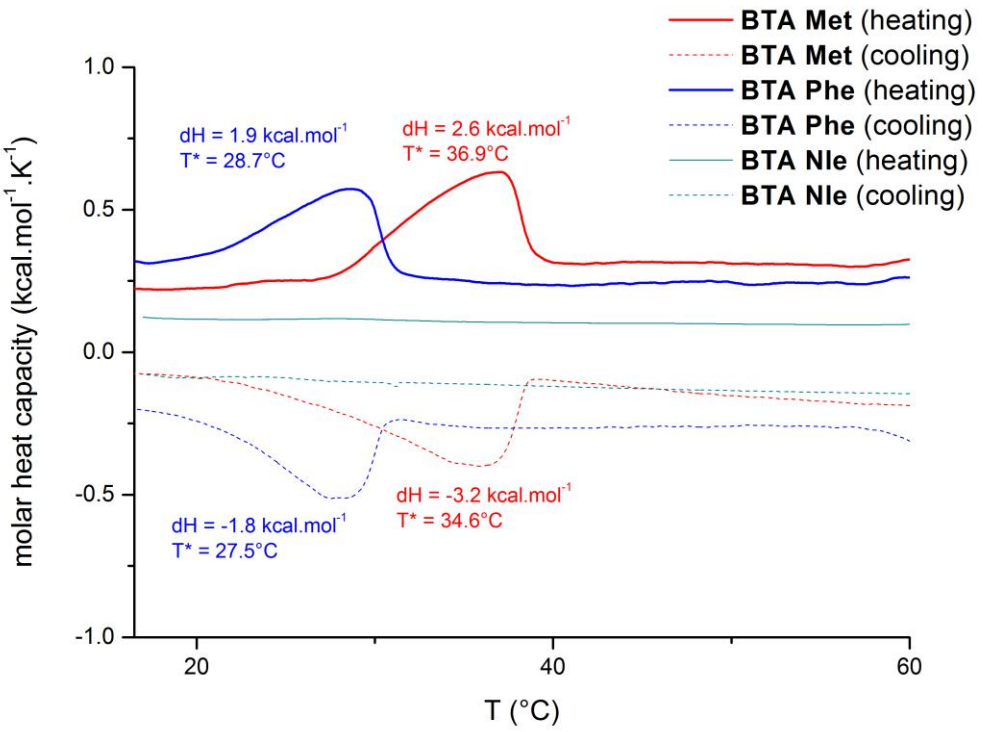



**Figure S3.** Variation of molar heat capacity with temperature obtained by high sensitivity DSC for ester BTA solutions in cyclohexane. **BTA Met** and **BTA Phe**: 1 mM; **BTA Nle**: 10mM.

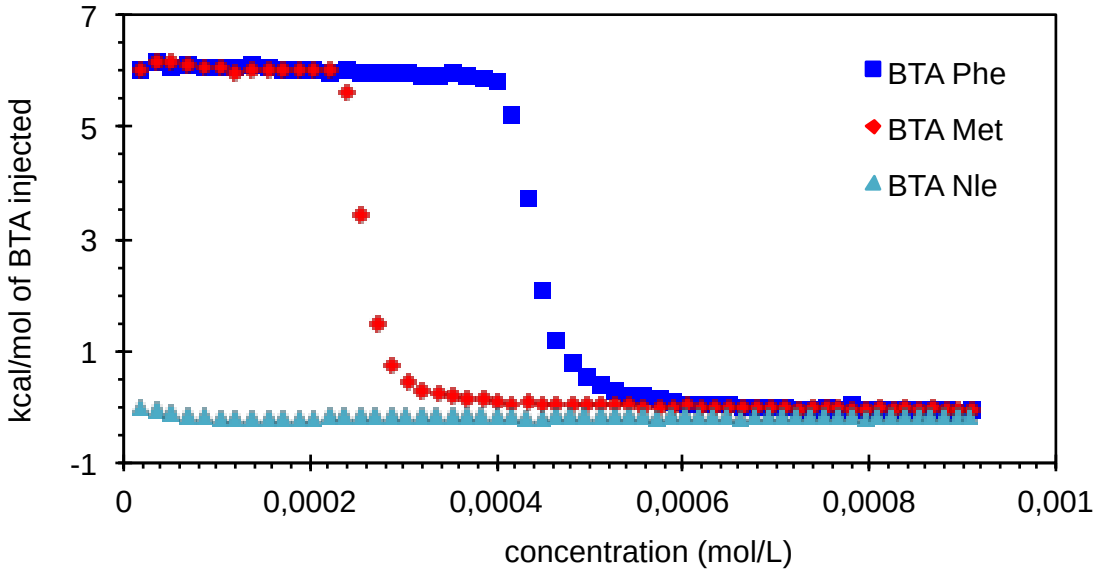



**Figure S4.** ITC enthalpograms for 5 mM ester BTA solutions in cyclohexane injected into pure cyclohexane, versus total ester BTA concentration in the cell at 20°C.
**BTA Met**: c* = 0.28 mM/0.03 wt%; **BTA Phe**: c* = 0.44 mM/0.05 wt%.

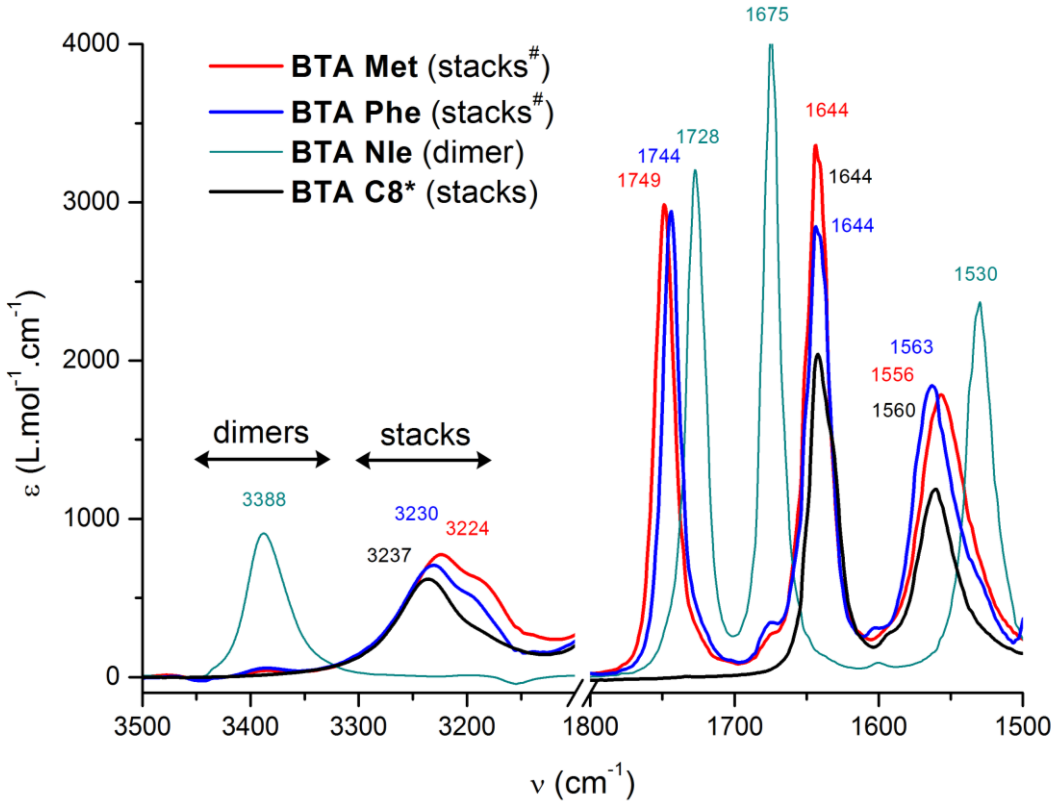



**Figure S5.** Infrared spectra of **BTA Met** at 9.6 mM (stacks, thick line), **BTA Phe** at 9.2 mM (stacks, thick line) and **BTA Nle** at 10.1 mM (dimer, thin line) in cyclohexane and of **BTA C8*** at 10.0 mM (stacks, thick line) in decaline (zoom on the regions associated with the amide and ester frequencies). Frequencies corresponding to the maximum absorbances of the NH and CO bands are indicated.
# At these concentrations **BTA Met** and **BTA Phe** predominantly exist as stacks, but 5% and 7% of the dimers can be detected from their IR spectra, respectively.

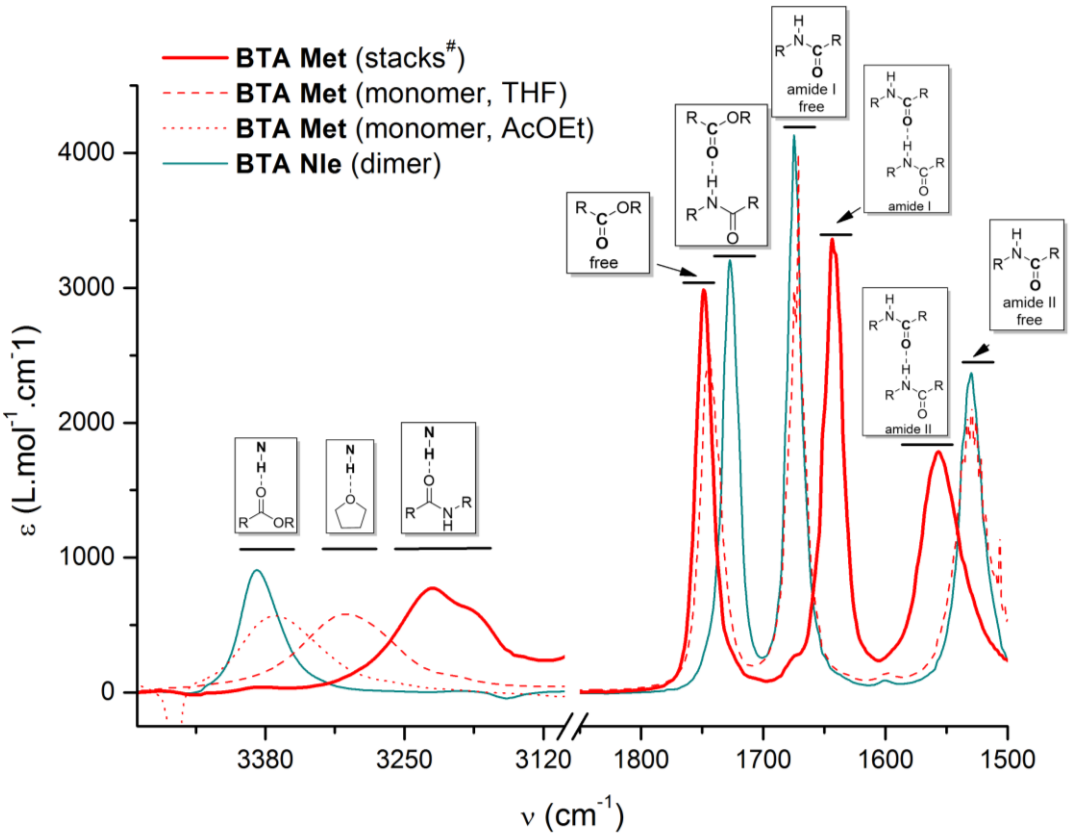



**Figure S6.** Infrared spectra of **BTA Met** at 9.6 mM in cyclohexane (stacks, thick line), in THF (monomer, dashed line), in AcOEt (monomer, dotted line) and of **BTA Nle** at 10.1 mM in cyclohexane (dimer, thin line). Assignment of the NH and ester and amide CO bands.
# At this concentration **BTA Met** predominantly exists as stacks, but 5% of the dimer can be detected from its IR spectrum.

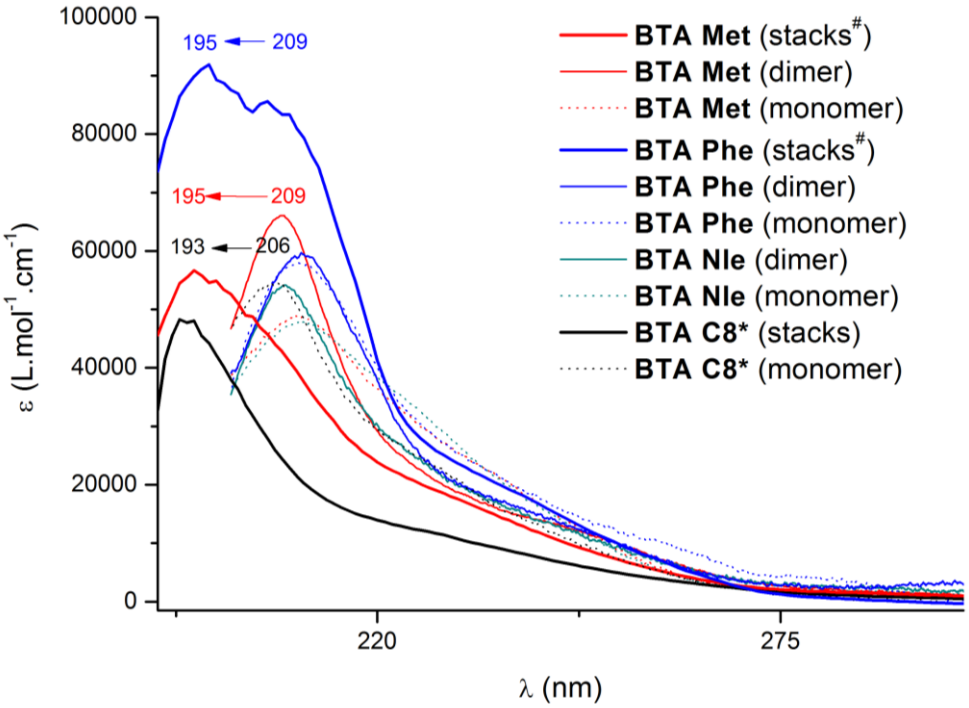



**Figure S7.** UV spectra of ester BTAs and **BTA C8***. The spectra were recorded at 0.125 mM (dimer, thin line) and 2.0 mM (stacks, thick line) in cyclohexane for the ester BTAs and in decaline for **BTA C8***. Spectra of the monomers (dotted lines) were recorded at 0.125 mM in absolute ethanol for ester BTAs and at 0.04 mM in acetonitrile for **BTA C8***.

# At these concentrations **BTA Met** and **BTA Phe** predominantly exist as stacks, but 22% and 28% of dimers are also present, respectively (see Fig. S9 and S10).

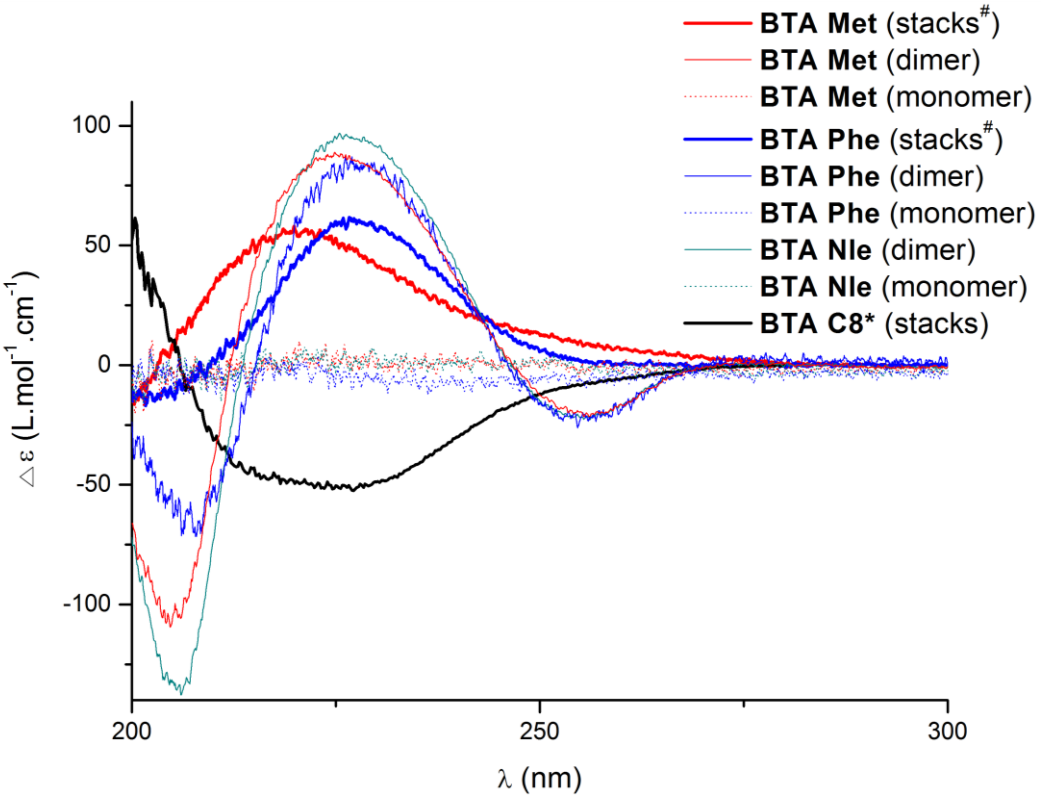



**Figure S8.** CD spectra of ester BTAs and **BTA C8*** in cyclohexane. The spectra were recorded at 0.125 mM (dimer, thin line) and 2.0 mM (stacks, thick line). Monomers are not CD active (spectra in absolute ethanol at 0.125 mM, dotted line).

# At these concentrations **BTA Met** and **BTA Phe** predominantly exist as stacks, but 22% and 28% of dimers are also present, respectively (see Fig. S9 and S10).

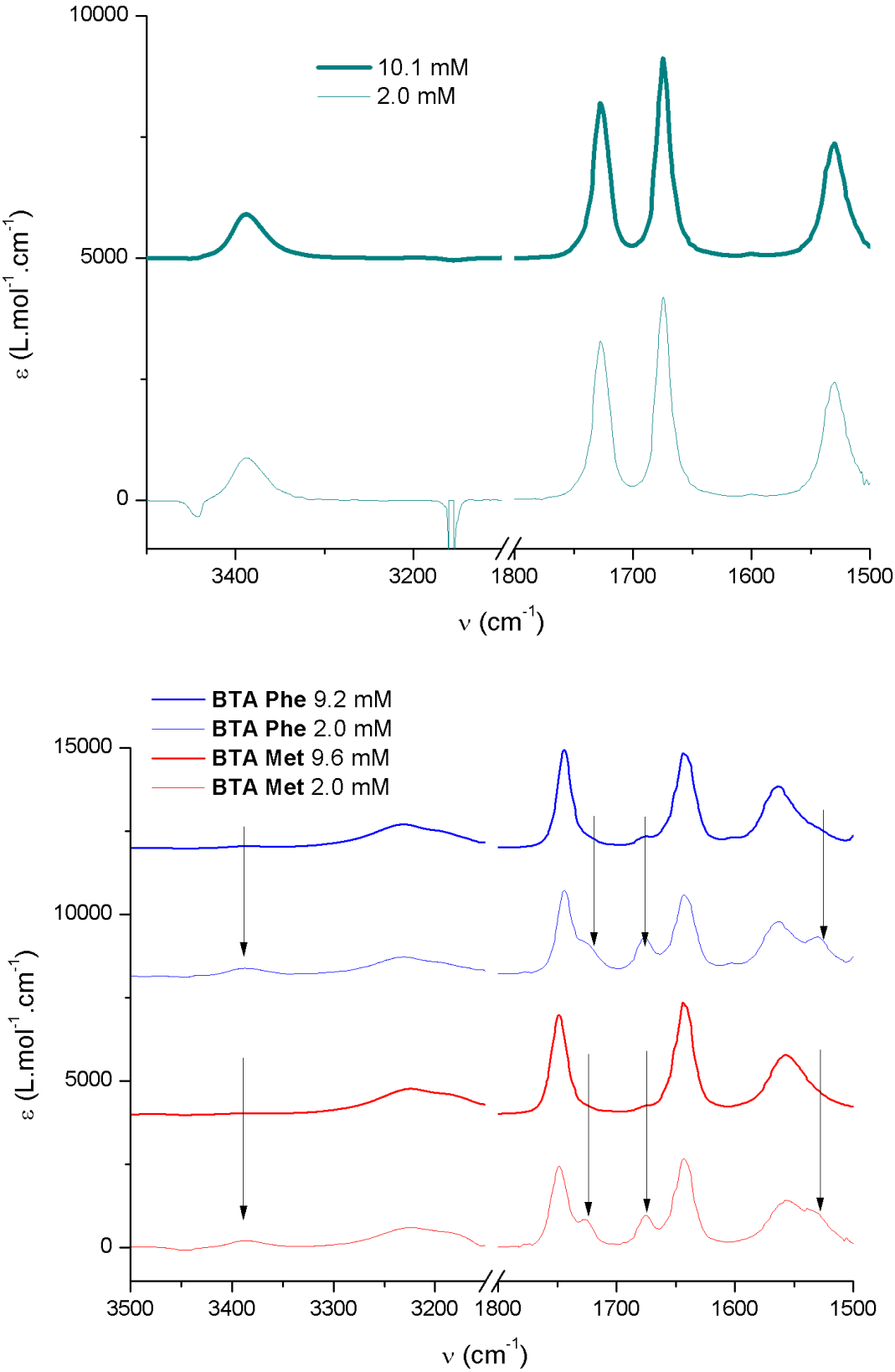



**Figure S9. a)** IR spectra of **BTA Nle** at 2.0 and 10.1 mM in cyclohexane (dimer in both cases, shown with an offset of 5000 $L.mol^{-1}.cm^{-1}$). **b)** IR spectra of **BTA Phe** (at 2.0 and 9.2 mM) and **BTA Met** (at 2.0 and 9.6 mM) shown with an offset of 4000 $L.mol^{-1}.cm^{-1}$. Arrows indicate an increased amount of dimer. At 2.0 mM, 22% and 28% of the dimers of **BTA Met** and **BTA Phe** can be detected from their IR spectra, respectively.

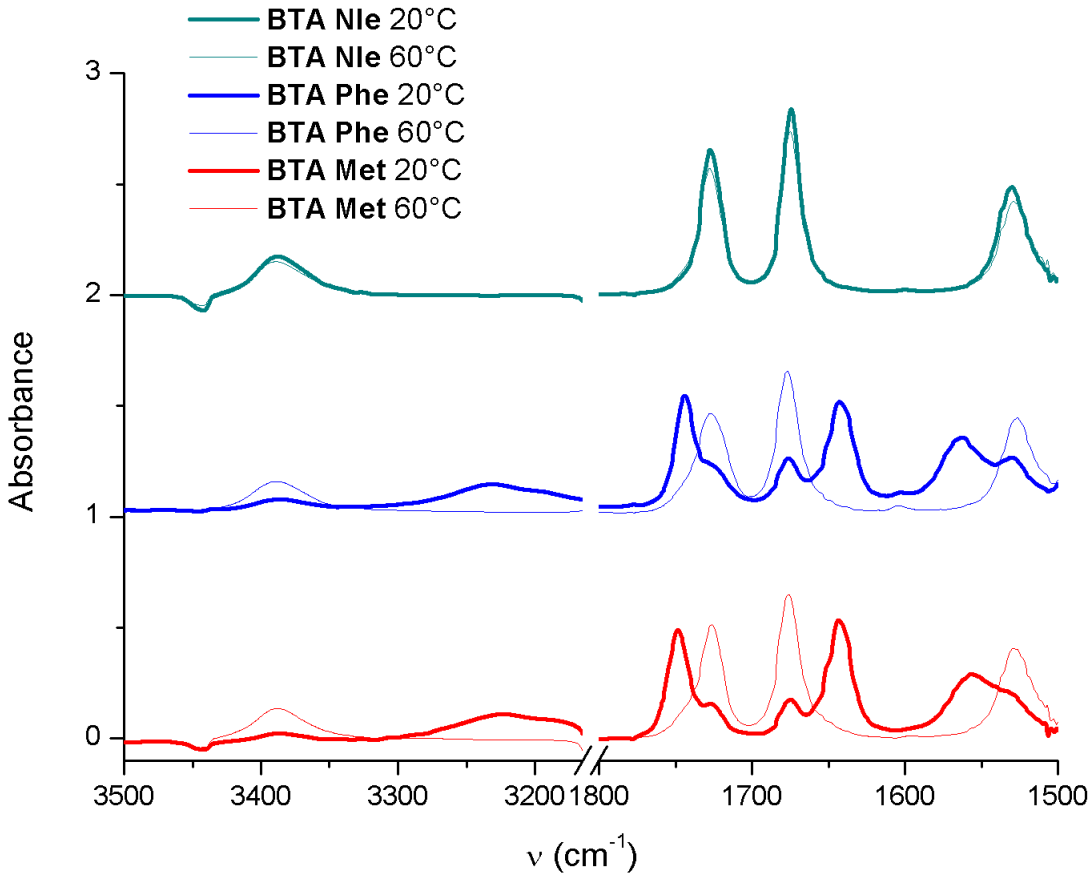



**Figure S10.** IR spectra of ester BTAs at 2.0 mM in cyclohexane at 20°C (thick line) and 60°C (thin line) shown with an offset of one absorbance unit. At 20°C **BTA Met** and **BTA Phe** predominantly exists as stacks (with 22% and 28% of their dimer, respectively) while only dimers are present at 60°C. **BTA Nle** only exists as dimer at 20°C and 60°C.

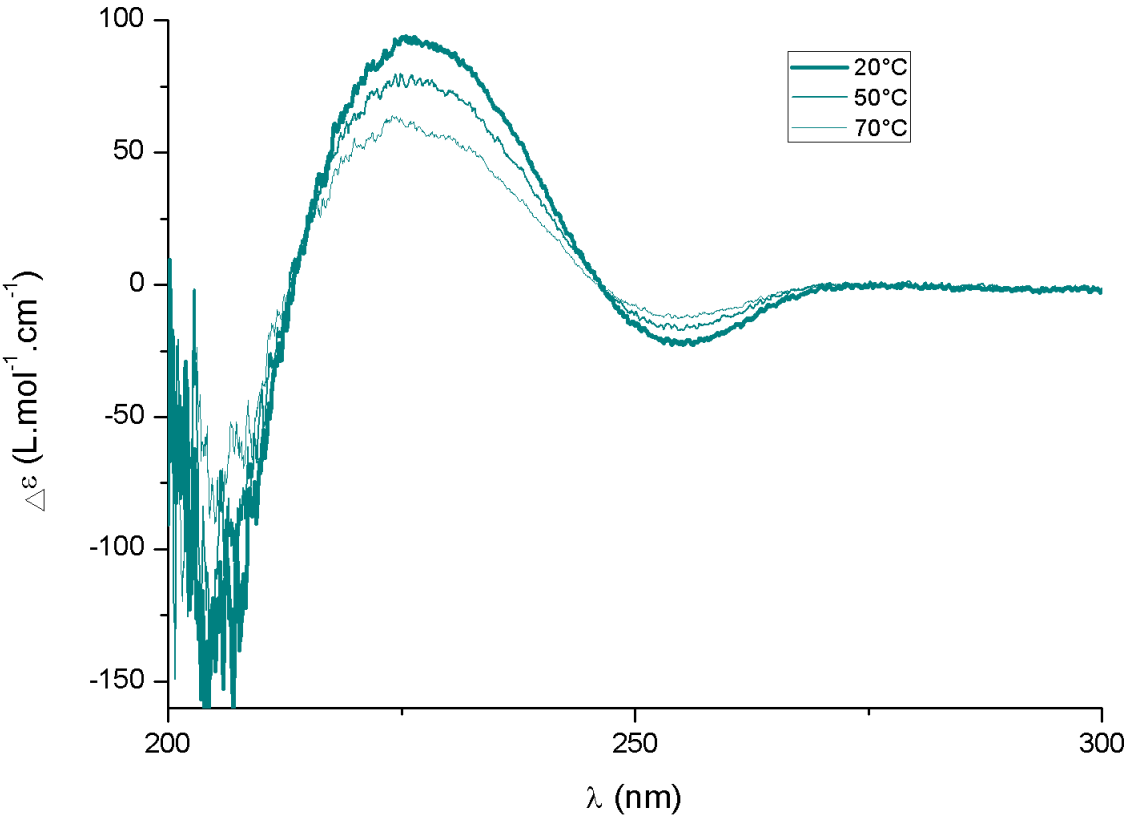



**Figure S11.** CD spectra of **BTA Nle** at 20, 50 and 70°C in cyclohexane at 0.0125 mM.
Based on the Δε at 225 nm, only 30% of the signal is lost at 70°C highlighting the high stability of the dimer formed by **BTA Nle**.

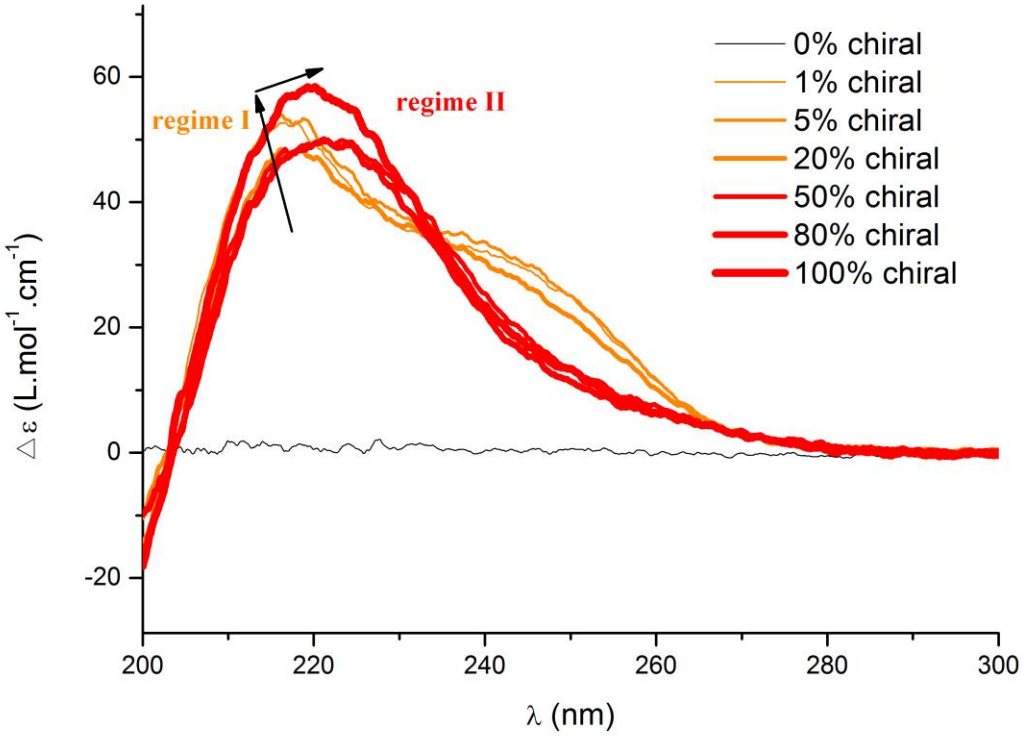



**Figure S12.** CD spectra of mixtures of **BTA Met:BTA C8** at 2.0 mM total BTA concentration in cyclohexane (20°C).

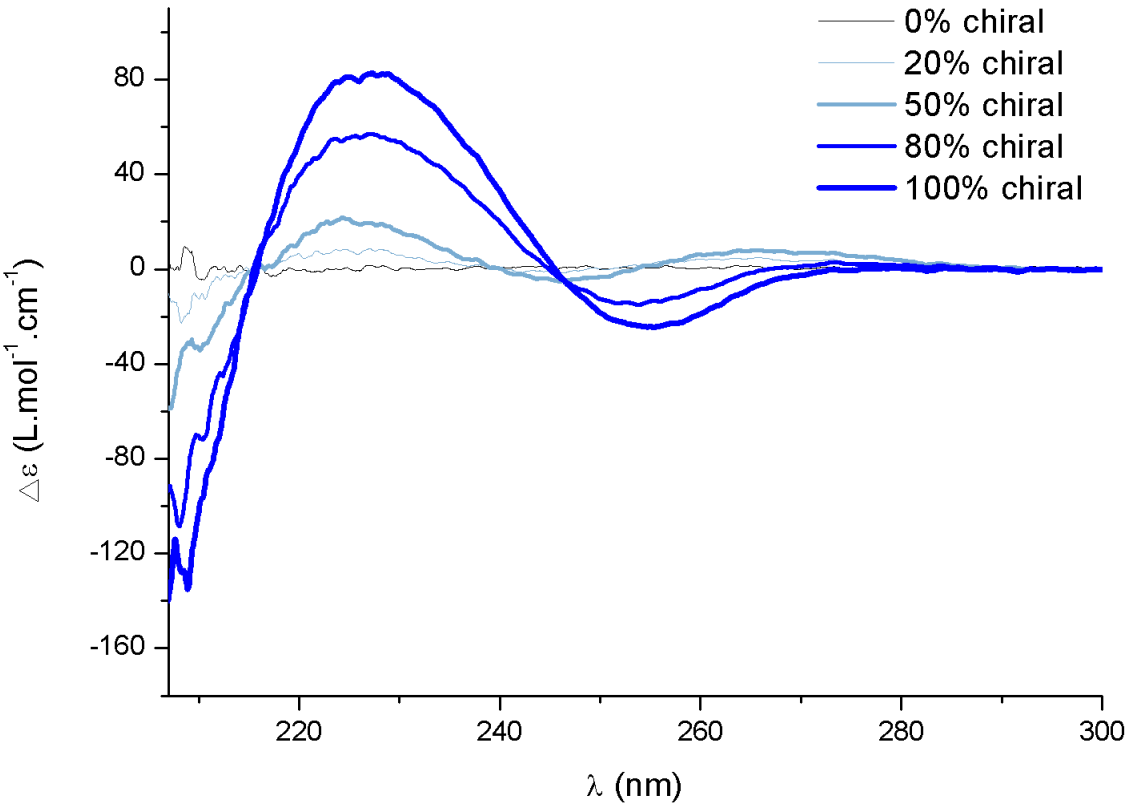



**Figure S13.** CD spectra of mixtures of **BTA Phe:BTA C8** at 0.01 mM total BTA concentration in cyclohexane (20°C).

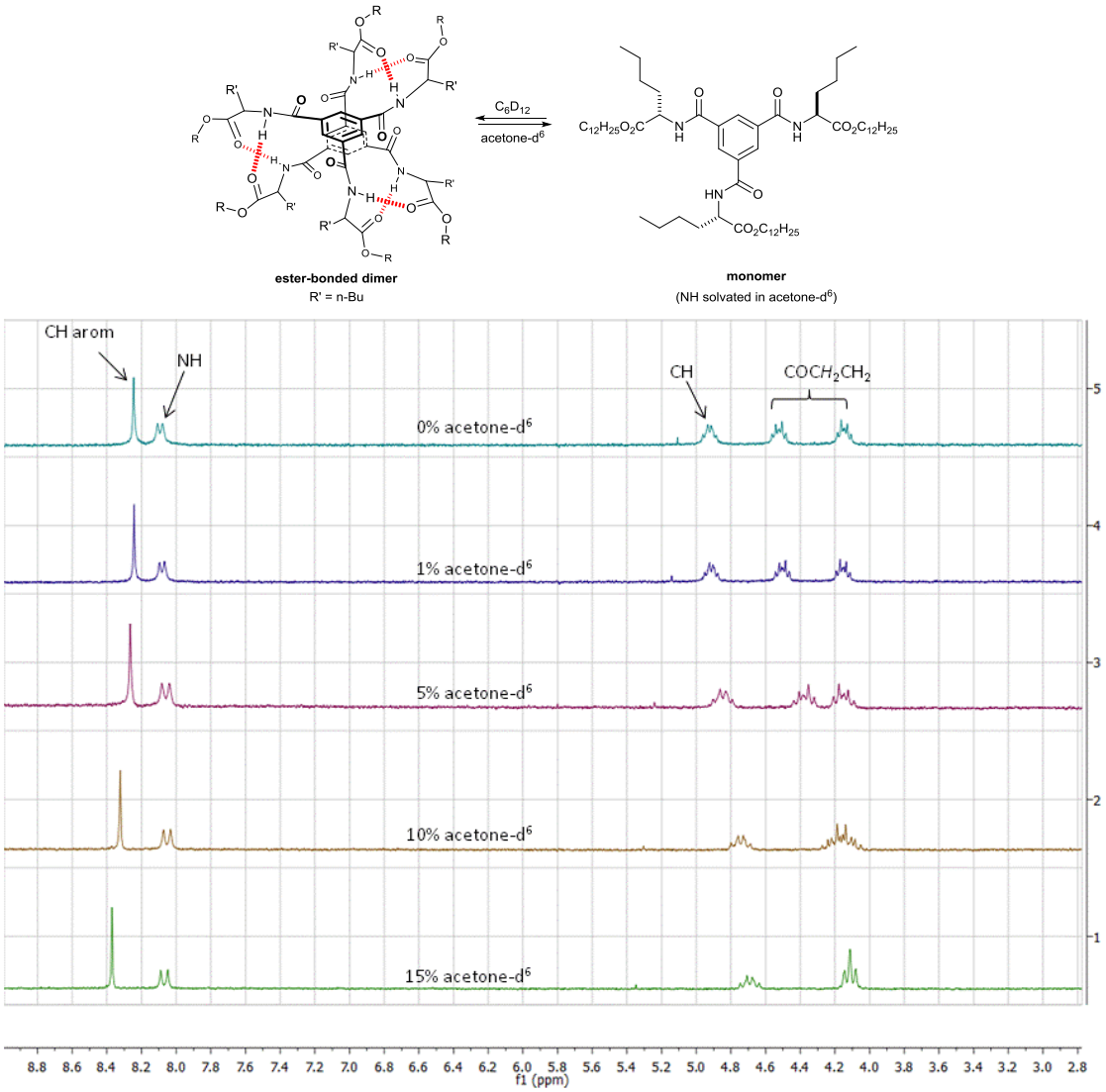



**Figure S14.** NMR spectra of **BTA Nle** at 2.0 mM in $C_6D_{12}$ with incremental amounts of acetone-$d^6$ (1%, 5%, 10% and 15%). The singlet corresponding to the aromatic protons is upfield shifted in $C_6D_{12}$ compared to $C_6D_{12}$/acetone mixtures as a possible result of aromatic interactions between the phenyl rings of the dimer.

**References.**


1. P. J. M. Stals, M. M. J. Smulders, R. Martin-Rapun, A. R. A. Palmans and E. W. Meijer, *Chem. Eur. J.*, 2009, **15**, 2071-2080.
2. F. Lortie, S. Boileau, L. Bouteiller, C. Chassenieux, B. Deme, G. Ducouret, M. Jalabert, F. Laupretre and P. Terech, *Langmuir*, 2002, **18**, 7218-7222.
3. M. Bergstrom and J. S. Pedersen, *Phys. Chem. Chem. Phys.*, 1999, **1**, 4437-4446.

## Experimental procedures.

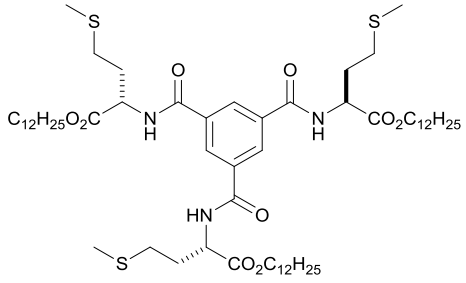
**General Method A: Synthesis of ammonium ester tosylate salts:**

In a Dean-Stark apparatus-mounted two-neck flask, the amino acid (1 eq., 15.0 mmol) is suspended in toluene (0.1 M, 150 mL), and *p*-TsOH.$H_2O$ (1.2 eq., 18.0 mmol) is added at room temperature. Dodecanol (1.1 eq., 16.5 mmol) is then added, and the resulting slurry is stirred at reflux temperature overnight. After cooling the reaction mixture to room temperature, the crude reaction mixture is evaporated under reduced pressure to give a thick oil or a solid. This residue is taken up in ca. 70 mL of $Et_2O$, gently heated to 35°C, and let cool in an ice bath. The resulting precipitate is filtered under vacuum and rinced with ice-cold ether to remove residual reactants. The white solid is then dried under vacuum to yield the pure ammonium tosylate (81-97% yield).

When the ammonium tosylate is somewhat soluble in $Et_2O$ (**BTA Phe**), the crude reaction mixture is filtered with heptane instead, and used as such for the next step.

**General Method B: Synthesis of ester BTAs:**

In a flame-dried round-bottom flask under argon atmosphere, benzene-1,3,5-tricarbonyl chloride (1eq., 3.0 mmol) is dissolved in dry DCM (100 mL) at room temperature. The ammonium tosylate (3.3 eq.; 9.9 mmol) is then added in one portion, and the resulting mixture is cooled to 0°C with an ice/water bath. Dry $Et_3N$ is then added dropwise, the reaction is let warm to room temperature and stirred for 36-42h. Brine is then added to the flask, and the crude mixture is extracted thrice with DCM. The combined organic phases are dried over $MgSO_4$, filtered, and the solvent is evaporated under reduced pressure. The resulting solid is taken up as a slurry in a little DCM, and purified on a short column of silica gel (elution: DCM/EtOAc 6:1) to remove salts and impurities ($R_f$ ~ 0.85-0.9). When necessary, the product is repurified by column chromatography on silica gel, eluting with petroleum ether/EtOAc 95:5 – 80:20 gradient ($R_f$ ~ 0.1–0.2). The pure BTA is isolated as a sticky white gum (80-96% yield).



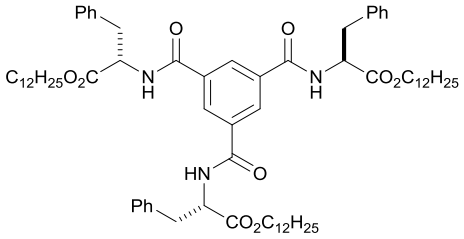

**BTA Met**

**Tri-N-[(*L*)-(3-thio-butyl)dodecyloxycarbonymethyl]benzene-1,3,5-tricarboxamide (BTA Met)**

Obtained from (L)-methionine using General Method A, then B, with an overall yield of 85%, as a white gum.

**$^1$H NMR** (300 MHz, DMSO-d6): δ 9.08 (d, *J* = 7.5 Hz, 3H), 8.51 (s, 3H), 4.61 (td, *J* = 8.1, 5.9 Hz, 3H), 4.17–3.97 (m, 6H), 2.71–2.49 (m, 6H), 2.13–2.00 (m, 15H), 1.56 (p, *J* = 6.3 Hz, 6H), 1.35–1.07 (m, 54H), 0.87–0.81 (m, 9H); **$^{13}$C{$^1$H} NMR** (75 MHz, DMSO-d6): δ 171.8, 165.9, 134.2, 129.5, 64.5, 51.9, 39.5, 31.3, 30.0, 29. 9, 29.0, 28.9, 28.9, 28.7, 28.6, 28.1, 25.3, 22.1, 14.5, 13.9; **HRMS:** Calculated for

$C_{60}H_{106}N_3O_9S_3$ $[M+H]^+$: 1108.7086, found: 1108.7082. IR (solid, ATR diamond) see page S19. ee>99%, de = 86%.

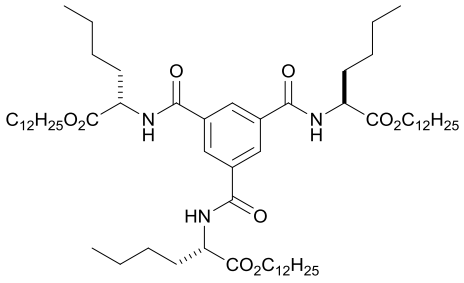



**BTA Phe**

**Tri-N-[(*L*)-(benzyl)dodecyloxycarbonymethyl]benzene-1,3,5-tricarboxamide (BTA Phe)**

Obtained from (L)-phenylalanine using General Method A, then B, with an overall yield of 90%, as a white gum.

**$^1$H NMR** (300 MHz, DMSO-d6) δ 9.11 (d, *J* = 7.7 Hz, 3H), 8.40 (s, 3H), 7.31–7.22 (m, 10H), 7.27–7.11 (m, 5H), 4.69 (q, *J* = 7.6, 6.9 Hz, 3H), 4.02 (t, *J* = 6.4 Hz, 6H), 3.20–3.07 (m, 6H), 1.49 (t, *J* = 6.6 Hz, 6H), 1.31–1.13 (m, 54H), 0.86–0.80 (m, 9H); **$^{13}$C{$^1$H} NMR** (75 MHz, DMSO-d6) δ 171.5, 165.5, 137.5, 134.2, 129.2, 129.0, 128.2, 126. 5, 64.5, 54.6, 39.5, 36.3, 31.3, 29.0, 28.9, 28.9, 28.7, 28.6, 28.0, 25.2, 22.1, 13.9; **HRMS** Calculated for $C_{72}H_{106}N_3O_9$ $[M+H]^+$: 1156.7924, found: 1156.7920. IR (solid, ATR diamond) see page S19. ee>99%, de> 97%.




**BTA Nle**

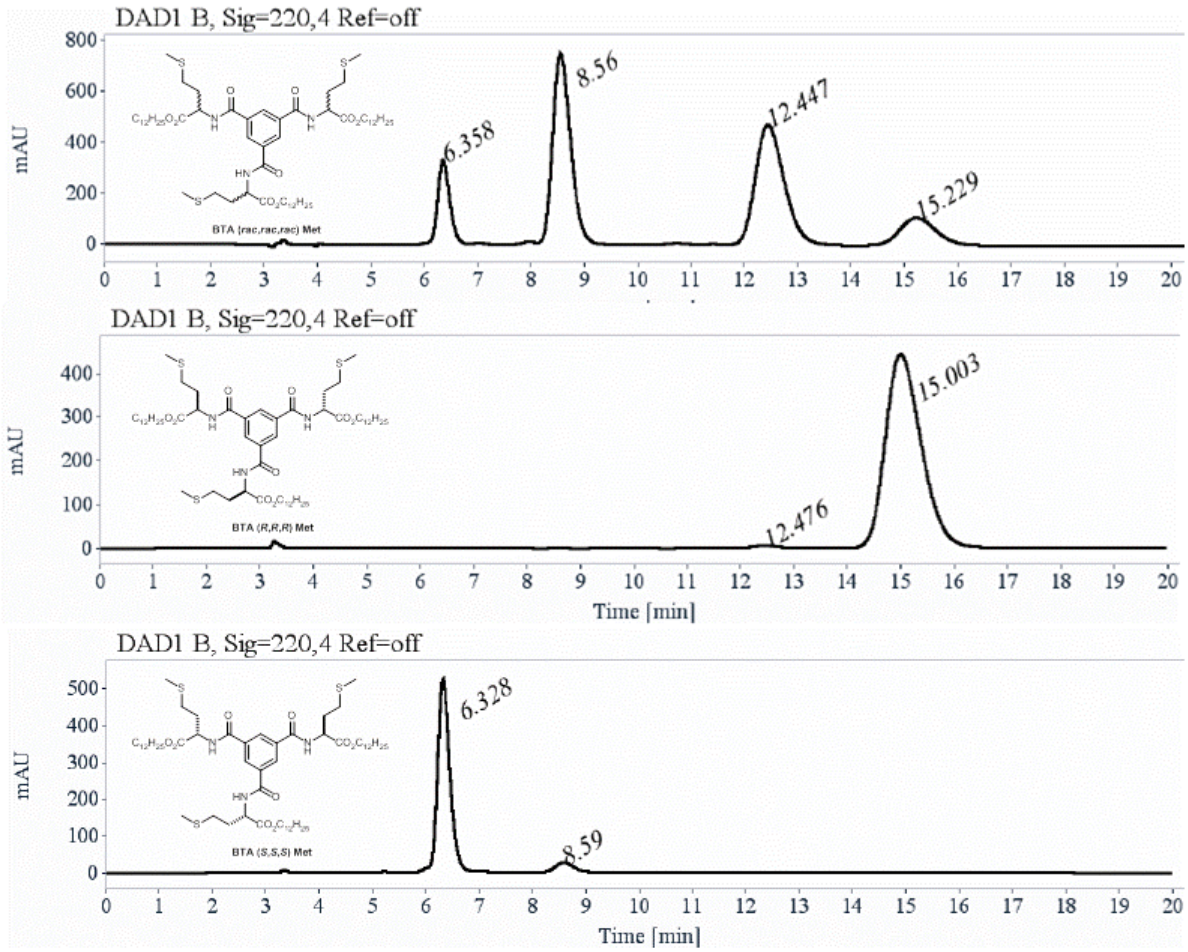
**Tri-N-[(*L*)-(butyl)dodecyloxycarbonymethyl]benzene-1,3,5-tricarboxamide (BTA Nle)**

Obtained from (L)-norleucine using General Method A, then B, with an overall yield of 80%, as a white gum.

**$^1$H NMR** (300 MHz, acetone-d6) δ 8.47 (s, 3H), 8.22 (d, *J* = 7.7 Hz, 3H), 4.64 (td, *J* = 8.2, 5.5 Hz, 3H), 4.13 (hept, *J* = 6.2, 5.3 Hz, 6H), 2.03–1.78 (m, 6H), 1.73–1.58 (m, 6H), 1.28 (s, 66H), 0.94–0.86 (m, 18H); **$^{13}$C{$^1$H} NMR** (75 MHz, acetone-d6) δ 173.0, 166.6, 135.9, 129.8, 65.6, 54.2, 32.7, 32.1, 30.4, 30.3, 30.0, 29.8, 29.4, 29.0, 26.7, 23.4, 23.0, 14.4, 14.2; **HRMS:** Calculated for $C_{63}H_{112}N_3O_9$ $[M+H]^+$: 1054.8393, found: 1054.8391. IR (solid, ATR diamond) see page S19. ee>99%, de> 95%.

**Determination of the optical purity of BTA Met by chiral HPLC**

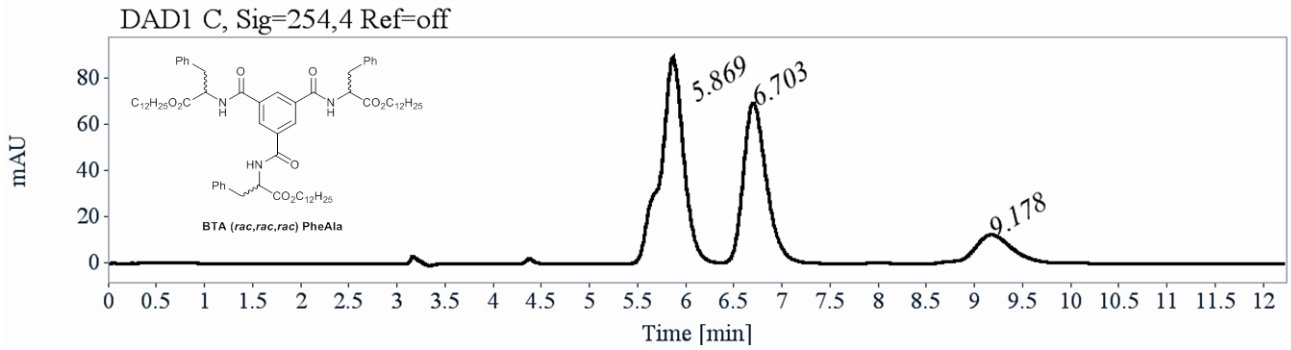
Method description: column = Chiralpak IC, heptane/ethanol 80/20, flow = 1 mL/min, detection at 254 nm.



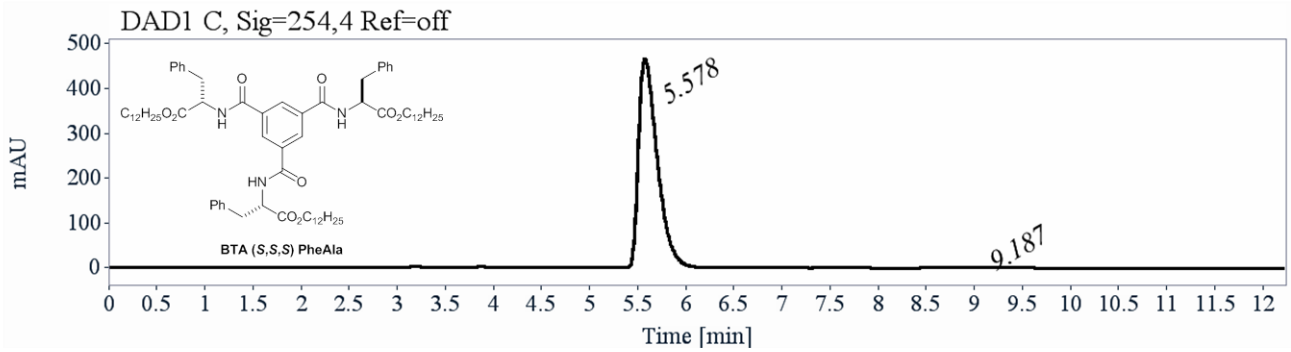

| RT [min] | Area | Area% |
|---|---|---|
| 6.33 | 8382 | 92.78 |
| 8.59 | 653 | 7.22 |
| Sum | 9034 | 100.00 |

**Determination of the optical purity of BTA Phe by chiral HPLC**

Method description: column = Lux-Cellulose-4, heptane/ethanol 90/10, flow = 1 mL/min, detection at 254 nm.



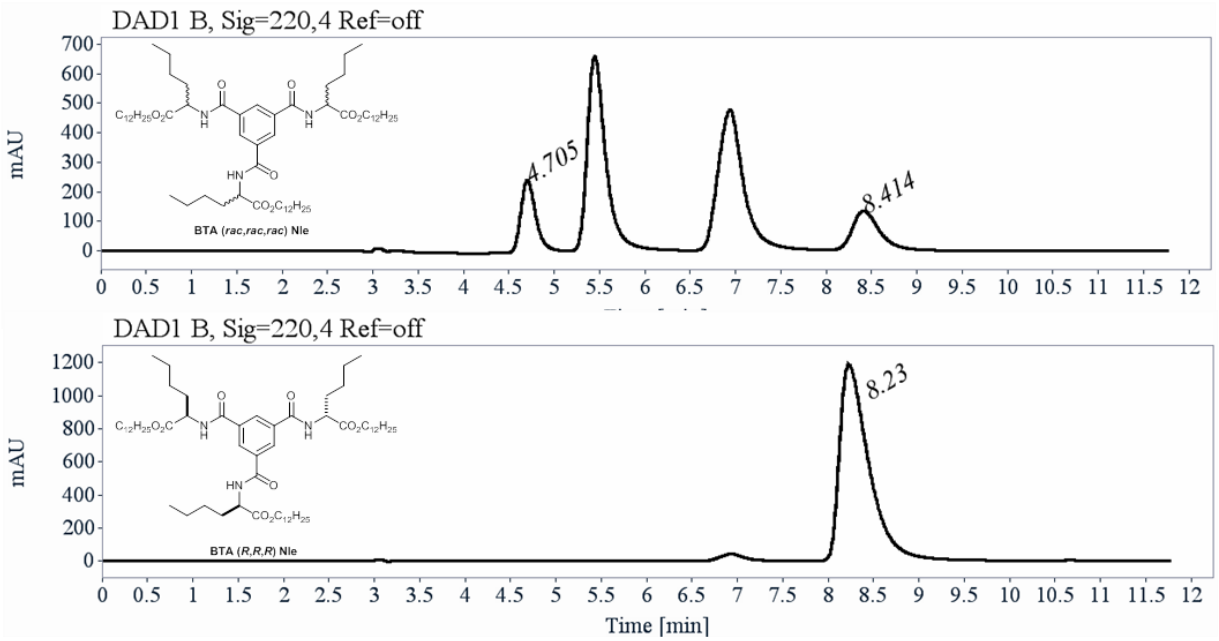

| RT [min] | Area | Area% |
|---|---|---|
| 5.87 | 1511 | 50.29 |
| 6.70 | 1163 | 38.71 |
| 9.18 | 330 | 11.00 |
| Sum | 3004 | 100.00 |

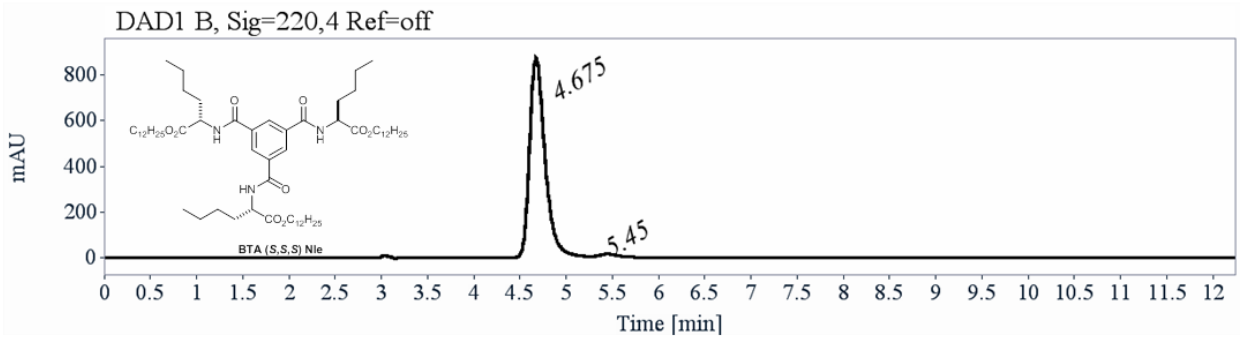



| RT [min] | Area | Area% |
|---|---|---|
| 5.58 | 6363 | 99.60 |
| 9.19 | 26 | 0.40 |
| Sum | 6389 | 100.00 |

**Determination of the optical purity of BTA Nle by chiral HPLC**

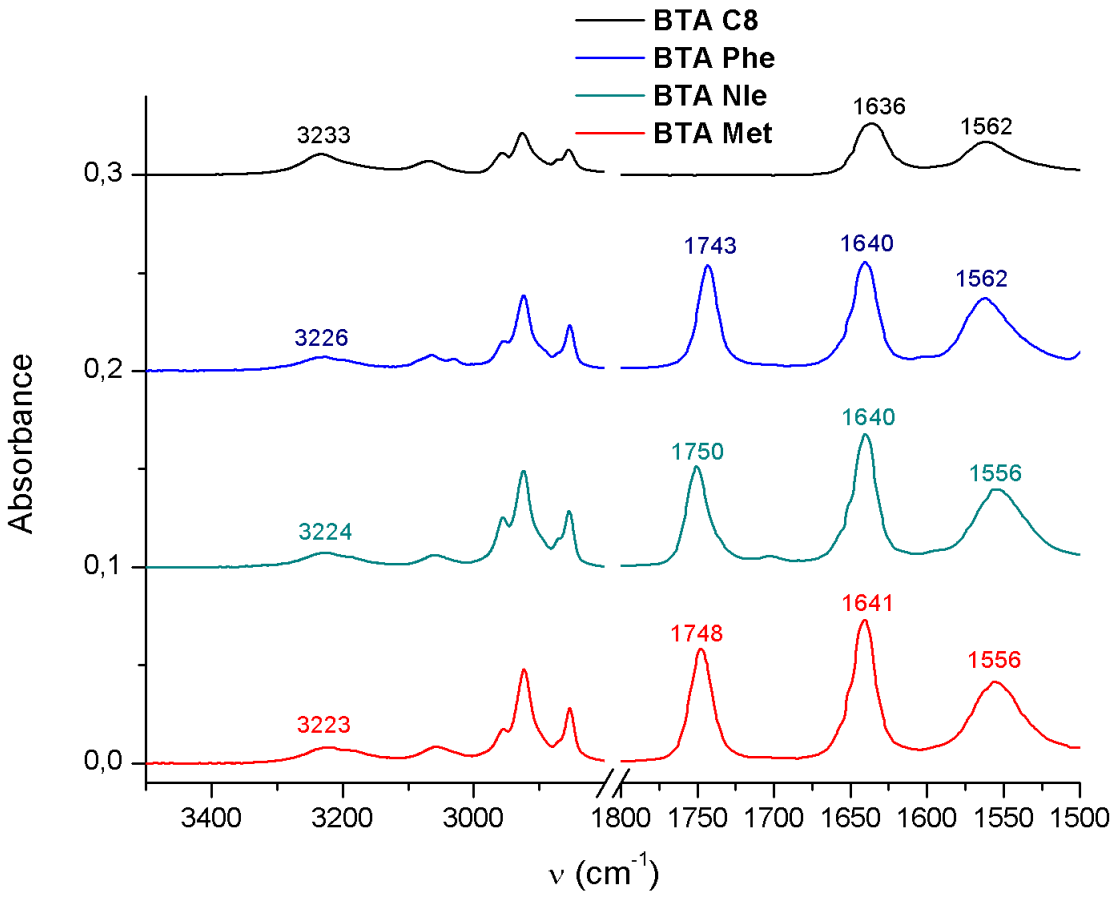
Method description: column = Lux-Amylose-4, heptane/ethanol 90/10, flow = 1 mL/min, detection at 220 nm.




| RT [min] | Area | Area% |
|---|---|---|
| 4.68 | 10272 | 97.61 |
| 5.45 | 252 | 2.39 |
| Sum | 10524 | 100.00 |

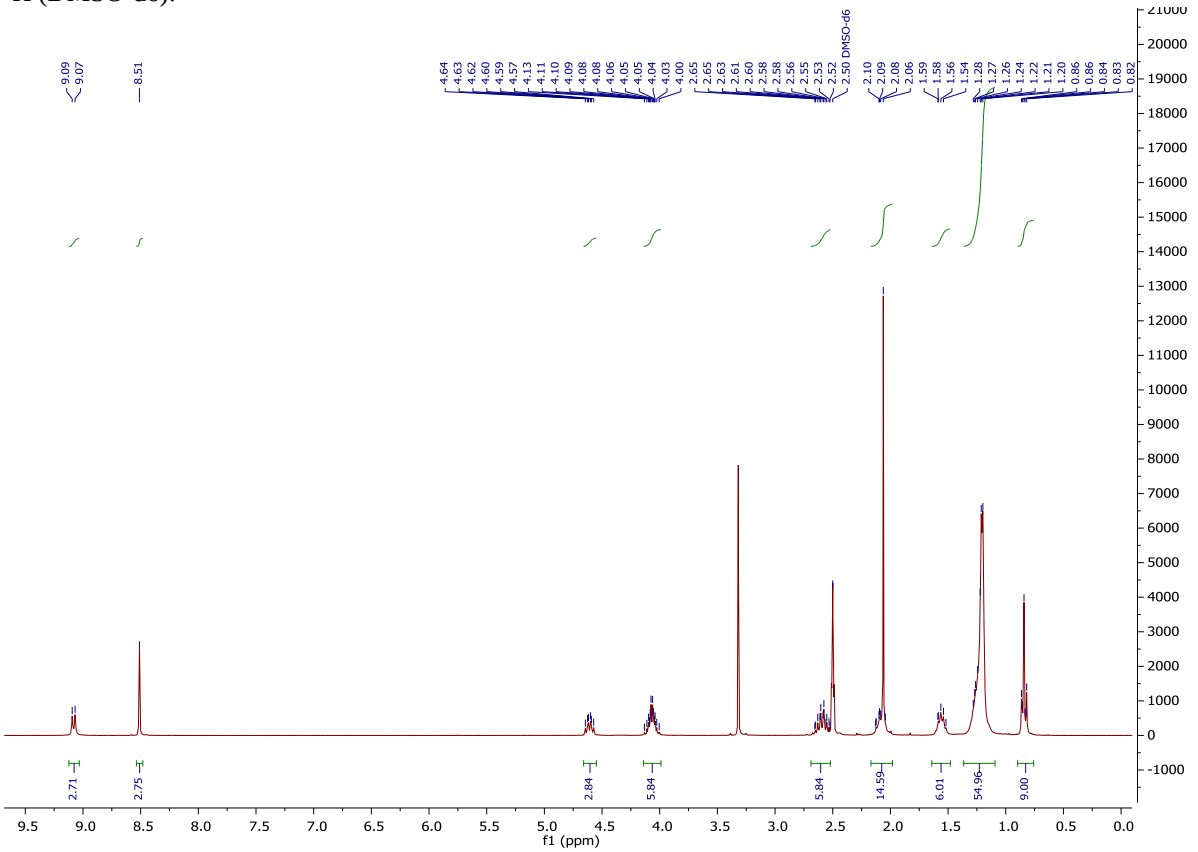
**Infrared spectra of ester BTAs and BTA C8 in the solid state**



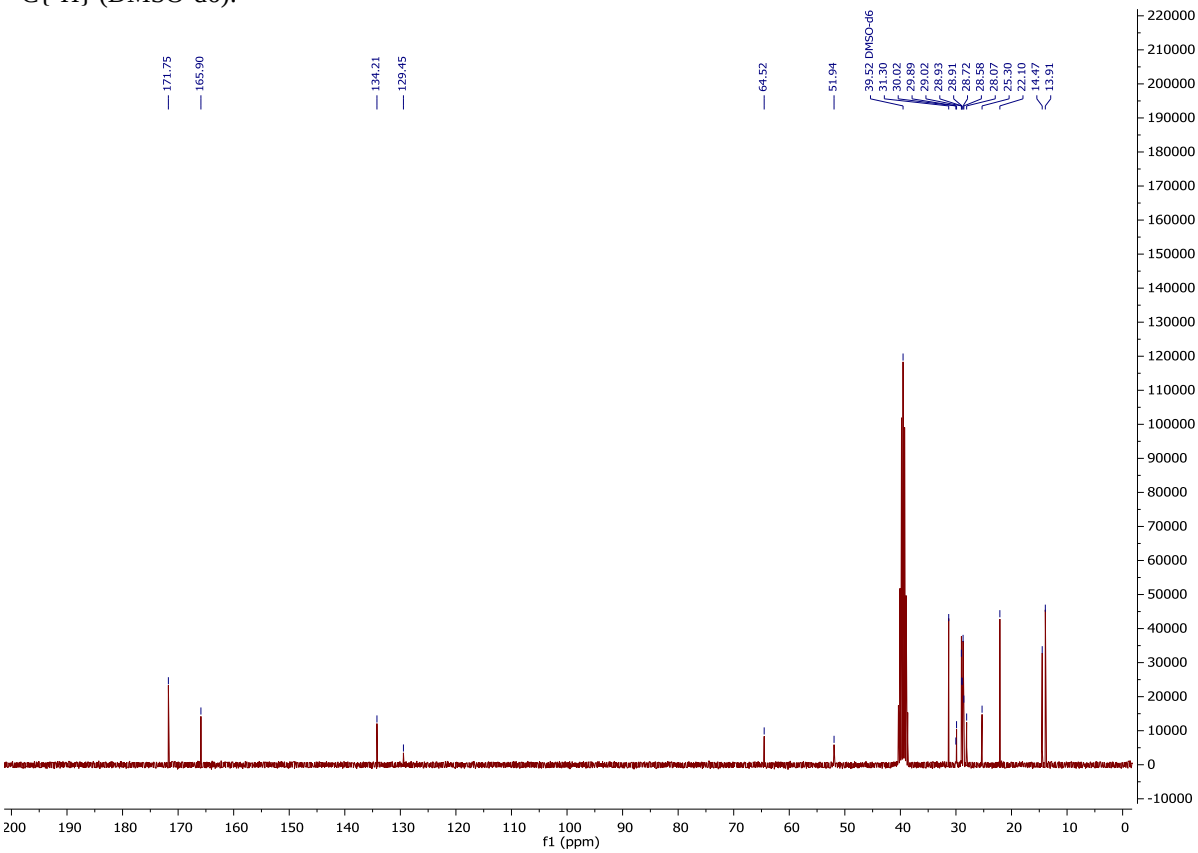

Zoom on the NH, CH, CO ester, amide I and amide II bands. Frequencies corresponding to the maximum absorbance of the NH and CO bands are indicated. The curves are shown with an absorbance offset of 0.1.

## BTA Met

$^{1}$H (DMSO-d6):

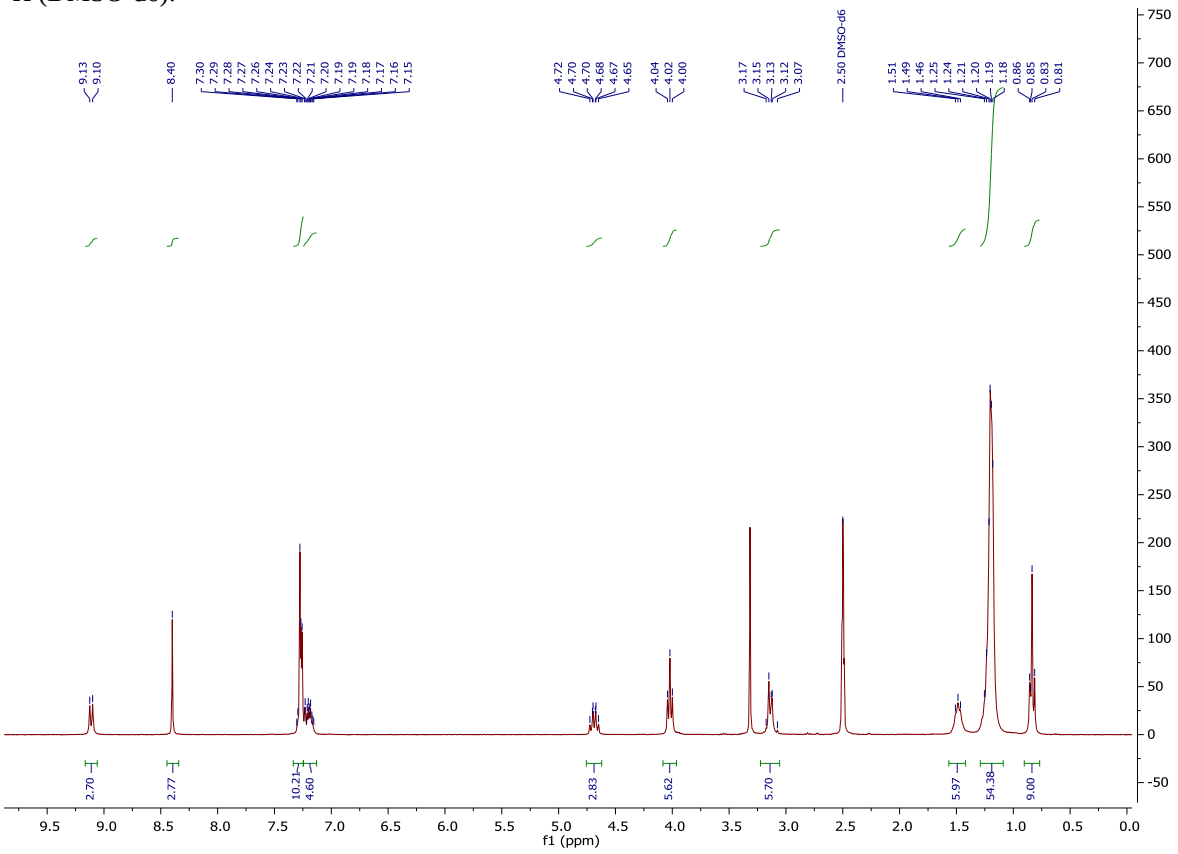



$^{13}$C{$^{1}$H} (DMSO-d6):

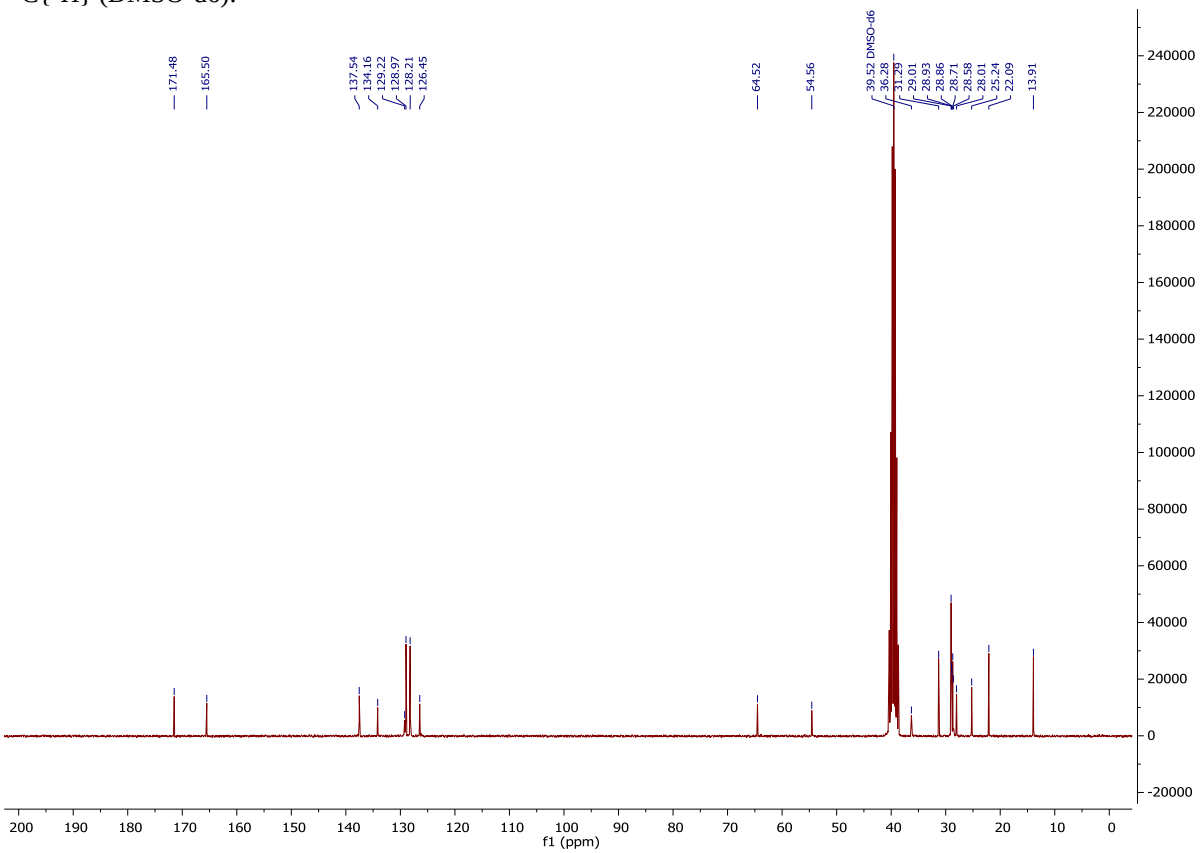


## BTA Phe

$^{1}$H (DMSO-d6):

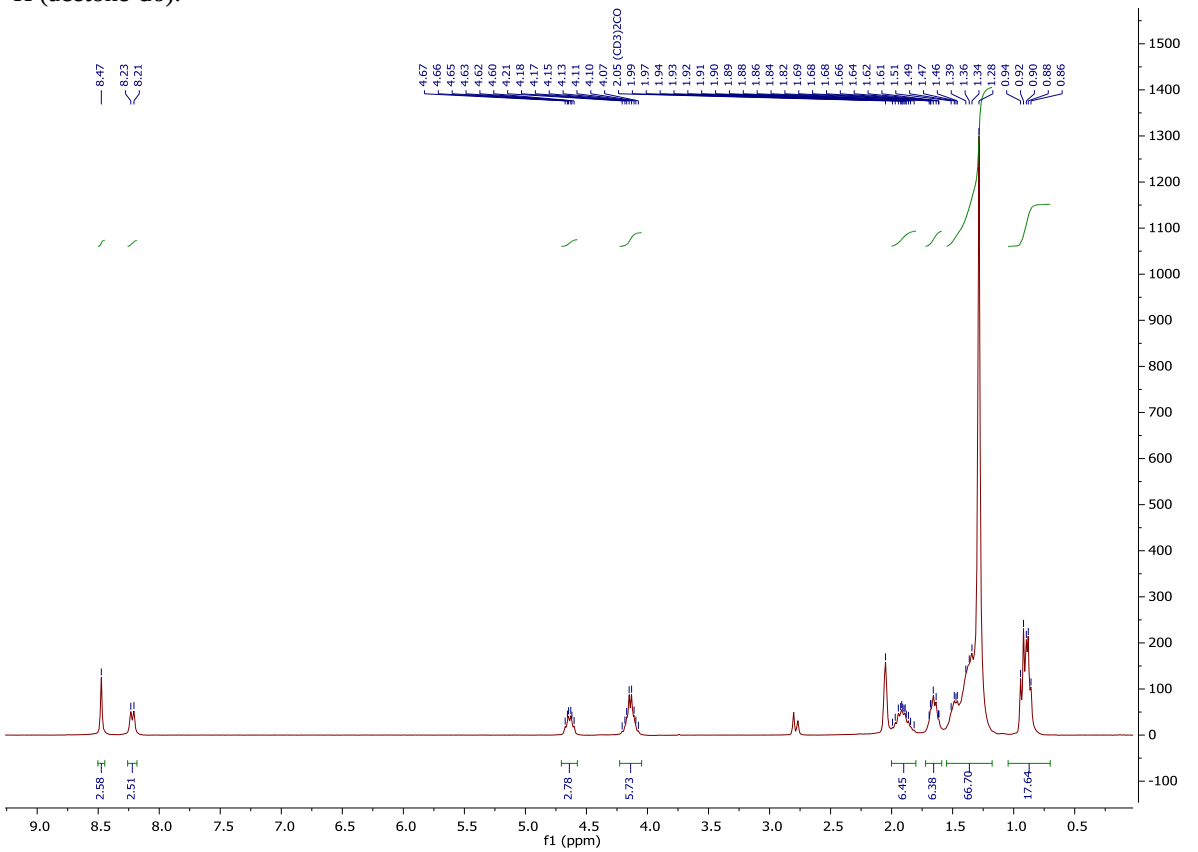



$^{13}$C{$^{1}$H} (DMSO-d6):



# BTA Nle

$^{1}$H (acetone-d6):




$^{13}$C{$^{1}$H} (acetone-d6):