Electronic Supporting Information

Extending Minisci chemistry to direct radical amidation through metal-assisted substrate activation

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Table of content

1 General remarks

All reactions involving moisture- or air-sensitive reagents were performed under an argon atmosphere using standard Schlenk techniques and pre-dried glassware. Syringes for handling dry solvents or liquid reagents were flushed with dry argon prior to use. All chemicals were purchased from commercial suppliers and used as received.

Solvents

Anhydrous CH_2Cl_2, anhydrous DMF, and anhydrous EtOH (reagent grade, AcroSeal®) were stored as received over activated molecular sieves and under inert atmosphere. MeCN (HPLC grade) was dried thoroughly over 3 Å activated molecular sieves and degassed by bubbling nitrogen through the MeCN prior to use. Solvents used for purification were of technical grade, distilled prior to use, and stored under otherwise ambient conditions.

Reagents

AgF_2 was purchased from BLDpharm and stored in a glove box. Note: The visual appearance of AgF_2 is crucial to secure success and reproducibility of the reaction. The AgF_2 must have an intense black appearance. Traces of AgF (yellow solid) lead to detoration in yield due to unproductive side reactions as discussed in Section 6.5 (Degradation of carbamoyl-substituted pyrazine carboxamides by silver(I) fluoride.).

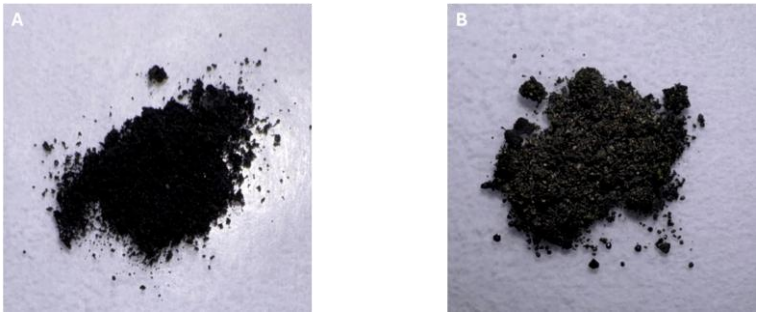


Figure S 1. A. AgF_2 as received from BLDpharm. B. AgF_2 (black) with AgF (yellow).[1]

Chromatography

Analytical TLC was performed on MERCK silica gel 60 F254 aluminum plates. Visualization was accomplished with UV light and/or $KMnO_4$ solution. Flash column chromatography was performed using MACHEREY-NAGEL Silica 60 (SiO_2, 40–63 μm particle size) or using an ADVION INTERCHIM puriFlash 5.030 flash purification system equipped with prepacked silica gel cartridges and UV detection. Crude reaction mixtures were adsorbed onto silica and loaded onto a pre-column. Purification was carried out using appropriate solvent gradients as indicated for each compound. Fractions containing the desired product were identified by UV absorption and/or TLC analysis, combined, and concentrated under reduced pressure to afford the purified compounds.

Melting Points

Melting points were measured on a METTLER TOLEDO MP 70 Melting Point System. Melting points marked with an asterisk (*) refer to the melting points of the recrystallized species.

Infrared Spectroscopy

Infrared spectra were recorded using an Alpha FT-IR-Spectrometer (BRUKER). Liquid and solid probes were measured neat, with absorption given in wave numbers (cm^{-1}), and were recorded in the range of 4000–400 cm^{-1}. The following abbreviations were used for characterization: s (strong), m (medium), w (weak).

Mass Spectrometry

Mass spectra were measured on an Agilent 6890/5973 (GC-MS), Agilent 1200/6210 Time-of-Flight (LC-MS), or Agilent 1260 Infinity/ 6125B (HPLC/MS). The respective mass-to-charge ratios are detected, and the intensities normalized to the base peak (I = 100) are given in brackets. High-resolution mass spectra (HRMS) were recorded on Thermo Electron MAT 95-XP (EI) or Agilent 1200/6210 Time-of-Flight (ESI). Electron ionization (GC-EI) spectra were performed at 70 eV using methane as the carrier gas, with a time-of-flight (TOF) mass analyzer.

Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) spectra were recorded at 300 or 400 MHz (^{1}H NMR) and 75 or 125 MHz (^{13}C NMR) on an AV 400 (BRUKER), AV 300 (BRUKER) or Fourier 300 (BRUKER) instrument. Chemical shifts are reported as δ-values in ppm relative to the residual solvent peak. All spectra were recorded at ambient temperature. The signals were assigned on the basis of experimental data (chemical shifts, coupling constants, integrals) and characterized using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, h = hextet, m = multiplet, dd = double doublet, ddd = double doublet of doublets, td = triple doublet, dt = double triplet, qt = quadruple triplet, dq = double quartet and reported as (multiplicity; coupling constant; integration). All coupling constants are given in Hertz (Hz).

X-Ray crystallography

Data were collected on a BRUKER Kappa APEX II Duo diffractometer using Cu-K_α radiation. The structures were solved by direct methods (SHELXS-97: Sheldrick, G. M. *Acta Cryst*. **2008**, A64, 112.) and refined by full-matrix least-squares procedures on F2 (SHELXL-2018: Sheldrick, G. M. *Acta Cryst.* **2015**, C71, 3.). Mercury (Macrae, C. F., Edgington, P. R., McCabe, P., Pidcock, E., Shields, G. P., Taylor, R., Towler, M., van de Streek, J. *J. Appl. Cryst.* **2006**, 39, 453.) was used for graphical representation. Displacement ellipsoids correspond to 30% probability. Hydrogen atoms are omitted for clarity.

Specific optical rotation

Specific rotation values were recorded using an ANTON PAAR MCP 200 polarimeter at the sodium D-line (589 nm) using a cell of 10 mm or 100 mm path length.

Photophysical Measurements

The absorption spectra were measured with a UV/Vis absorption spectrophotometer UV5 Bio (METTLER TOLEDO). All emission spectra were recorded with a Cary Eclipse Fluorescence Spectrophotometer (Varian) with an excitation wavelength (λ_{Ex}) of 350 nm. The samples were dissolved in acetonitrile (MeCN; UVASOL®-Quality) and placed in 10 mm quartz cuvettes (HELLMA ANALYTICS). All pyrimidopteridines (PPTs) were measured with an optical density of around 0.1 at 360 nm.

Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV)

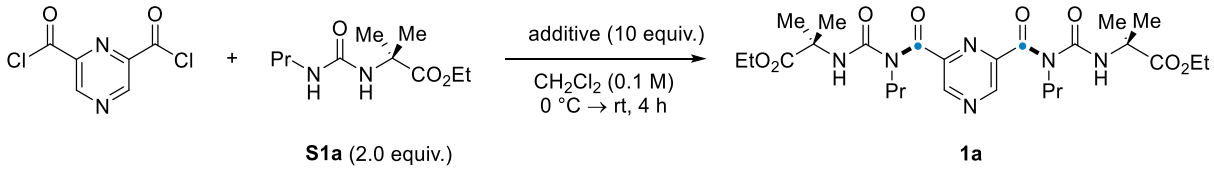
All electrochemical investigations were performed at room temperature in dried acetonitrile p.A. (VWR) under an Argon atmosphere with 0.1 M tetrabutylammonium hexafluorophosphate (Fluka) as conducting salt using an Autolab (PGSTAT 204, Metrohm). A glassy carbon disk electrode (d = 2 mm) was used as the working electrode, a Pt-electrode as the counter electrode, and an Ag/AgCl/LiCl sat. in EtOH-system as the reference electrode (all electrodes: Metrohm). All potential mentioned in this paper were measured with respect to this reference system and were checked by using the ferrocenium/ferrocene-internal reference system (potential of Fc^+/Fc: 0.54 V [vs. Ag/AgCl/LiCl sat. in EtOH]. The potential reported relative to the Fc^+/Fc redox couple was converted to NHE by adding 0.63 V. The CV scans were done three times at a scan rate of 50, 100 & 250 mV s^{-1}. The measurements were performed with a 10 mM compound dissolved in the electrolyte. Differential pulse voltammetry was measured using a step potential of 5 mV, modulation amplitude of 25 mV, modulation time of 0.05 s, and interval time of 0.05 s.

2 Optimization of reaction conditions

2.1 Amide bond formation of heteroaryl carboxylic acids and ureas

Amide bond formation reactions of heteroaryl carboxylic acids and ureas were optimized on a 0.1 mmol scale using pyrazine-2,6-dicarboxylic acid and ethyl 2-methyl-2-(3-propylureido)propanoate (**S1a**). The pyrazine-2,6-dicarboxylic acid (1.0 mmol, 1.0 equiv.) was suspended in CH_2Cl_2 (0.1 M) Subsequently, oxalyl chloride (3.6 mmol, 3.6 equiv.) and catalytic amounts of DMF were added, and the reaction was stirred for 2 h at ambient temperature, resulting in a clear solution. The solvent and unreacted oxalyl chloride were removed under reduced pressure. The crude acid chloride was redissolved in CH_2Cl_2 before urea **S1a** (2.0 equiv.), and the indicated additive (10 equiv.) were added at 0 °C. Unless otherwise stated, reported yields correspond to isolated yields of the desired carbamoyl-substituted pyrazine carboxamide **1a**. In selected cases, yields were determined by ^{1}H NMR spectroscopy using 4-phenylpyridine as an internal standard.

Table S 1. Screening of the coupling between the urea **S1a** and 2,6-pyrazinedicarbonyl dichloride.[a]



S1a (2.0 equiv.) **1a**

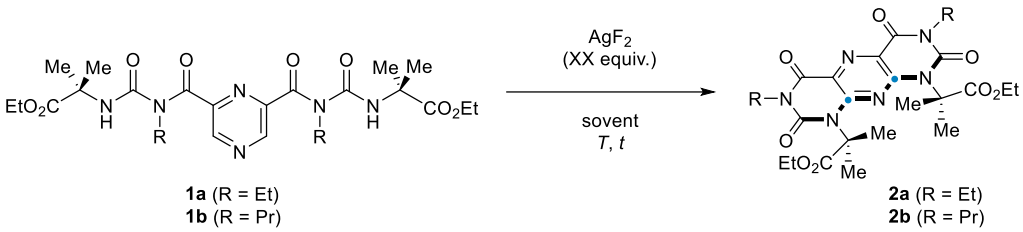
Entry	additive	Yield of 1a [%][b]
1	**pyridine**	**65**
2	4-phenylpyridine	66[c]
3	4-methoxypyridine	21
4	4-trifluoromethylpyridine	37
5	4-cyanopyridine	49
6	pyridine-*N*-oxide	16
7	dimethylaminopyridine	-
8	*N*-methylmorpholine	-
9	1-methylimidazole	-
10	pyridazine	-

[a]Reactions were run at 0.1 mmol scale using carboxylic acid (1.0 equiv.), $(COCl)_2$ (3.6 equiv.) and DMF (cat.). The corresponding acyl dichloride (1.0 equiv.) was used without further purification in the subsequent step using **S1a** (2.0 equiv.) and additive (10 equiv.). [b] Yields given refer to isolated yields if not otherwise noted. [c] 4-phenylpyridine was detected as impurity.

2.2 Silver(II) fluoride-mediated cyclization

The AgF_2-mediated radical cyclization was optimized on a 0.1 mmol scale using carbamoyl-substituted pyrazine carboxamide **1a** or **1b** (1.0 equiv.) and AgF_2 (4.5 equiv.) in MeCN (0.13 M). Reported yields correspond to isolated yields of the desired pyrimidopteridine **2a** and **2b**, respectively.

Table S 2. Screening of the AgF_2-mediated cyclization of using carbamoyl-substituted pyrazine carboxamide.[a]



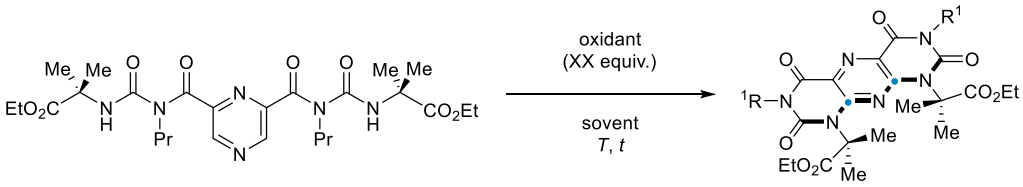
1a (R = Et)
1b (R = Pr)

2a (R = Et)
2b (R = Pr)

Entry	R[1]	AgF_2 [equiv.]	Additive (equiv.)	Solvent (M)	*T* [°C]	*t* [min]	Yield [%][b]
1	Et	4.5		MeCN (0.05)	30	30	59
2	**Et**	**4.5**		**MeCN (0.13)**	**30**	**30**	**62**
3	Et	4.5		MeCN (0.05)	30	120	59
4	Et	4.5		MeCN (0.05)	50	30	43
5	Et	4.5	collidine (1.0)	MeCN (0.05)	30	30	54
6	Pr	6.0		MeCN (0.05)	r.t.	90	34
7	Pr	4.0		$MeNO_2$ (0.05)	r.t.	90	nd.
8	Pr	4.0		CH_2Cl_2 (0.05)	r.t.	90	nd.
9	Pr	4.0		$MeCN/CH_2Cl_2$	r.t.	90	traces
10	Pr	2.0		MeCN (0.05)	r.t.	90	6[a]
11	Pr	4.0		MeCN (0.05)	r.t.	90	42
12	Pr	4.0		MeCN (0.05)	−15-(−10)	90	33[c]
13	**Pr**	**4.5**		**MeCN (0.13)**	**30**	**30**	**61**

[a]Reaction were performed on a 0.1 mmol-scale.[b]yields refer to isolated yields.

Table S 3. Screening of alternative oxidants.[a]



1b **2b**

Entry	R^1	Oxidant [equiv.]	Additive (equiv.)	Solvent (M)	*T* [°C]	*t* [h]	Scale [mmol]	Yield [%][b]
1	Pr	NBS (5.0)	K_2CO_3 (5.0)	DCE (0.1)	60	5	0.12	n.d
2	Pr	NBS (2.2)	-	MeOH (0.1)	r.t.	3	0.75	n.d.
3	Et	$K_2S_2O_8$ (2.2)	$AgNO_3$(0.20) TFA (0.10)	MeCN (0.05)	40	18	0.1	n.d.
4	Et	$K_2S_2O_8$ (5.0)	$AgNO_3$ (1.0) TFA (0.1)	MeCN:H_2O (1:1, 0.05)	40	18	0.1	n.d.
5	Pr	$Ag^{II}bpyS_2O_8$	-	MeCN (0.05)	30	18	0.05	n.d.
6	Et	CuF_2 (4.5)	-	MeCN (0.13)	30	18	0.1	n.d.
7	Et	$Cu(OTf)_2$ (4.5)	-	MeCN (0.13)	30	18	0.1	n.d.
8	Et	$Cu(EH)_2$ (4.5)	-	MeCN (0.13)	30	18	0.1	n.d.

[a]Reaction were performed on a 0.1 mmol-scale.

To probe the role of the metal fluoride oxidant, CuF_2 was evaluated under otherwise identical reaction conditions in place of AgF_2. In contrast to AgF_2, CuF_2 resulted in only trace conversion and no significant formation of the desired product. This pronounced difference in reactivity can be attributed to the distinct electronic properties of the two transition-metal fluoride complexes. While both transition metal fluoride complexes formally contain a d^9 metal center, the enhanced reactivity of AgF_2 relative to CuF_2 likely originates from the combination of the high Ag(II/I) reduction potential and the comparatively covalent Ag–F bond,[ref] which together provide a substantially more favorable effective BDFE for concerted proton-coupled electron transfer (PCET) and H-atom abstraction processes. In contrast, the Cu-F bond in CuF_2 is more ionic and CuF_2 is orders of magnitude less oxidizing than AgF_2 and therefore much less capable of engaging in high-energy dissociative PCET or H-atom abstraction pathways.

Table S 4. Calculated prices per mmol for selected oxidants.

Oxidant	Batch Size [g]	Price [€]	Price per mmol [€][a]
Silver(II)-fluoride	10	125	1.8
Silver(II)-fluoride[b]	**25**	**256**	**1.5**
Selectfluor	25	141	2.0
Copper(II)-trifluormethanesulfonate	25	385	5.6
Copper(II)-2-ethylhexanoate	25	222	3.1
Copper(II)-fluoride	20	218	1.1

Unless otherwise noted, prices were retrived from https:/www.sigmaaldrich.com (12.05.2026). [a]Prices per mmol were rounded to 1 decimal place. [b]Purchased from https://www.bldpharm.com/ (25.02.2026) and used in this work.

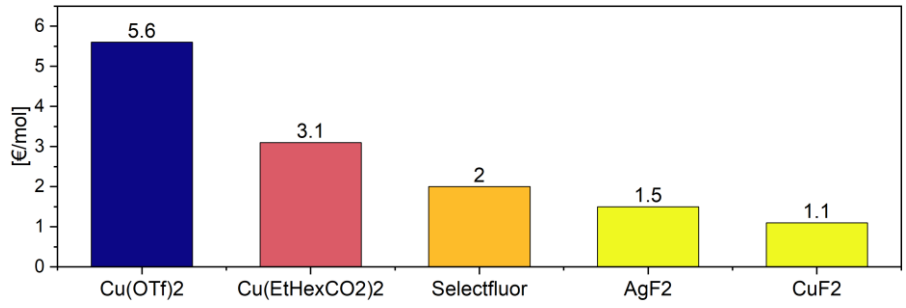
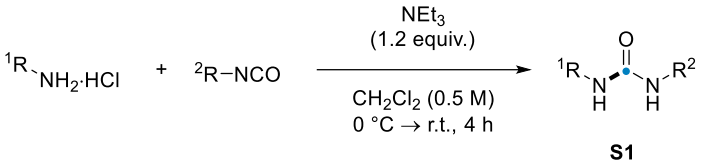


Figure S 2. Calculated prices per mmol for selected oxidants.

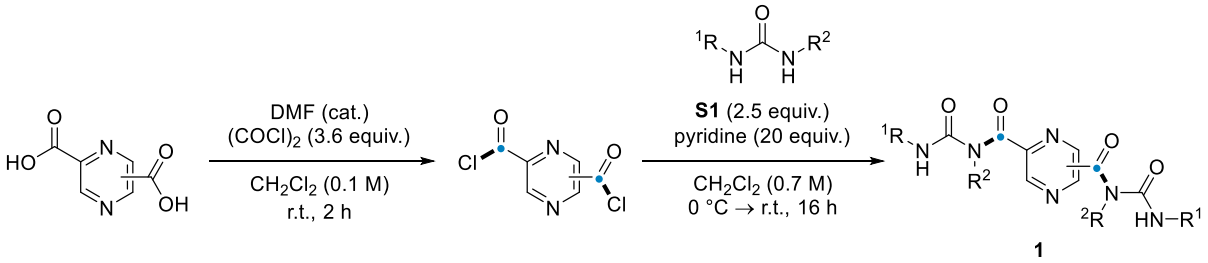
3 General procedures

3.1 General procedure for the synthesis of the urea derivatives (GP1)

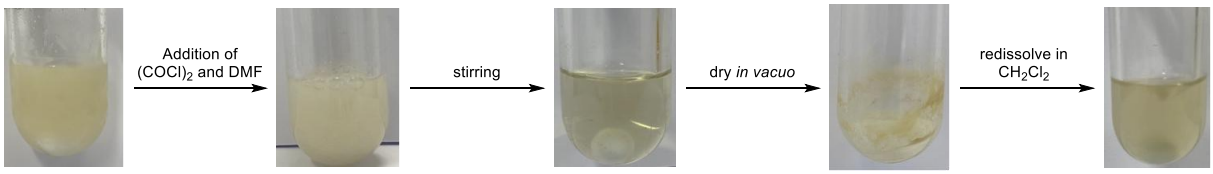


GP1 is based on a literature-known procedure.[2] A flask was charged with the corresponding amino hydrochloride salt (10 mmol, 1.0 equiv.) and dissolved in dichloromethane (CH_2Cl_2) (20 ml, 0.5 M) under ambient atmosphere. The reaction mixture was cooled to 0 °C using an ice bath, and triethylamine (12 mmol, 1.2 equiv.) was added while stirring. Alkyl isocyanate (11 mmol, 1.1 equiv.) was then added dropwise to the cooled reaction mixture. The mixture was stirred for 4 h, gradually warming to ambient temperature. After completion, the reaction was quenched by adding 1 M (20 mL) aqueous HCl solution, and the mixture was extracted three times with CH_2Cl_2 (20 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate (Na_2SO_4), and filtered. The solvent was removed under reduced pressure using a rotary evaporator, yielding the desired ureas **S1a**-**S1x**.

3.2 General procedure for the synthesis of carbamoyl-substituted pyrazine carboxamides (GP2)



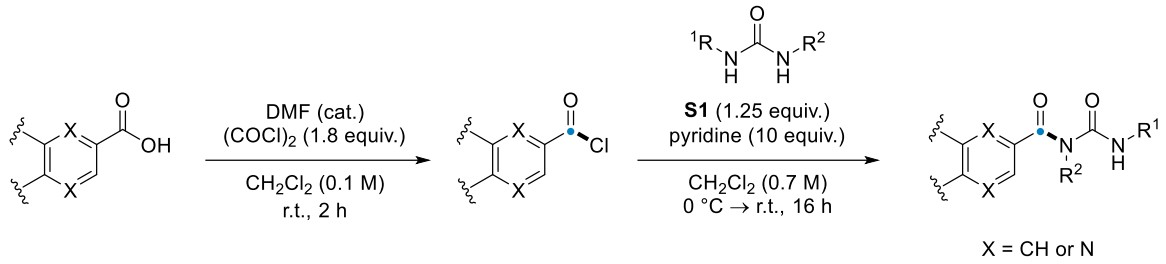
The corresponding pyrazinedicarbonyl dichlorides were synthesized according to a literature-known procedure.[3] The corresponding pyrazinyl dicarboxylic acid (1.0 mmol, 1.0 equiv.) was suspended in CH_2Cl_2 (0.1 M) Subsequently, oxalyl chloride (3.6 mmol, 3.6 equiv.) and catalytic amounts of DMF were added, and the reaction was stirred for 2 h at ambient temperature, resulting in a clear solution. The solvent and unreacted oxalyl chloride were removed under reduced pressure. The crude acid chloride was redissolved in CH_2Cl_2 (0.1 M).



Scheme S 1. Visual appearance of the reaction for heteroarylacyl dichloride formation.

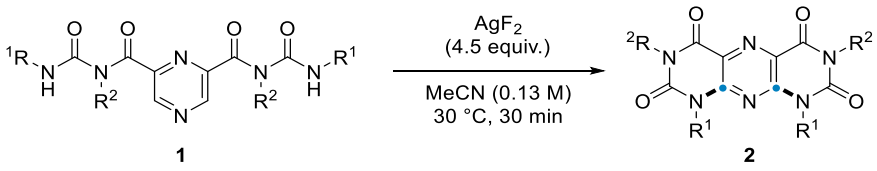
The acid chloride solution was cooled to 0 °C, and pyridine (20 mmol, 20 equiv.) was added. Subsequently, the corresponding urea (2.5 mmol, 2.5 equiv.) was dissolved in CH_2Cl_2 (0.5 M) under ambient atmosphere in and added dropwise at 0 °C. The reaction mixture was allowed to warm to ambient temperature and stirred for 16 h. The crude mixture was then concentrated in vacuo and directly purified by flash column chromatography to yield the desired carbamoyl-substituted pyrazine carboxamides **1a**-**1y**, **15** and **16**.

3.3 General procedure for the synthesis of mono carbamoyl-substituted azine carboxamides (GP2‘)



The corresponding azine carboxylic acid (1.0 mmol, 1.0 equiv.) was suspended in CH_2Cl_2 (0.1 M) Subsequently, oxalyl chloride (1.8 mmol, 1.8 equiv.) and catalytic amounts of DMF were added, and the reaction was stirred for 2 h at ambient temperature. The solvent and unreacted oxalyl chloride were removed under reduced pressure. The crude acid chloride was redissolved in CH_2Cl_2 (0.1 M). The acid chloride solution was cooled to 0 °C, and pyridine (10 mmol, 10 equiv.) was added. Subsequently, the corresponding urea (1.25 mmol, 1.25 equiv.) was dissolved in CH_2Cl_2 (0.5 M) under ambient atmosphere in and added dropwise at 0 °C. The reaction mixture was allowed to warm to ambient temperature and stirred for 16 h. The crude mixture was then concentrated in vacuo and directly purified by flash column chromatography on silica gel to yield the desired carbamoyl-substituted pyrazine carboxamides **3**, **17**, **18** and **19**.

3.4 General procedure for the synthesis of the pyrimodopteridinetetraones (GP3)



1 **2**

A 4 ml-screw-capped vial equipped with a septum was charged with the corresponding pyrazine dicarboxamide **1** (0.25 mmol, 1.00 equiv.), which was subsequently dissolved in MeCN (1.2 ml, 0.13 M). The solution was stirred at 30 °C, and AgF_2 (1.13 mmol, 4.5 equiv.) was added under argon counterflow. After 30 min, the reaction was quenched with a saturated, aqueous $NaHCO_3$ solution

(10 mL) and extracted with EtOAc (3 x 10 ml). The combined organic phases were dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash column chromatography on silica gel.

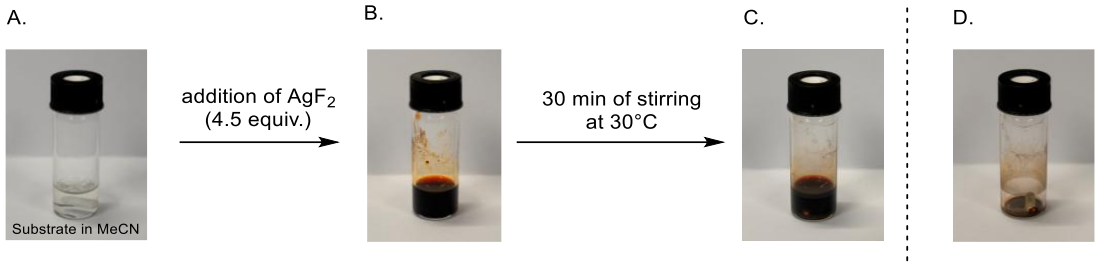
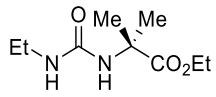


Figure S 3. Visual appearance of the reaction sequence of the synthesis of pyrimodopteridinetetraones **2**. **A.** Substrate **1** in MeCN (0.13 M). **B.** Reaction mixture after addition of AgF_2 (4.5 equiv.). **C.** Reaction mixture after stirring for 30 min at 30 °C. **D.** Residual precipitate after transfer of the liquid phase to the separatory funnel.

4 Compound Characterization

4.1 Synthesis of urea derivatives according to GP1

Synthesis of ethyl 2-(3-ethylureido)-2-methylpropanoate (S1a)



S1a

Following the general procedure GP1, using ethyl 2-amino-2-methylpropanoate hydrochloride (**H-Aib-OEt·HCl**) (4.0 mmol), afforded the title compound **S1a** (796 mg, 3.94 mmol, 98% yield) as a colorless crystalline solid.

R_f = 0.21 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 70-72°C

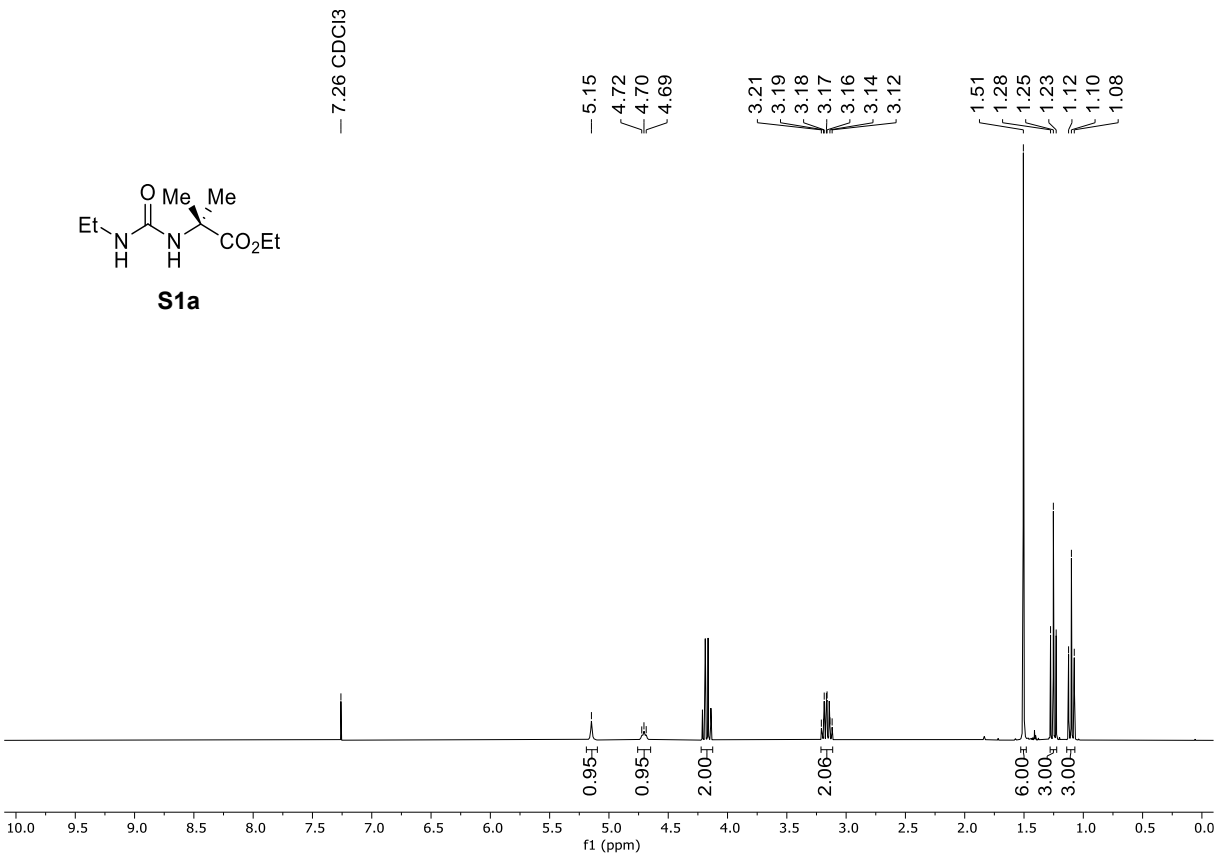
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.15 (s, 1H), 4.70 (t, *J* = 5.6 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.16 (qd, *J* = 7.2, 5.6 Hz, 2H), 1.51 (s, 6H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.10 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 175.9, 157.4, 61.4, 56.2, 35.2, 25.8, 15.6, 14.3.

MS (EI): *m/z* (%) = 129 (42), 58 (100).

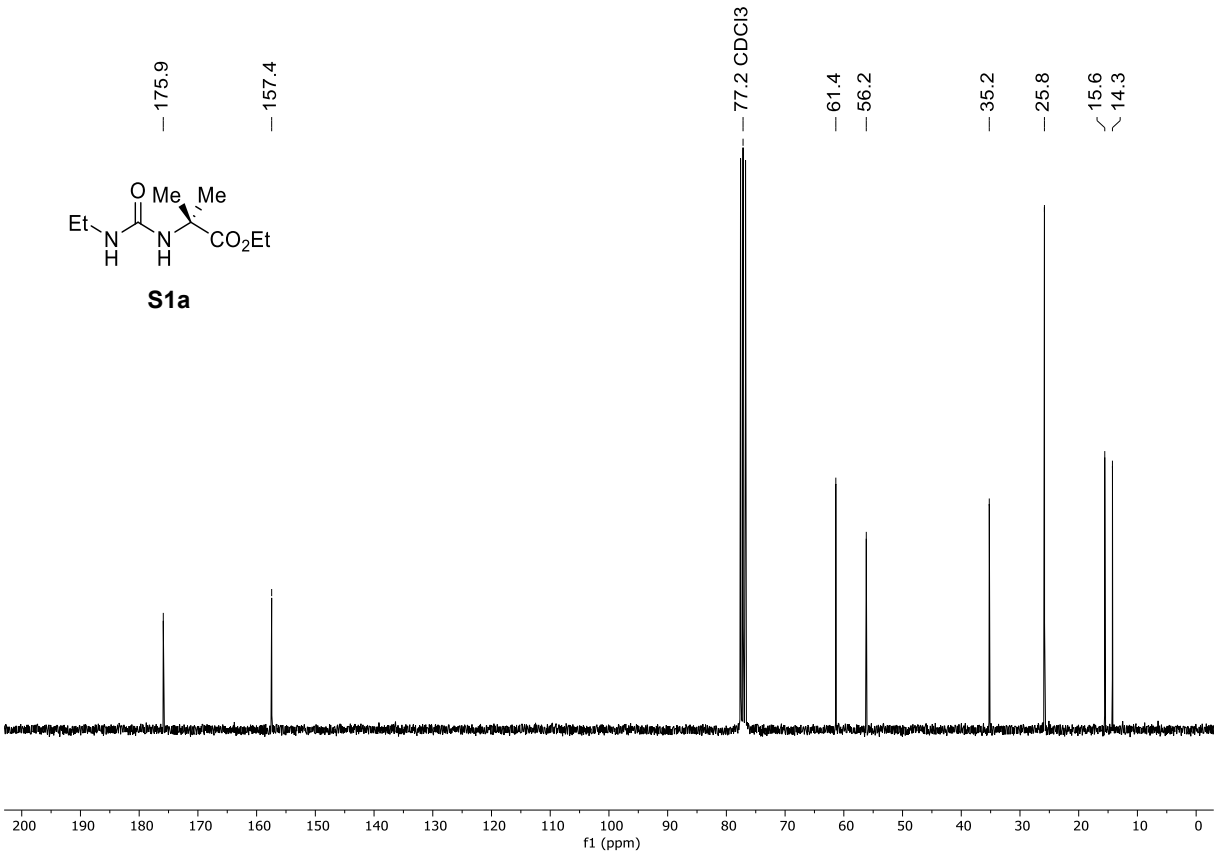
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_9H_{18}N_2O_3Na]^+$: 225.1210; found: 225.1217, (Error ppm: 3.1).

IR (ATR, $\tilde{\nu}$) = 3350 (w), 3001 (w), 2973 (w), 2930 (w), 2908 (w), 2871 (vw), 1733 (vs), 1676 (w), 1625 (vs), 1566 (vs), 1521 (w), 1470 (m), 1456 (m), 1440 (m), 1395 (w), 1380 (w), 1362 (m), 1289 (s), 1272 (s), 1223 (vs), 1166 (vs), 1146 (vs), 1113 (m), 1081 (w), 1024 (s), 971 (w), 938 (w), 901 (w), 877 (w), 844 (w), 816 (vw), 775 (w), 763 (w), 701 (w), 616 (s), 602 (s), 565 (m), 459 (w), 418 (m) cm^{-1}.



S1a

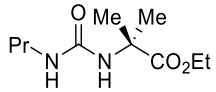
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



S1a

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl 2-methyl-2-(3-propylureido)propanoate (S1b)



S1b

Following the general procedure GP1, using ethyl 2-amino-2-methylpropanoate hydrochloride (**H-Aib-OEt·HCl**) (10 mmol), afforded the title compound **S1b** (2.13 g, 9.8 mmol, 98% yield) as a colorless crystalline solid.

R_f = 0.39 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 69-72 °C

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.29 (bs, 2H) 4.17 (q, *J* = 7.1 Hz, 2H), 3.13 – 3.04 (m, 2H), 1.66 – 1.47 (m, 8H), 1.25 (t, *J* = 7.1 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H).

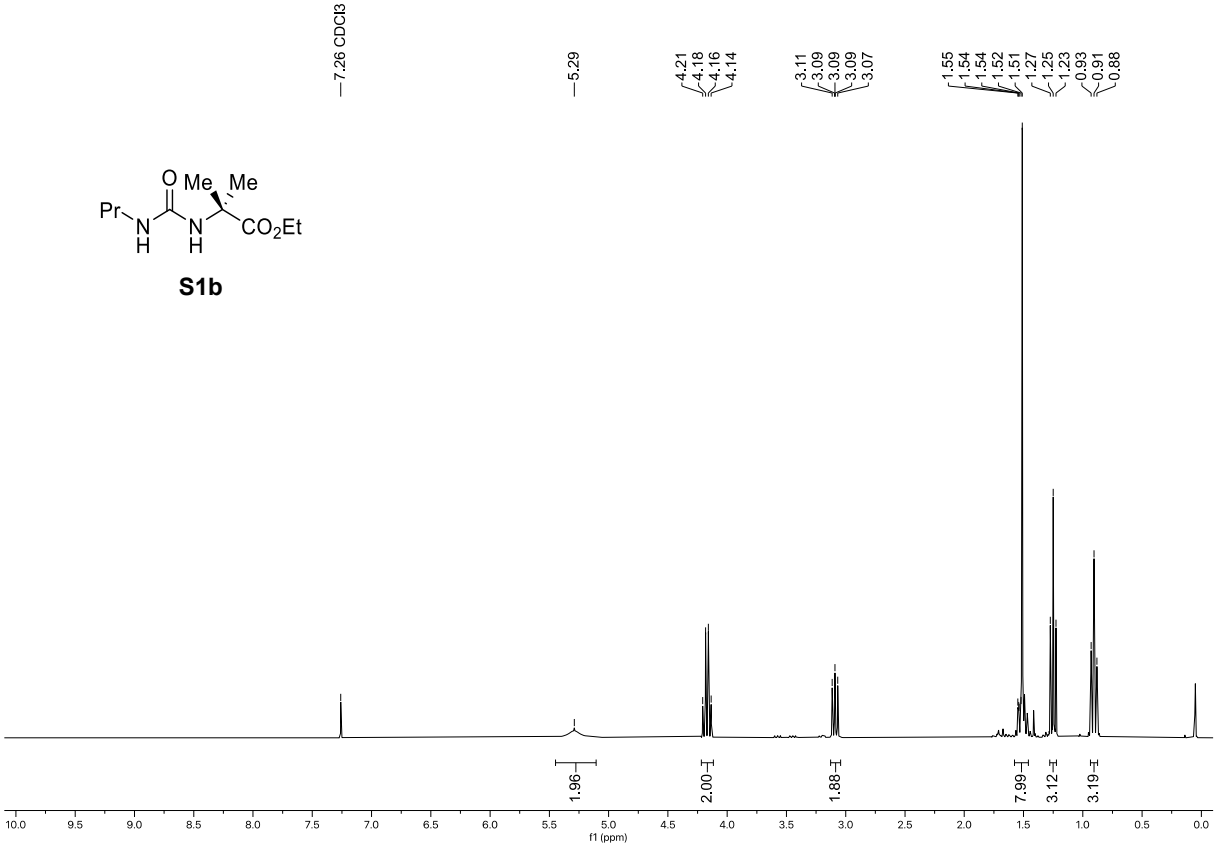
^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 175.7, 157.9, 61.5, 56.4, 42.4, 25.8, 23.4, 14.2, 11.4.

MS (EI): *m/z* (%) = 143 (51), 84 (11), 58 (100).

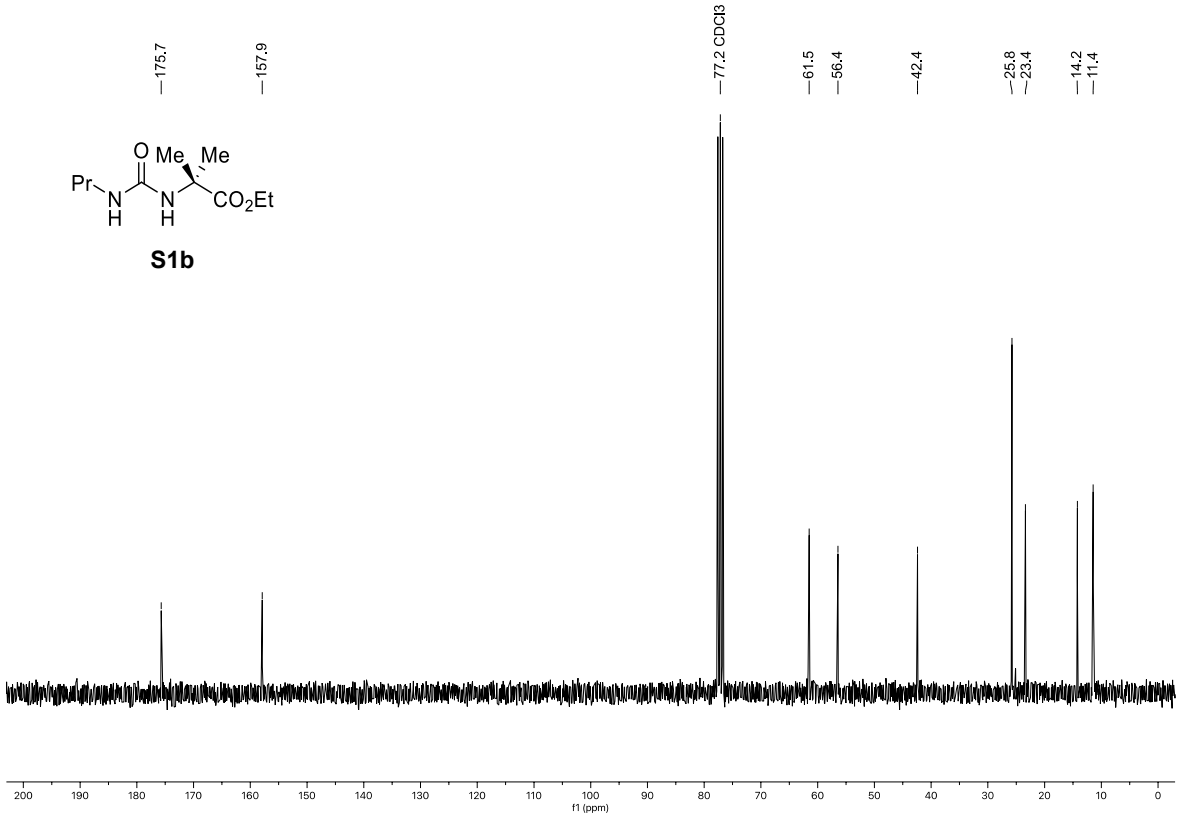
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{10}H_{20}N_2O_3Na]^+$: 239.1366; found: 239.1366 (Δppm: 0.0).

IR (ATR, neat, cm^{-1}): 3335 (w), 2965 (w), 2930 (w), 2874 (w), 1730 (m), 1677 (w), 1626 (s), 1562 (s), 1455 (m), 1382 (w), 1362 (w), 1284 (m), 1223 (s), 1144 (s), 1112 (m), 1024 (m), 880 (w), 844 (w), 775 (w), 619 (m), 467 (w), 405 (w).

The analytic data agrees with those reported in literature.[2]

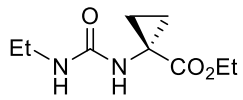


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl 1-(3-benzylureido) cyclopropane-1-carboxylate (S1c)



S1c

Following the general procedure GP1, using ethyl 1-aminocyclopropane-1-carboxylate hydrochloride (**H-Acpc-OEt·HCl**) (5.0 mmol), afforded the title compound **S1c** (859 mg, 4.29 mmol, 86% yield) as a colorless crystalline solid.

$\mathbf{R}_f$ = 0.13 (*n*-pentane:ethyl acetate = 1:2, Vanillin stain)

m.p. = 116-118 °C

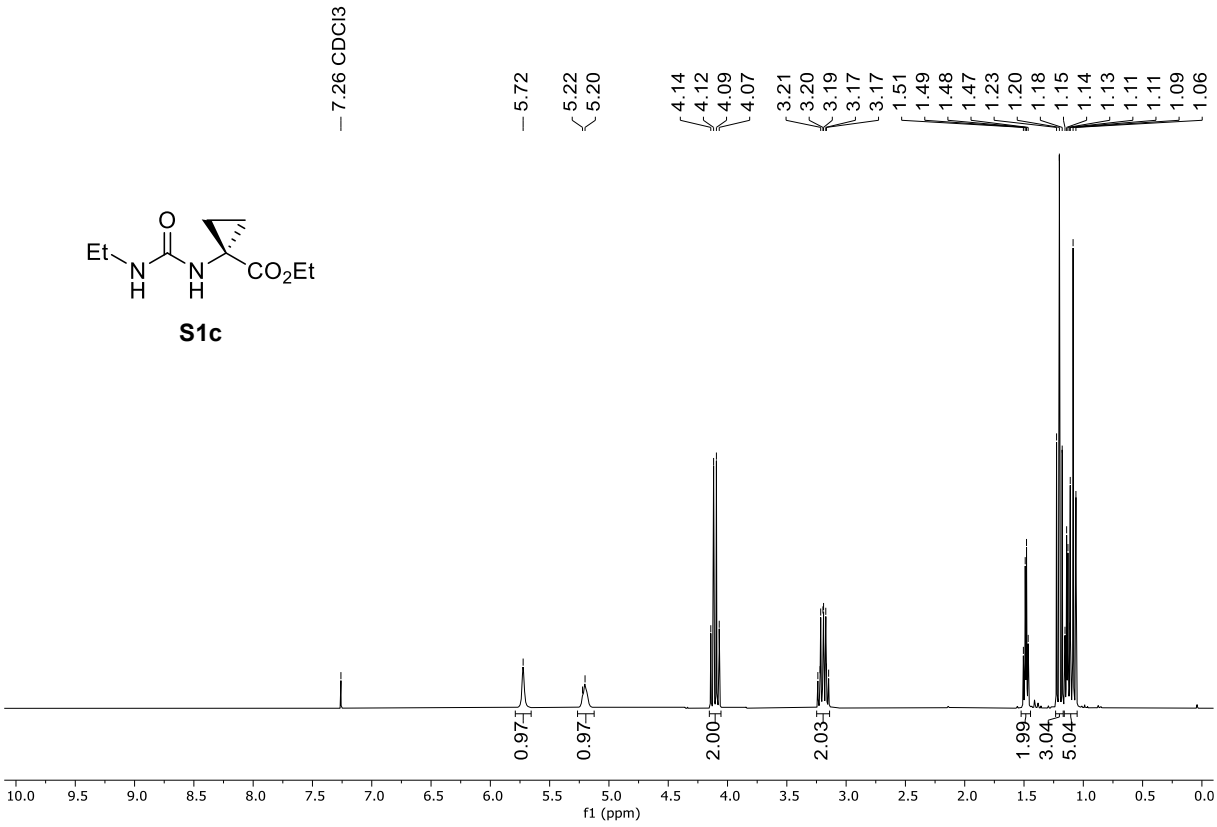
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.72 (bs, 1H), 5.20 (m, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.19 (qd, *J* = 7.2, 5.6 Hz, 2H), 1.52 – 1.43 (m, 2H), 1.20 (t, *J* = 7.1 Hz, 3H), 1.16 – 1.11 (m, 2H), 1.09 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 173.6, 158.9, 61.4, 35.1, 34.3, 18.1, 15.6, 14.3.

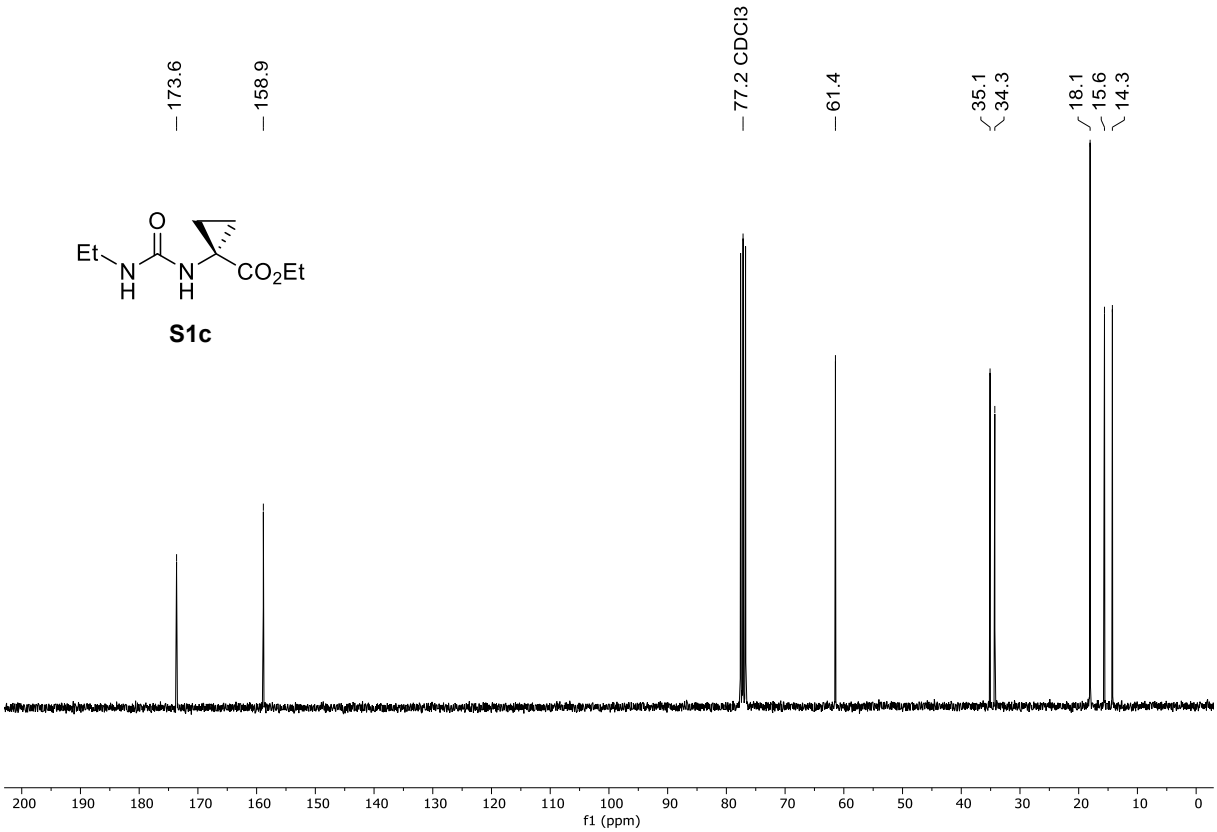
MS (EI): *m/z* (%) = 154 (14), 129 (34), 100 (100), 83 (27), 55 (22).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_9H_{16}N_2O_3Na]^+$: 223.1053; found: 223.1067 (Error ppm: 1.8).

IR (ATR, $\tilde{\nu}$) = 3320 (m), 2969 (w), 2938 (w), 2871 (vw), 1731 (vs), 1635 (vs), 1570 (vs), 1478 (w), 1450 (m), 1413 (w), 1368 (m), 1340 (w), 1289 (s), 1262 (vs), 1179 (vs), 1140 (vs), 1113 (s), 1081 (w), 1032 (s), 1020 (vs), 936 (m), 895 (w), 873 (w), 828 (w), 799 (w), 777 (w), 752 (m), 712 (w), 652 (vs), 604 (vs), 516 (s), 453 (m), 412 (m) cm^{-1}.

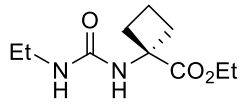


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl 1-(3-ethylureido)cyclobutane-1-carboxylate (S1d)



S1d

Following the general procedure GP1, using ethyl 1-aminocyclobutane-1-carboxylate hydrochloride (**H-Acbc-OEt·HCl**) (1.34 mmol), afforded the title compound **S1d** (271 mg, 1.26 mmol, 94% yield) as a colorless crystalline solid.

R_f = 0.21 (*n*-pentane:ethyl acetate = 1:2, Vanillin stain)

m.p. = 73-75 °C

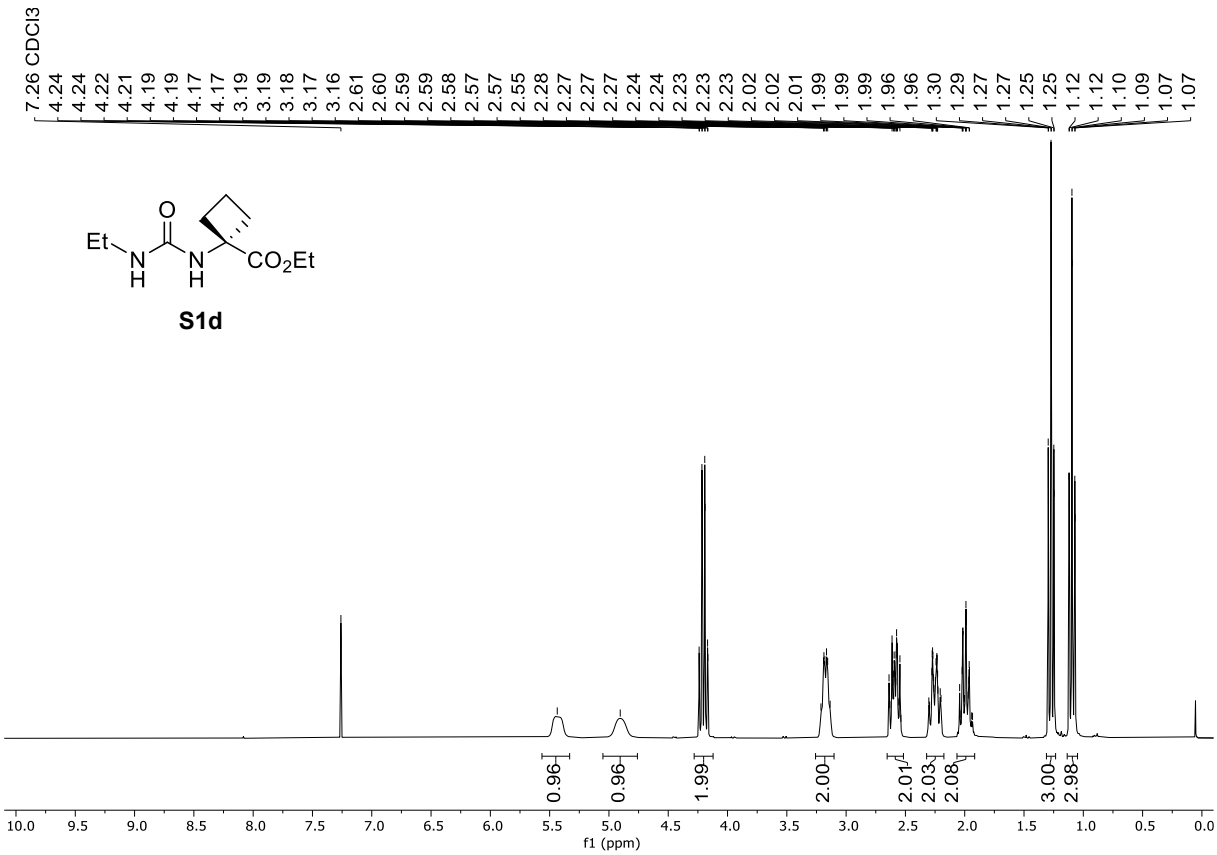
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.44 (s, 1H), 4.90 (s, 1H), 4.20 (qd, *J* = 7.1, 0.8 Hz, 2H), 3.26 – 3.10 (m, 2H), 2.65 – 2.52 (m, 2H), 2.32 – 2.18 (m, 2H), 2.07 – 1.92 (m, 2H), 1.27 (td, *J* = 7.1, 0.7 Hz, 3H), 1.09 (td, *J* = 7.2, 0.7 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.9, 157.7, 61., 58.45, 35.17, 31.75, 15.56, 15.4, 14.3.

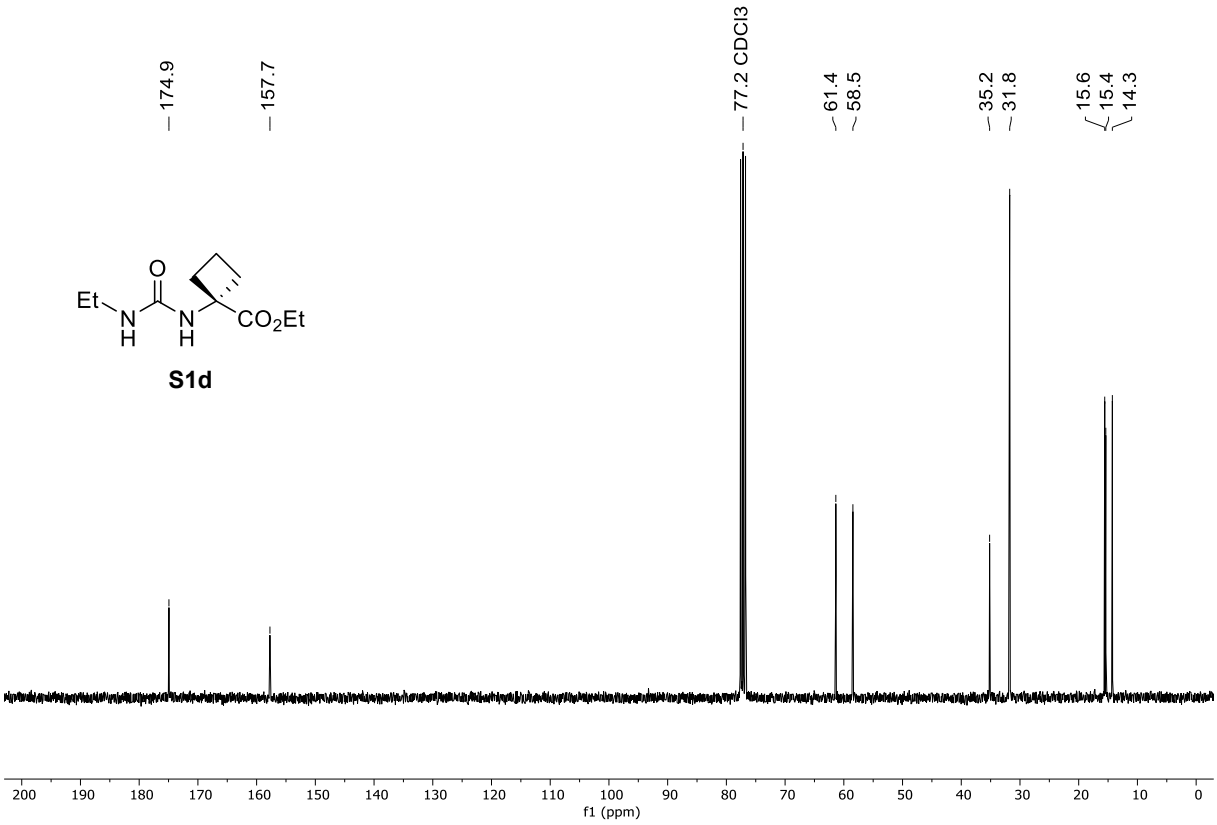
MS (EI): *m/z* (%) = 214 (5, $[M]^+$), 186 (90), 140 (76), 115 (100), 71 (90).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{10}H_{18}N_2O_3Na]^+$: 237.1210; found: 237.1208 (Error appm: -0.8).

IR (ATR, ṽ) = 3342 (m), 2989 (w), 2965 (m), 2950 (w), 2895 (vw), 2873 (w), 1723 (vs), 1684 (w), 1674 (w), 1625 (vs), 1562 (vs), 1507 (m), 1495 (m), 1460 (s), 1452 (m), 1427 (w), 1387 (w), 1374 (m), 1366 (m), 1307 (vs), 1254 (vs), 1217 (vs), 1195 (vs), 1172 (m), 1148 (s), 1115 (vs), 1095 (vs), 1077 (vs), 1062 (s), 1042 (s), 1011 (s), 991 (m), 962 (w), 920 (w), 899 (w), 873 (w), 858 (w), 824 (w), 805 (w), 785 (w), 775 (w), 742 (w), 669 (s), 616 (vs), 520 (w), 514 (w), 459 (m), 442 (s) cm^{-1}.

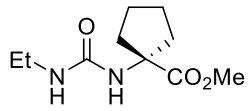


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of methyl 1-(3-ethylureido)cyclopentane-1-carboxylate (S1e)



S1e

Following the general procedure GP1, using methyl 1-aminocyclopentane-1-carboxylate hydrochloride (**H-Acp-OEt·HCl**) (6.00 mmol), afforded the title compound **S1e** (1.28 g, 5.98 mmol, 100% yield) as a colorless crystalline solid.

R_f = 0.11 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 97-99 °C

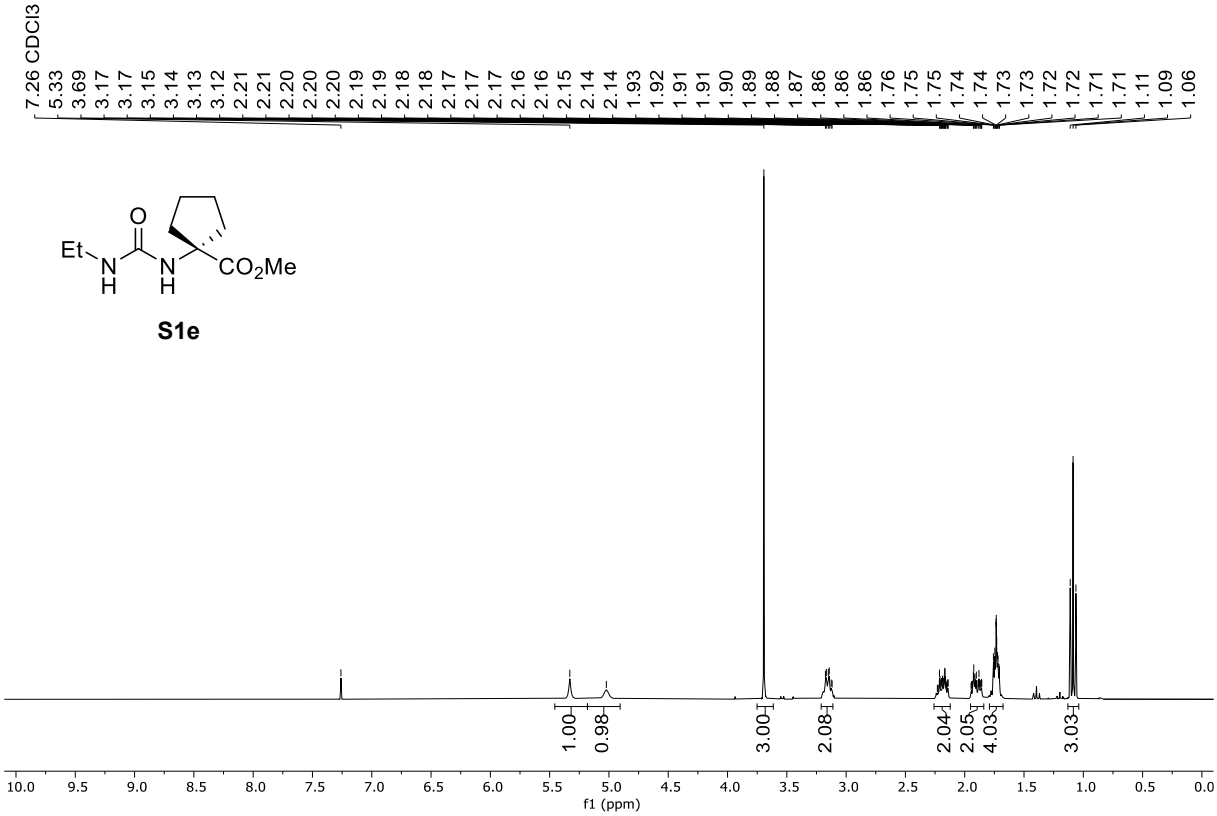
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.33 (bs, 1H), 5.02 (bs, 1H), 3.69 (s, 3H), 3.21 – 3.11 (m, 2H), 2.26 – 2.12 (m, 2H), 1.95 – 1.84 (m, 2H), 1.79 – 1.68 (m, 4H), 1.09 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 176.3, 158.2, 65.9, 52.4, 37.8, 35.1, 24.6, 15.6.

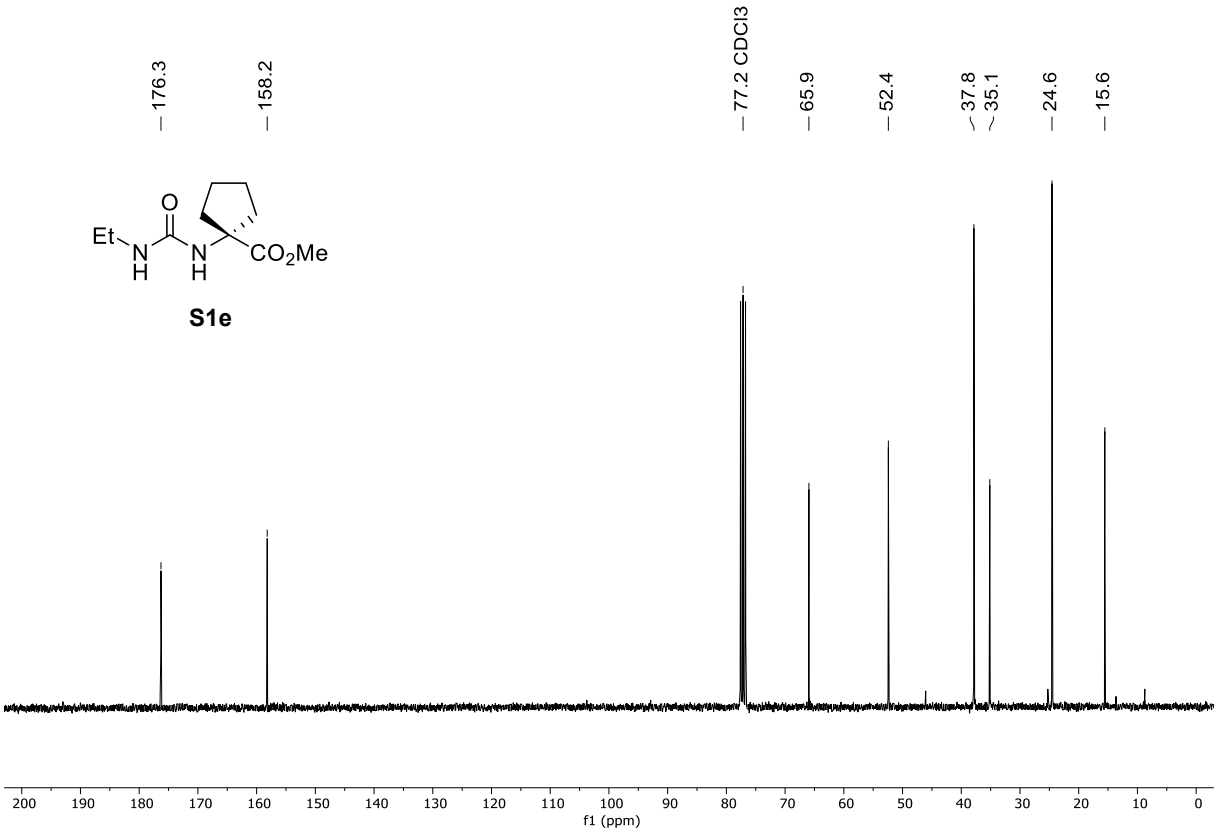
MS (EI): *m/z* (%) = 214 (5, $[M]^+$), 182 (26), 155 (100), 141 (31), 114 (35), 85 (94), 67 (96), 54 (98).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{10}H_{18}N_2O_3Na]^+$: 237.1210; found: 237.1209 (Error ppm: 0.4).

IR (ATR, neat, $\tilde{\nu}$) = 3358 (w), 3309 (w), 2957 (w), 2873 (w), 1729 (vs), 1625 (vs), 1560 (vs), 1513 (s), 1446 (m), 1433 (s), 1378 (w), 1321 (m), 1295 (s), 1262 (vs), 1232 (vs), 1189 (s), 1164 (vs), 1115 (m), 1073 (s), 1050 (m), 1020 (m), 956 (w), 913 (w), 775 (w), 750 (w), 708 (w), 622 (vs), 461 (w) cm^{-1}.

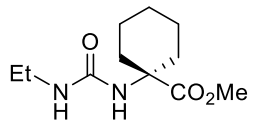


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of methyl 1-(3-ethylureido)cyclohexane-1-carboxylate (S1f)



S1f

Following the general procedure GP1, using methyl 1-aminocyclohexane-1-carboxylate hydrochloride (**H-Ach-OMe·HCl**) (5.99 mmol), washing with brine and drying over anhydrous sodium sulfate afforded the title compound **S1f** (1.34 g, 5.85 mmol, 98% yield) as a colorless crystalline solid.

$\mathbf{R}_f$ = 0.12 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 112-115 °C

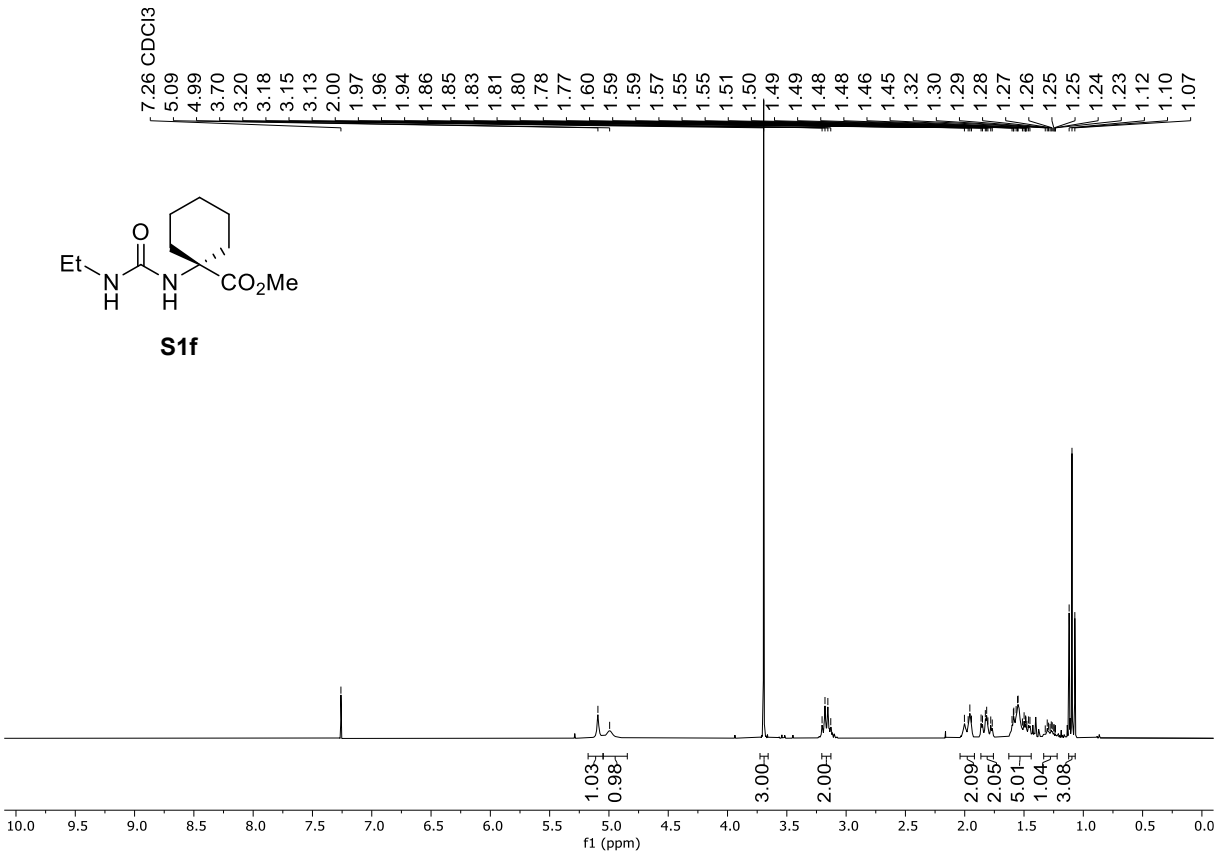
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.09 (s, 1H), 4.99 (s, 1H), 3.70 (s, 3H), 3.17 (q, *J* = 7.3 Hz, 2H), 2.04 – 1.92 (m, 2H), 1.86 – 1.76 (m, 2H), 1.63 – 1.44 (m, 5H), 1.33 – 1.22 (m, 1H), 1.10 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 176.3, 157.7, 58.6, 52.3, 35.2, 33.3, 25.5, 21.6, 15.5.

MS (EI): *m/z* (%) = 228 (2, $[M]^+$), 196 (35), 169 (100), 141 (52), 114 (34), 99 (88), 89 (87), 81 (91), 69 (43), 54 (82).

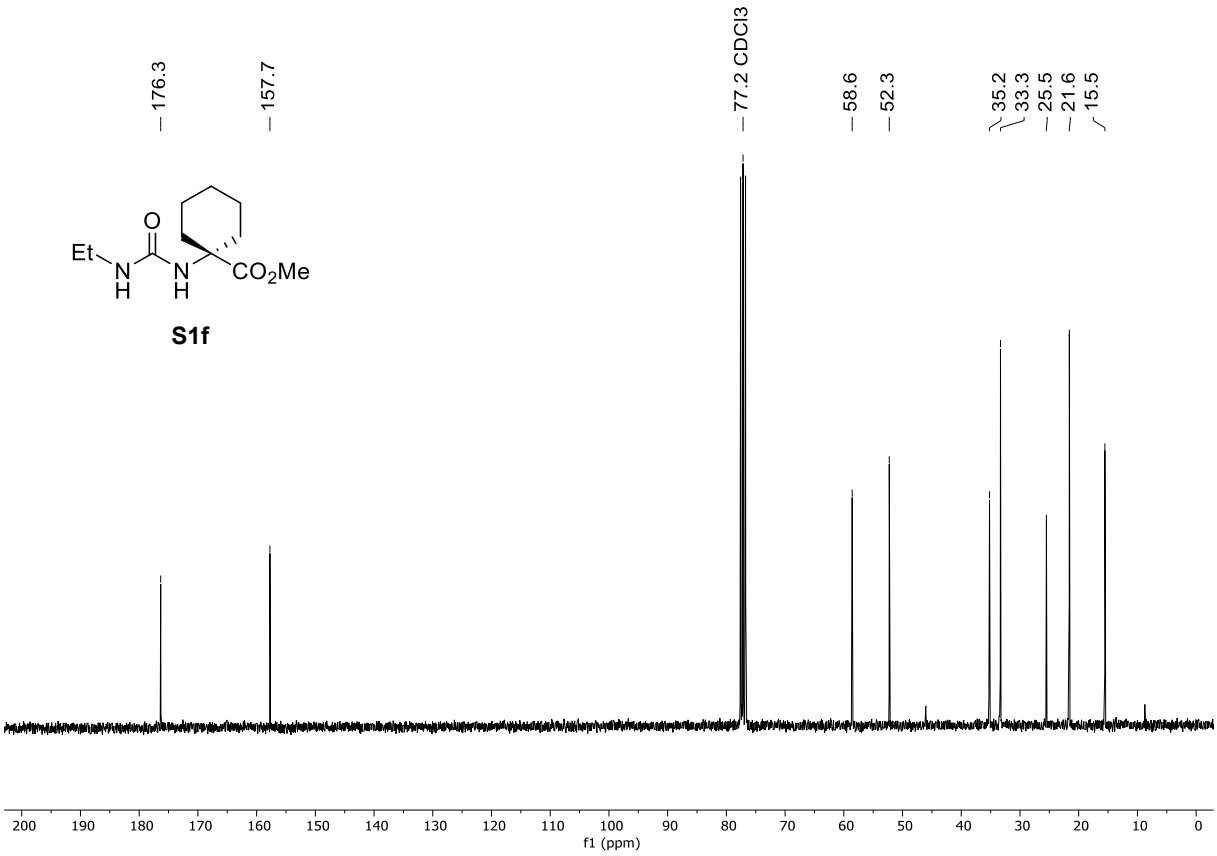
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{11}H_{20}N_2O_3Na]^+$: 251.1366; found: 251.1380 (Error PPM: 1.6).

IR (ATR, neat, $\tilde{\nu}$) = 3362 (w), 3334 (w), 2963 (w), 2932 (m), 2857 (w), 1735 (vs), 1674 (w), 1625 (vs), 1564 (vs), 1509 (m), 1464 (w), 1444 (m), 1378 (w), 1358 (w), 1321 (w), 1287 (s), 1262 (s), 1227 (vs), 1191 (m), 1172 (s), 1142 (vs), 1068 (m), 1044 (w), 1022 (w), 979 (m), 932 (w), 903 (w), 858 (vw), 838 (vw), 809 (w), 773 (w), 738 (w), 693 (m), 610 (m), 546 (w), 516 (w), 477 (w), 432 (w), 408 (w) cm^{-1}.



S1f

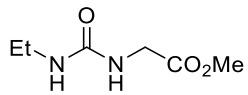
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



S1f

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of methyl (ethylcarbamoyl)glycinate (S1g)



S1g

Following the general procedure GP1, using methyl glycinate hydrochloride (**H-Gly-OMe·HCl**) (10.0 mmol), afforded the title compound **S1g** (1500 mg, 9.37 mmol, 94% yield) as a colorless crystalline solid.

R_f = 0.10 (*n*-pentane:ethyl acetate = 1:3, Vanillin stain)

m.p. = 59-62 °C

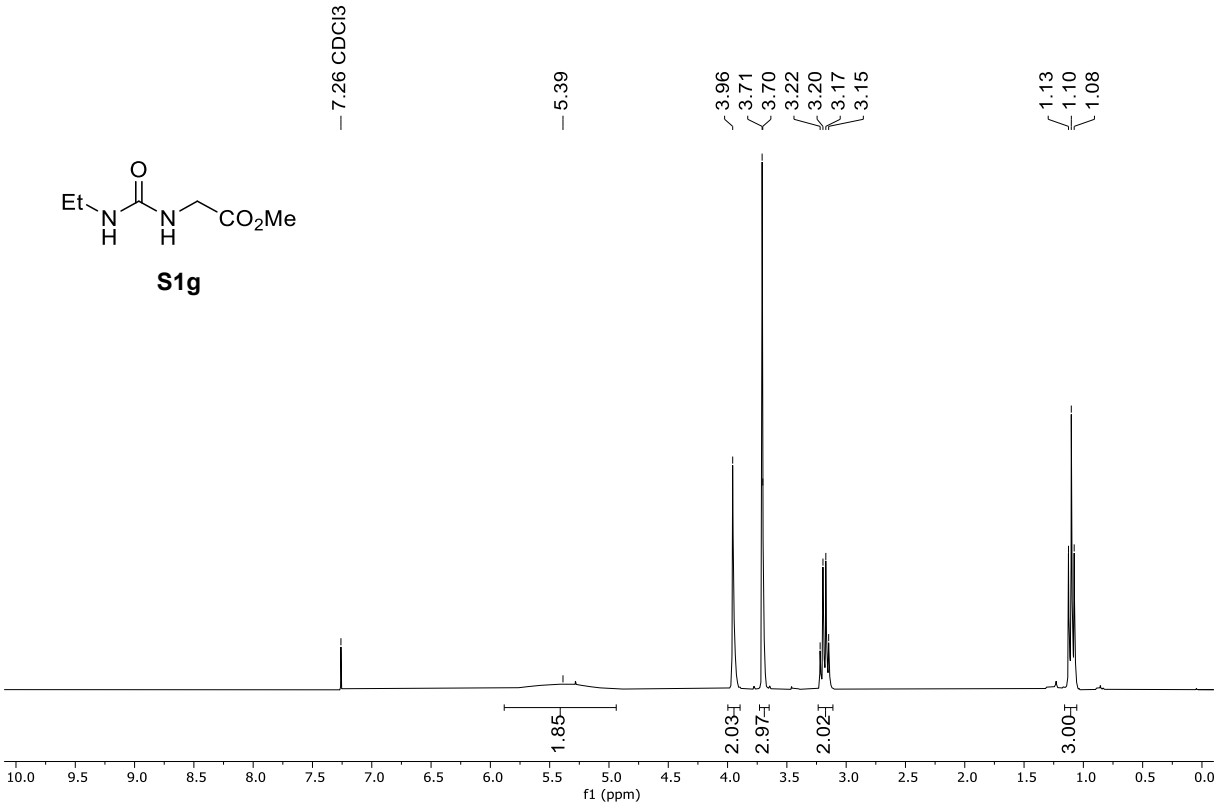
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.39 (bs, 2H), 3.96 (s, 1H), 3.71 (s, 1H), 3.18 (q, *J* = 7.2 Hz, 1H), 1.10 (t, *J* = 7.2 Hz, 1H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 172.1, 158.5, 52.2, 42.1, 35.3, 15.4.

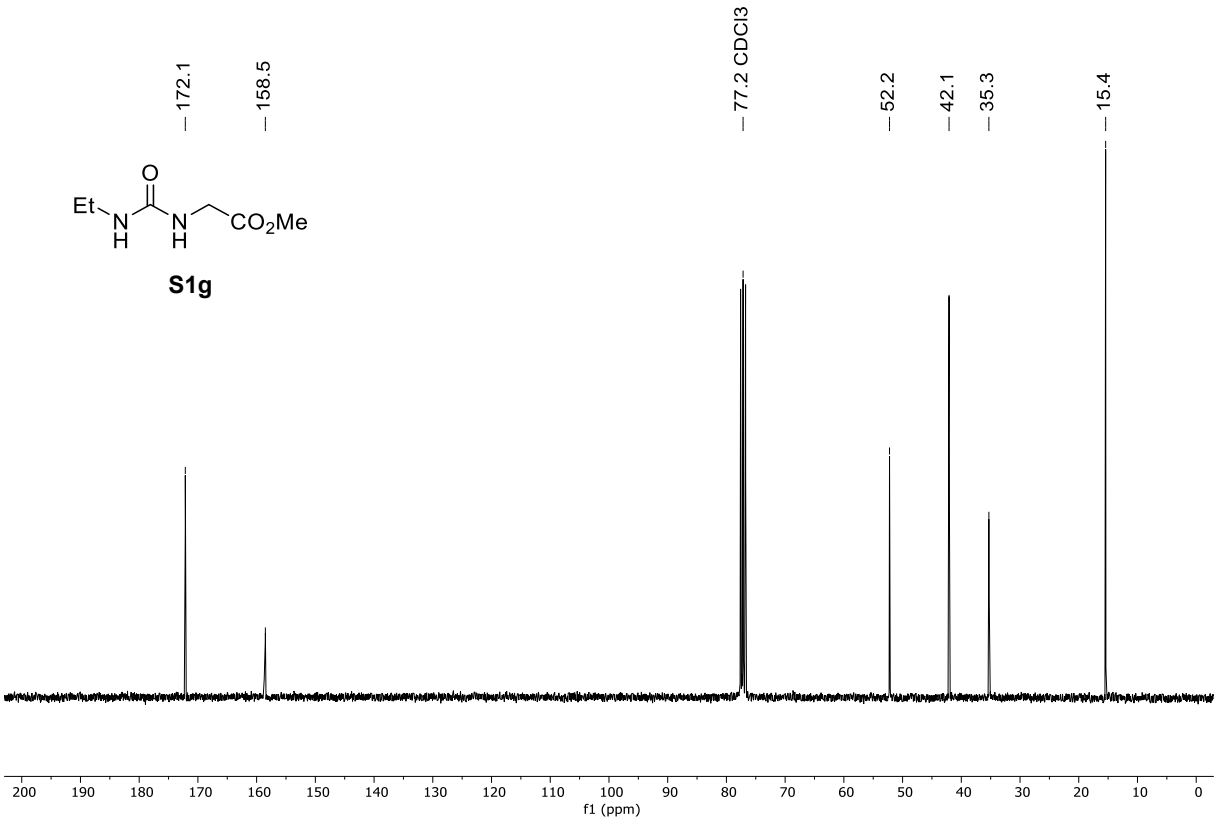
MS (EI): *m/z* (%) = 160 (38, $[M]^+$), 128 (100), 90 (47), 85 (76), 72 (56), 56 (60).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_6H_{12}N_2O_3Na]^+$: 183.0740; found: 183.0743 (Error PPM: 1.6).

IR (ATR, neat, $\tilde{\nu}$) = 3346 (m), 3317 (w), 2971 (w), 2950 (w), 2938 (w), 2877 (w), 1745 (s), 1690 (w), 1586 (vs), 1580 (vs), 1535 (s), 1484 (m), 1464 (w), 1454 (w), 1438 (s), 1409 (s), 1374 (m), 1354 (w), 1285 (m), 1264 (m), 1201 (vs), 1177 (vs), 1144 (vs), 1134 (vs), 1075 (s), 1040 (s), 993 (vs), 944 (m), 916 (w), 889 (w), 775 (w), 699 (w), 620 (s), 612 (vs), 569 (vs), 555 (vs), 408 (w) cm^{-1}.

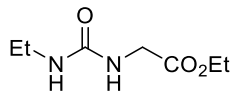


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl (ethylcarbamoyl)glycinate (S1h)



S1h

Following the general procedure GP1, using ethyl glycinate hydrochloride (**H-Gly-OEt·HCl**) (4.99 mmol), afforded the title compound **S1h** (747 mg, 4.29 mmol, 86% yield) as a colorless crystalline solid.

R_f = 0.37 (ethyl acetate, Vanillin stain)

m.p. = 92-94 °C

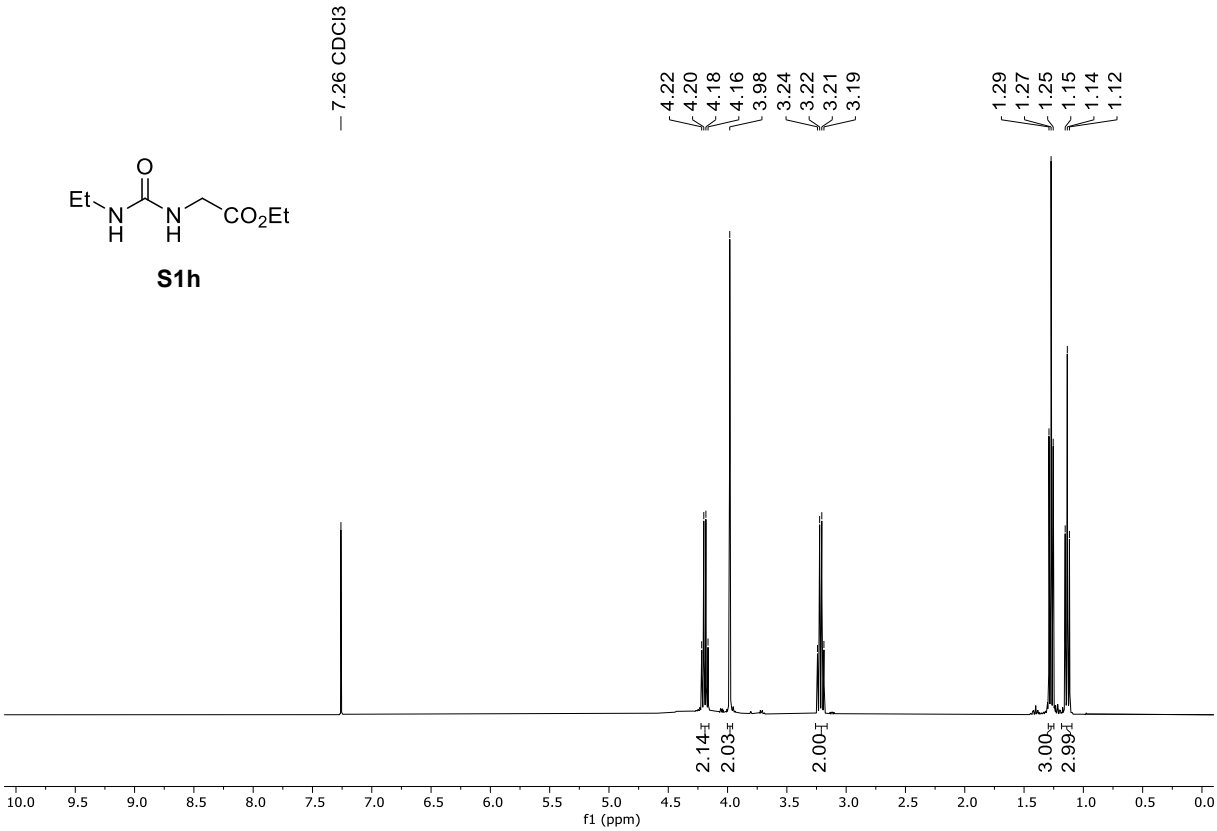
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.19 (q, *J* = 7.2 Hz, 2H), 3.98 (s, 2H), 3.21 (q, *J* = 7.2 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.14 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 171.5, 158.2, 61.5, 42.3, 35.6, 15.4, 14.3.

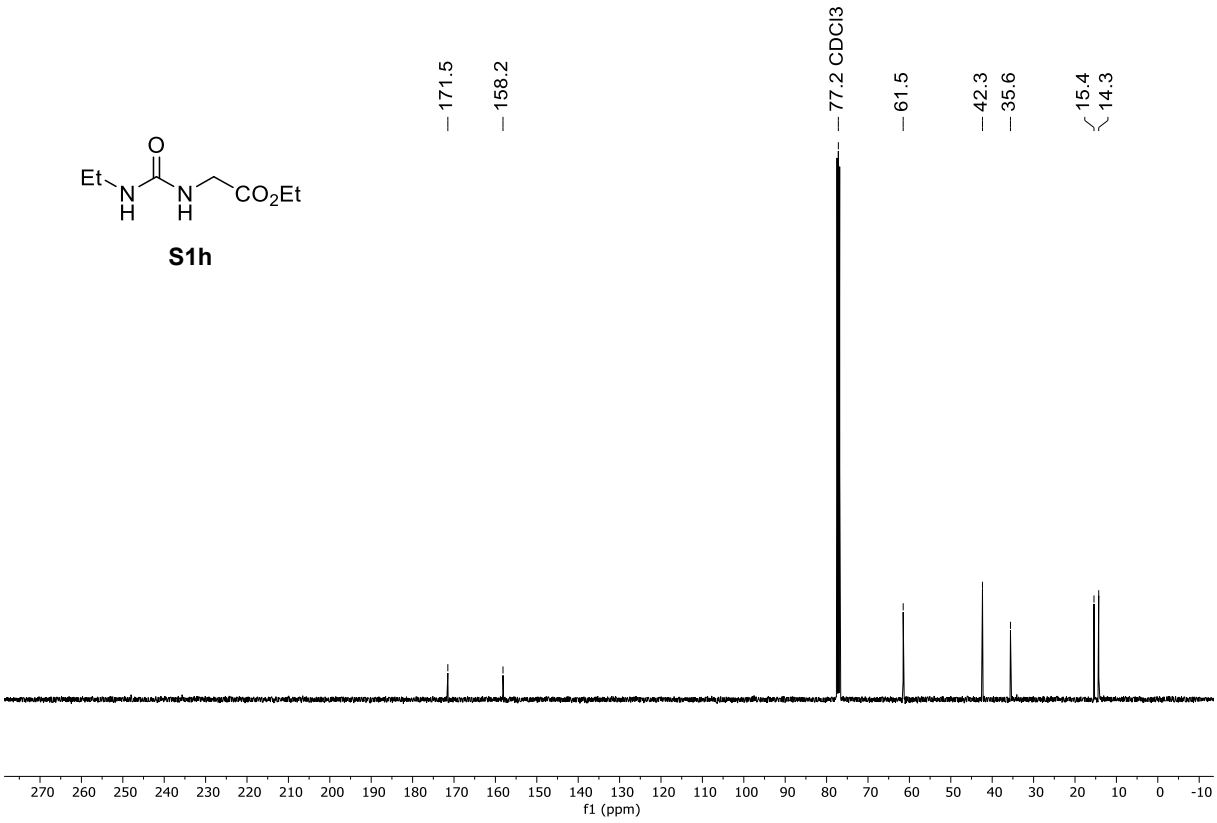
MS (EI): *m/z* (%) = 174 (45, $[M]^+$), 128 (41), 104 (80), 101 (100), 73 (60), 56 (28).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_7H_{14}N_2O_3Na]^+$: 197.0897; found: 197.0902 (Error PPM: 2.5).

IR (ATR, neat, $\tilde{\nu}$) = 3336 (m), 2979 (w), 2940 (w), 1747 (vs), 1631 (vs), 1576 (vs), 1533 (m), 1478 (w), 1454 (m), 1409 (m), 1393 (w), 1374 (s), 1354 (w), 1307 (w), 1283 (m), 1262 (w), 1183 (vs), 1113 (m), 1093 (s), 1068 (m), 1036 (w), 1020 (vs), 942 (w), 920 (w), 903 (w), 869 (w), 779 (w), 724 (w), 693 (w), 632 (s), 567 (vs), 557 (s), 442 (m) cm^{-1}.

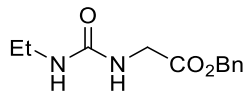


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of benzyl (ethylcarbamoyl)glycinate (S1i) (TT1069)



S1i

Following the general procedure GP1, using benzyl glycinate hydrochloride (**H-Gly-OBn·HCl**) (5.10 mmol), afforded the title compound **S1i** (1176 mg, 4.98 mmol, 98% yield) as a colorless crystalline solid.

R_f = 0.25 (*n*-pentane:ethyl acetate = 1:4, Vanillin stain)

m.p. = 78-80 °C

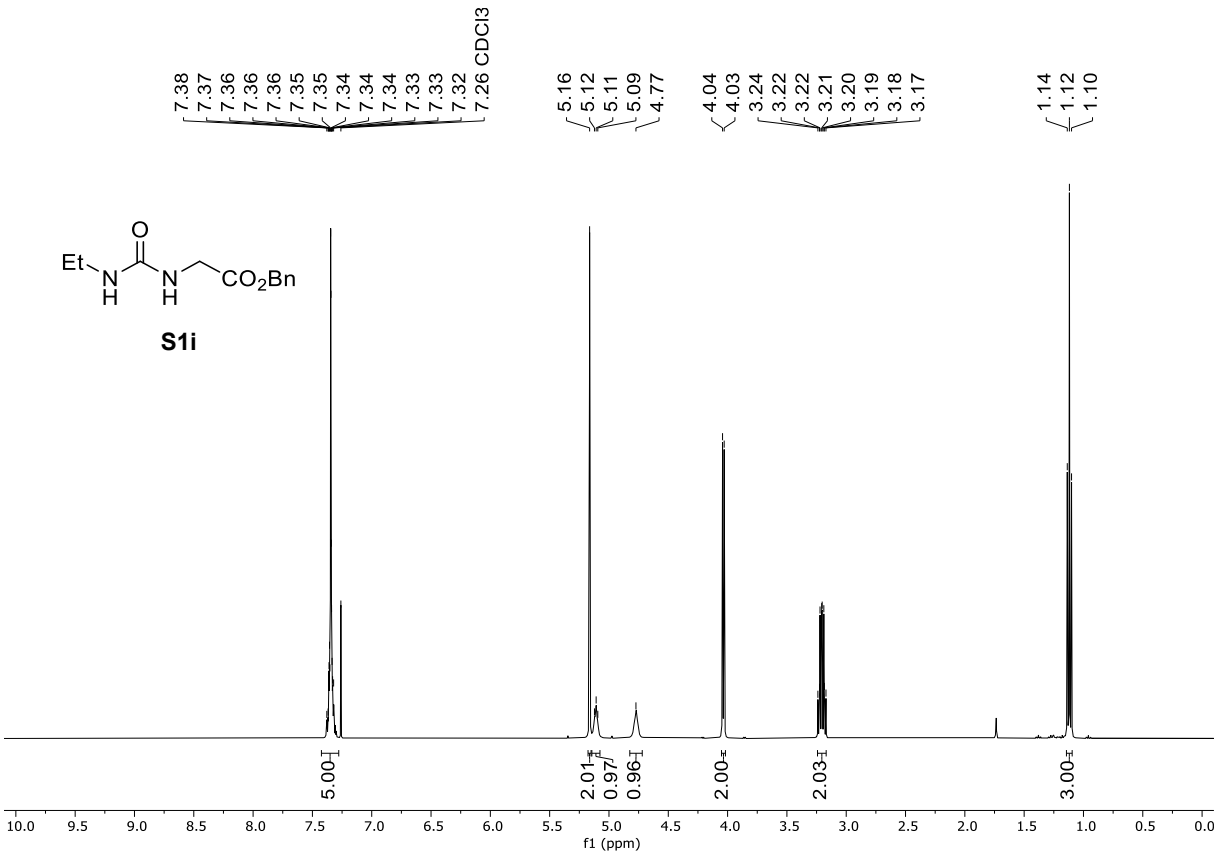
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.42 – 7.28 (m, 5H), 5.16 (s, 2H), 5.11 (t, *J* = 5.6 Hz, 1H), 4.77 (bs, 1H), 4.04 (d, *J* = 5.5 Hz, 2H), 3.20 (qd, *J* = 7.2, 5.6 Hz, 2H), 1.12 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 171.5, 158.4, 135.4, 128.7, 128.5, 128.3, 67.0, 42.3, 35.4, 15.4.

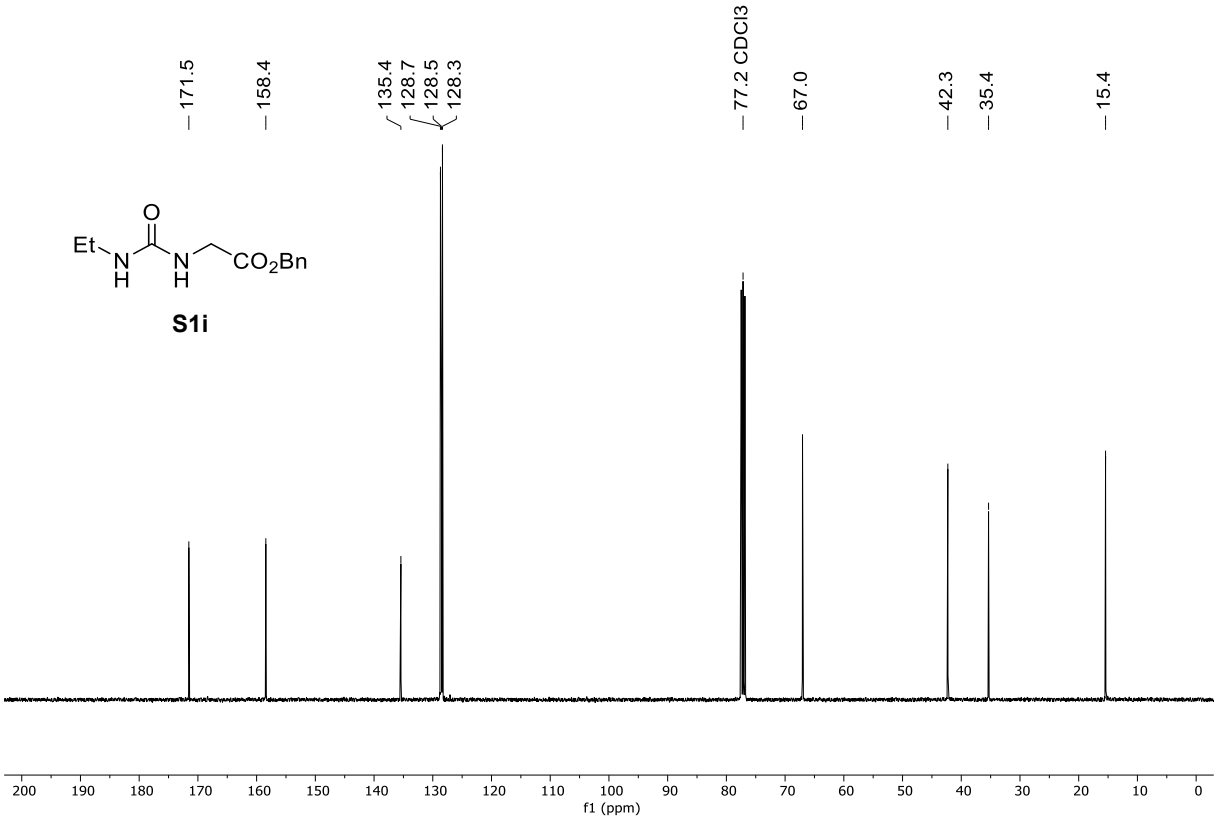
MS (EI): *m/z* (%) = 236 (11, $[M]^+$), 165 (100), 128 (32), 101 (70), 79 (38), 65 (53), 56 (17).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{12}H_{16}N_2O_3Na]^+$: 259.1053; found: 259.1055 (Error PPM: 0.8).

IR (ATR, neat, $\tilde{\nu}$) = 3334 (w), 2981 (w), 2932 (w), 1756 (s), 1631 (vs), 1578 (vs), 1527 (w), 1497 (w), 1450 (m), 1409 (m), 1378 (w), 1360 (m), 1307 (w), 1278 (m), 1183 (vs), 1172 (vs), 1158 (vs), 1093 (m), 1068 (w), 1038 (w), 1026 (w), 1001 (w), 989 (m), 944 (w), 934 (w), 899 (w), 777 (w), 726 (vs), 691 (s), 636 (s), 579 (s), 569 (vs), 553 (m), 461 (m), 422 (w) cm^{-1}.

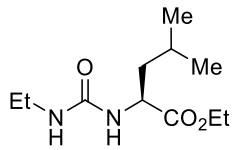


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl (ethylcarbamoyl)-*L*-leucinate ((*S*)-S1j)



(*S*)-**S1j**

Following the general procedure GP1, using ethyl *L*-leucinate hydrochloride (**H-Leu-OEt·HCl**) (6.00 mmol), afforded the title compound (*S*)-**S1j** (1.38 g, 5.99 mmol, quant. yield) as a colorless crystalline solid.

$\mathbf{R}_f$ = 0.41 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 56-58 °C

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23}}$ = -4.4 (c = 0.5 g/100 ml, DCM)

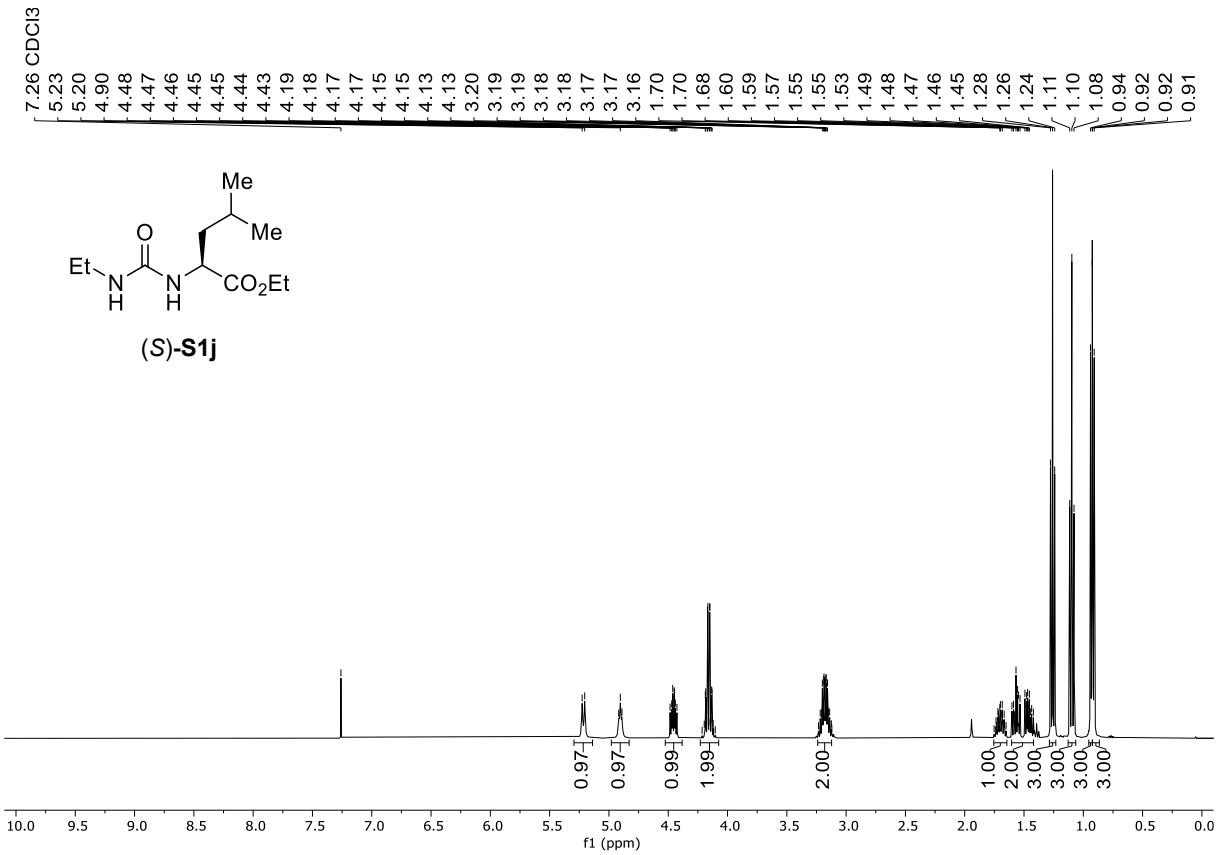
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.22 (d, *J* = 8.5 Hz, 1H), 4.90 (t, *J* = 5.5 Hz, 1H), 4.46 (td, *J* = 9.0, 5.4 Hz, 1H), 4.22 – 4.10 (m, 2H), 3.28 – 3.08 (m, 2H), 1.76 – 1.63 (m, 1H), 1.63 – 1.33 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.10 (t, *J* = 7.2 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H), 0.91 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (101 MHz, Chloroform-d [77.2 ppm], ppm) δ = 175.2, 158.0, 61.3, 51.7, 42.2, 35.3, 24.9, 23.0, 22.1, 15.5, 14.3.

MS (EI): *m/z* (%) = 230 (2, $[M]^+$), 174 (8), 157 (100), 128 (9), 102 (23), 86 (97), 74 (11), 56 (6).

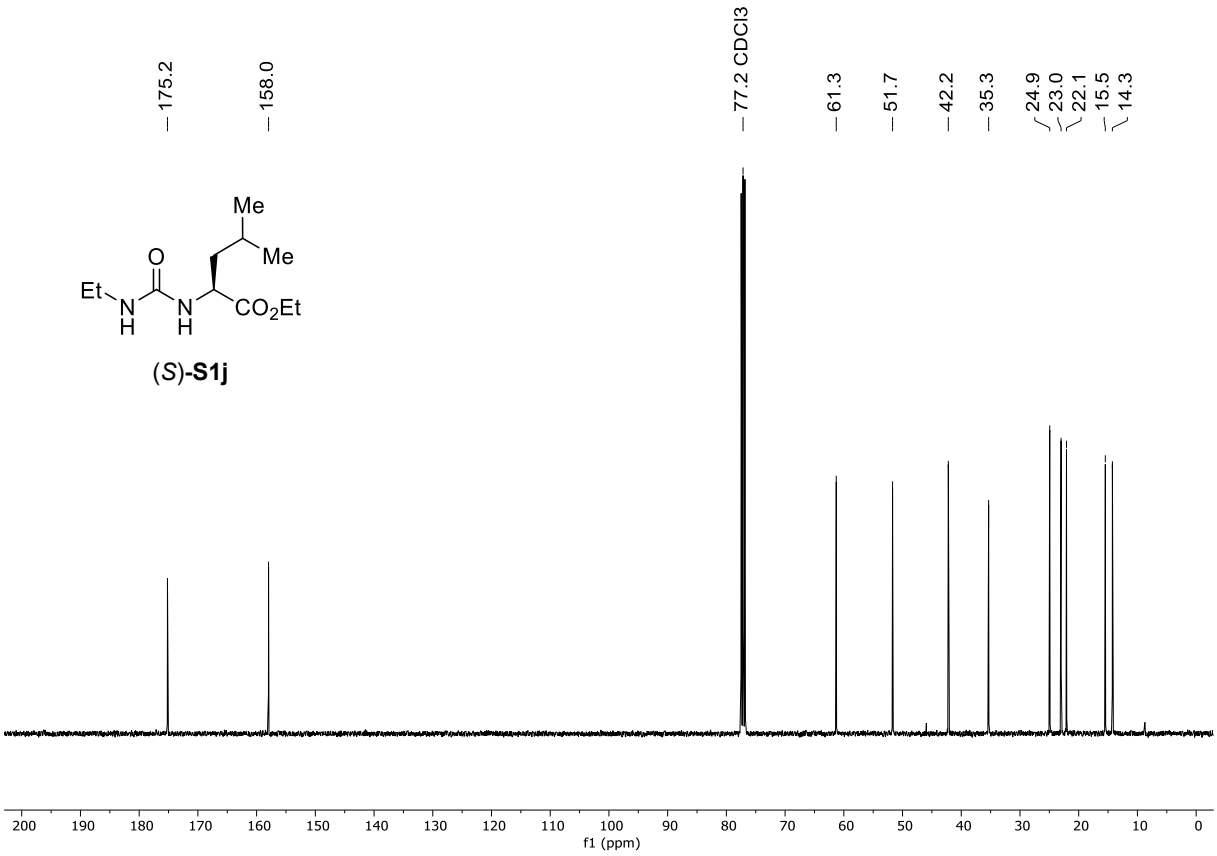
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{11}H_{22}N_2O_3Na]^+$: 253.1523; found: 253.1529 (Error PPM: 2.4).

IR (ATR, neat, $\tilde{\nu}$) = 3342 (w), 2965 (w), 2955 (w), 2936 (w), 2908 (w), 2873 (w), 1747 (vs), 1631 (vs), 1562 (vs), 1509 (m), 1474 (m), 1458 (m), 1384 (w), 1368 (w), 1338 (w), 1264 (s), 1230 (s), 1207 (m), 1168 (vs), 1138 (s), 1113 (m), 1087 (w), 1024 (s), 1011 (m), 934 (w), 871 (w), 836 (w), 775 (w), 748 (w), 699 (w), 614 (s), 569 (s), 528 (w), 516 (w), 487 (m), 451 (w), 428 (m) cm^{-1}.



(*S*)-**S1j**

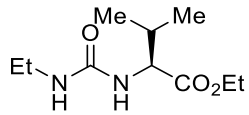
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



(*S*)-**S1j**

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl (ethylcarbamoyl)-*L*-valinate ((S**)-S1k)**



(*S*)-**S1k**

Following the general procedure GP1, using ethyl *L*-valinate hydrochloride (**H-Val-OEt·HCl**) (6.00 mmol), washing with brine and drying over anhydrous sodium sulfate afforded the title compound (S)-**S1k** (1.25 g, 5.79 mmol, 97% yield) as a transparent oil.

R_f = 0.38 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = N/A

$[\alpha]_D^{23}$ = 7.3 (c = 0.5 g/100 ml, DCM)

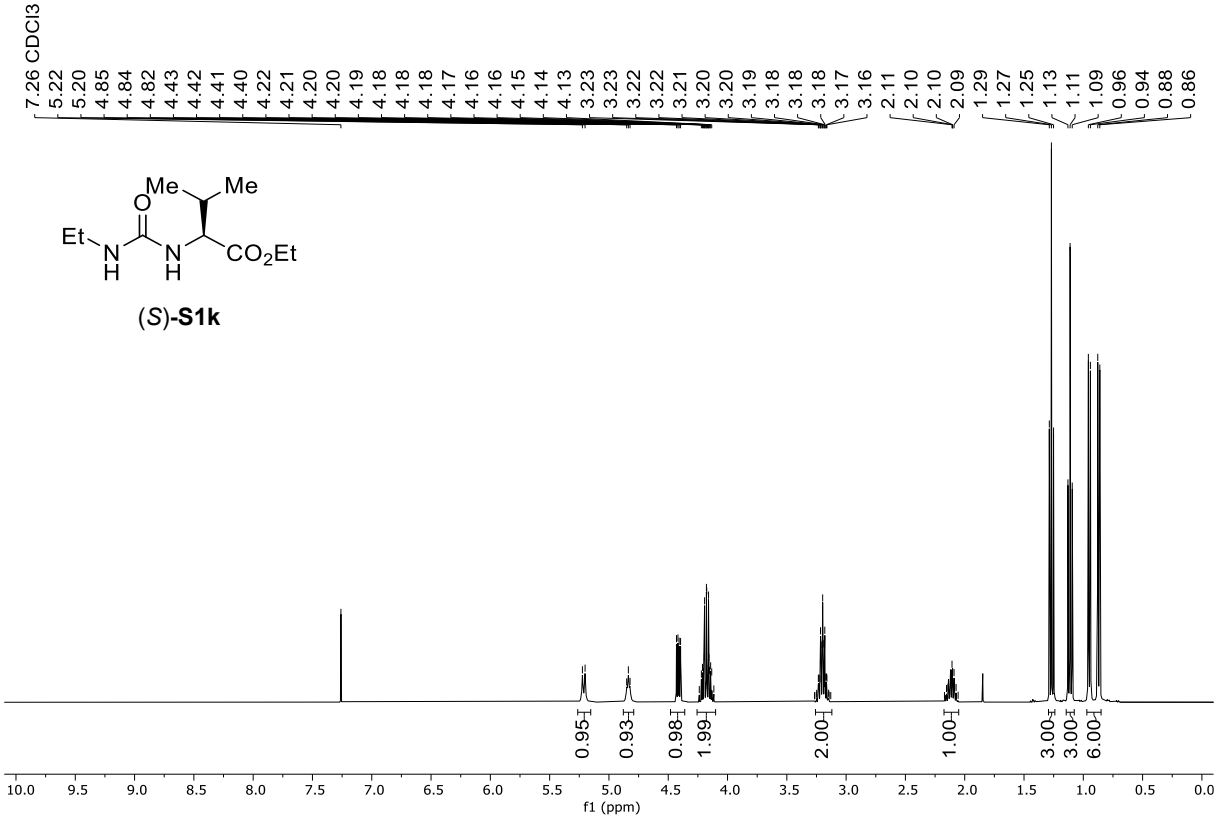
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.21 (d, *J* = 8.9 Hz, 1H), 4.84 (t, *J* = 5.5 Hz, 1H), 4.41 (dd, *J* = 8.9, 4.9 Hz, 1H), 4.27 – 4.08 (m, 2H), 3.34 – 3.00 (m, 2H), 2.11 (dq, *J* = 6.9, 4.9 Hz, 1H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.11 (t, *J* = 7.2 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 173.9, 158.1, 61.2, 58.0, 35.4, 31.5, 19.2, 17.8, 15.5, 14.3.

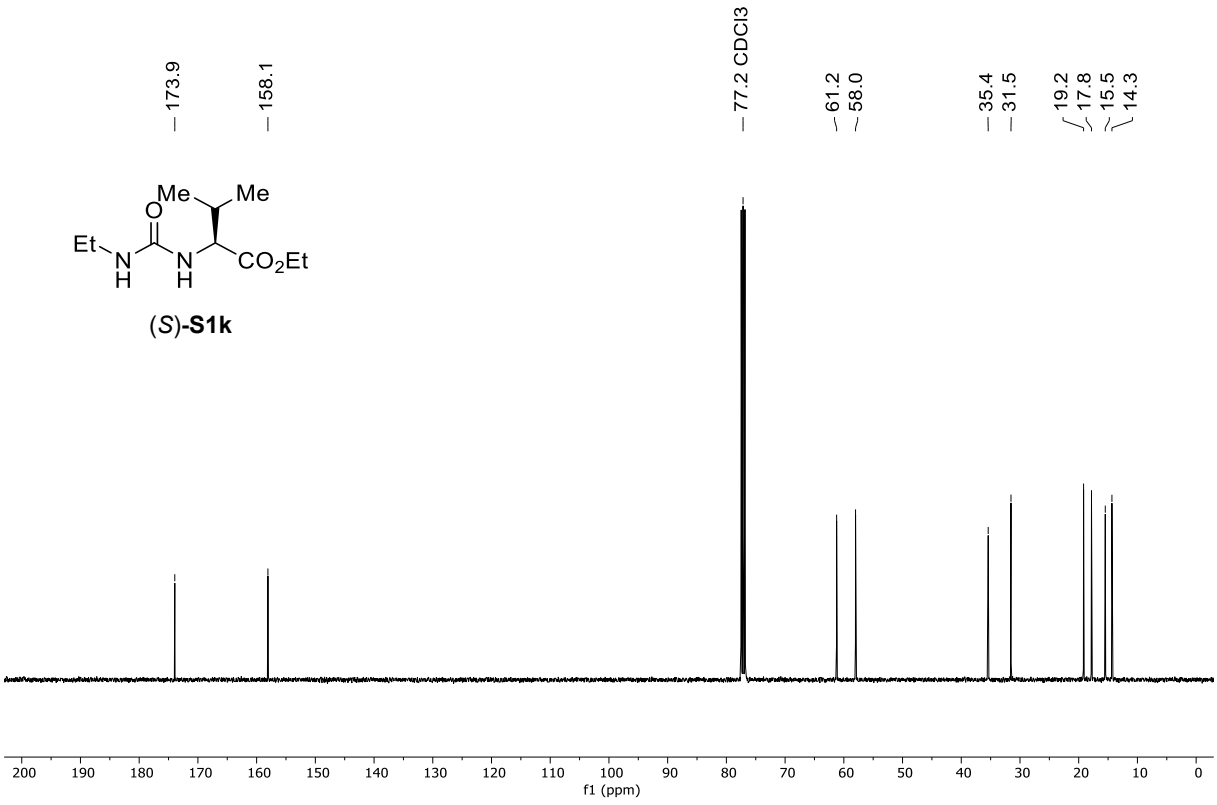
MS (EI): *m/z* (%) = 216 (2, $[M]^+$), 171 (3), 143 (100), 102 (89),72 (92), 55 (32).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{10}H_{20}N_2O_3Na]^+$: 239.1366; found: 239.1372 (Error PPM: 0.6).

IR (ATR, neat, $\tilde{\nu}$) = 3336 (w), 2967 (m), 2934 (w), 2910 (w), 2875 (w), 1737 (vs), 1631 (vs), 1560 (vs), 1466 (m), 1450 (m), 1391 (w), 1372 (m), 1346 (w), 1336 (w), 1305 (m), 1244 (s), 1187 (vs), 1134 (s), 1115 (m), 1095 (m), 1073 (w), 1046 (m), 1026 (s), 973 (w), 924 (w), 865 (w), 803 (w), 773 (w), 750 (w), 638 (m), 628 (m), 612 (m), 473 (w), 453 (w), 410 (w) cm^{-1}.

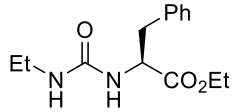


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl (ethylcarbamoyl)-L-phenylalaninate ((*S*)-S1n)



(*S*)-**S1n**

Following the general procedure GP1, using ethyl *L*-phenylalaninate hydrochloride (**H-Phe-OEt·HCl**) (6.0 mmol), afforded the title compound (*S*)-**S1n** (1.47 g, 5.56 mmol, 93% yield) as a colorless crystalline solid.

R_f = 0.38 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 74-75 °C

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23}}$ = +38.4 (c = 0.5 g/100 ml, DCM)

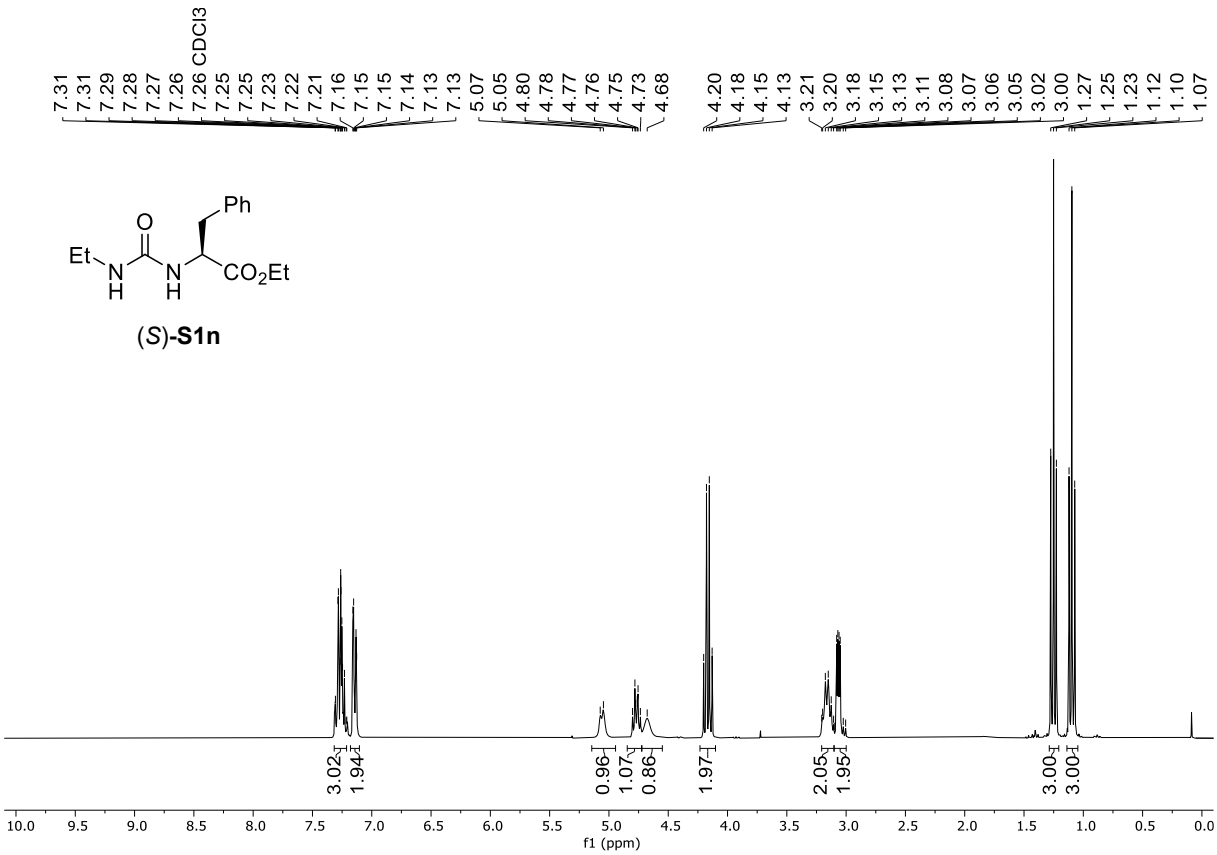
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.32 – 7.21 (m, 3H), 7.18 – 7.10 (m, 2H), 5.06 (d, *J* = 8.1 Hz, 1H), 4.77 (dt, *J* = 7.9, 5.9 Hz, 1H), 4.68 (s, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.16 (q, *J* = 7.2 Hz, 2H), 3.11 – 2.98 (m, 2H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.10 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 173.2, 157.4, 136.5, 129.5, 128.5, 127.0, 61.4, 54.1, 38.8, 35.4, 15.4, 14.2.

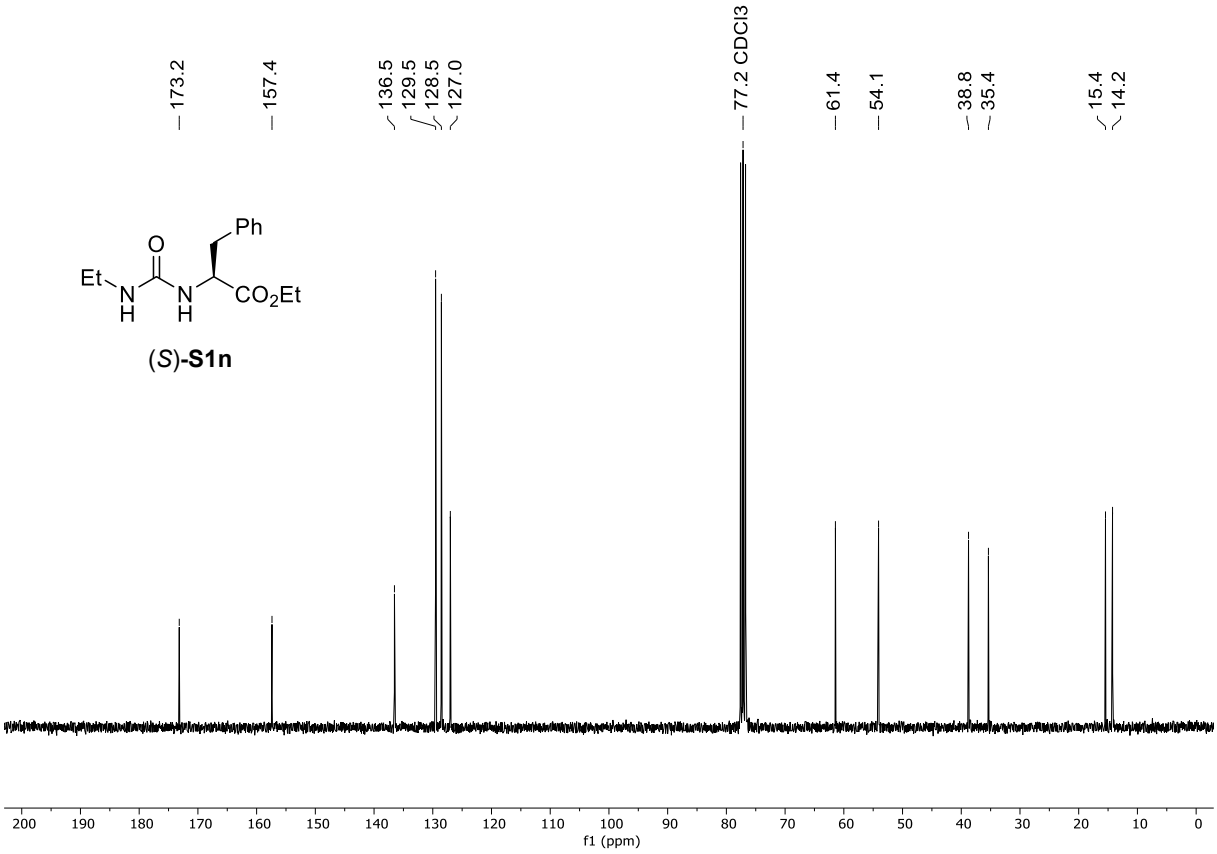
MS (EI): *m/z* (%) = 264 (13, $[M]^+$), 219 (5), 291 (25), 176 (100), 148 (20), 131 (37), 120 (96), 102 (95), 91 (75), 74 (70).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{14}H_{20}N_2O_3Na]^+$: 287.1366; found: 287.1363 (Error PPM: -1.0).

IR (ATR, $\tilde{\nu}$) = 3356 (w), 3332 (w), 3026 (w), 2971 (w), 2938 (w), 2873 (w), 1725 (vs), 1629 (vs), 1568 (vs), 1521 (s), 1495 (m), 1474 (m), 1452 (m), 1442 (s), 1395 (m), 1368 (vs), 1356 (s), 1342 (vs), 1331 (s), 1319 (s), 1262 (s), 1215 (vs), 1199 (vs), 1179 (s), 1154 (s), 1115 (s), 1091 (s), 1073 (s), 1032 (vs), 966 (w), 946 (w), 911 (w), 897 (w), 869 (m), 830 (w), 805 (w), 767 (m), 724 (w), 695 (vs), 624 (vs), 612 (vs), 602 (vs), 565 (vs), 518 (m), 485 (vs), 461 (m), 447 (m) cm^{-1}.

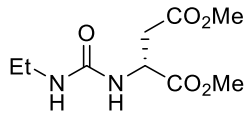


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dimethyl (ethylcarbamoyl)-*D*-aspartate ((*R*)-S1l)



(*R*)-**S1l**

Following the general procedure GP1, using dimethyl *D*-aspartate hydrochloride ((*R*)-**Asp(OMe)-OMe·HCl**) (9.52 mmol), afforded the title compound (*R*)-**S1l** (1.85 g, 7.97 mmol, 84%) as colorless crystalline solid.

R_f = 0.23 (*n*-pentane:ethyl acetate = 1:2, Vanillin stain)

m.p. = 72-74°C

$[\alpha]_D^{25}$ = -49.2 (c = 0.5 g/100 ml, DCM)

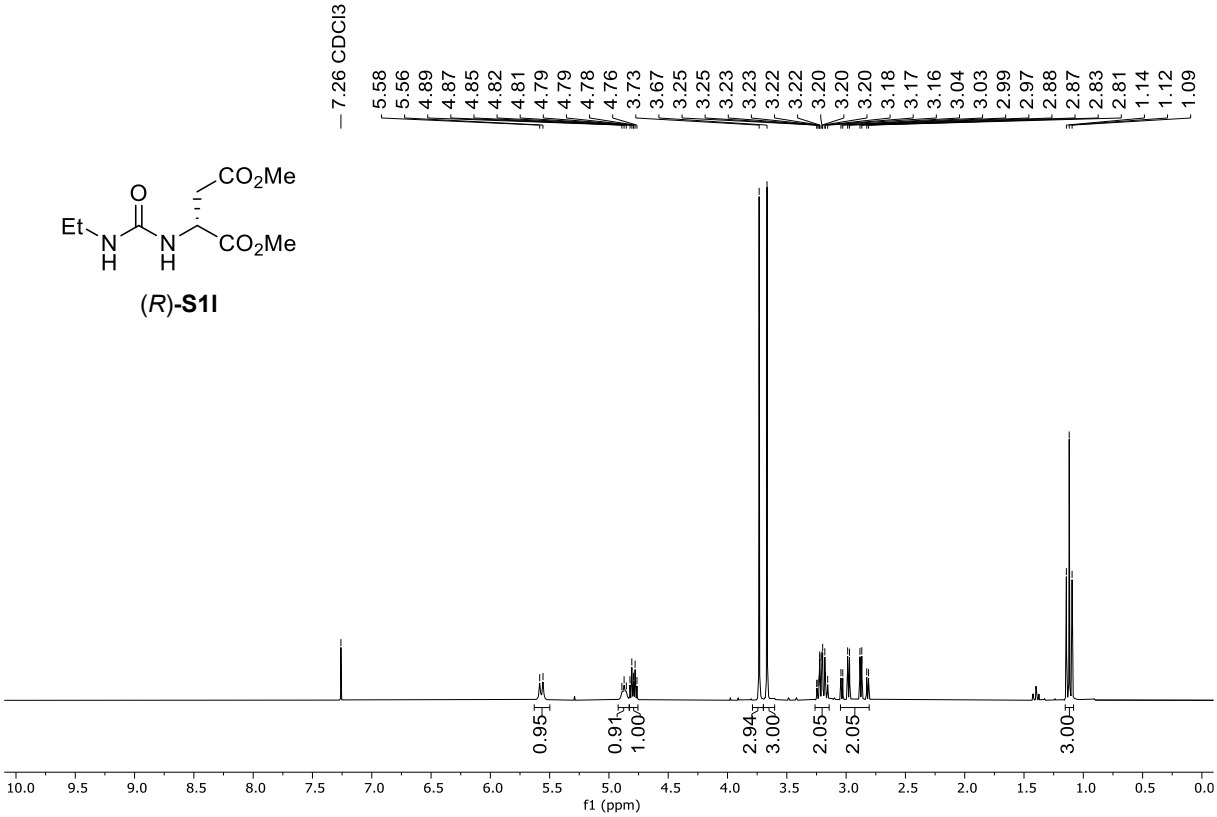
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.57 (d, *J* = 8.3 Hz, 1H), 4.87 (t, *J* = 5.6 Hz, 1H), 4.79 (dt, *J* = 8.3, 4.6 Hz, 1H), 3.73 (s, 3H), 3.67 (s, 3H), 3.28 – 3.14 (m, 2H), 3.09 – 2.76 (m, 2H), 1.12 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 172.7, 172.1, 157.5, 52.8, 52.1, 49.5, 37.1, 35.4, 15.4.

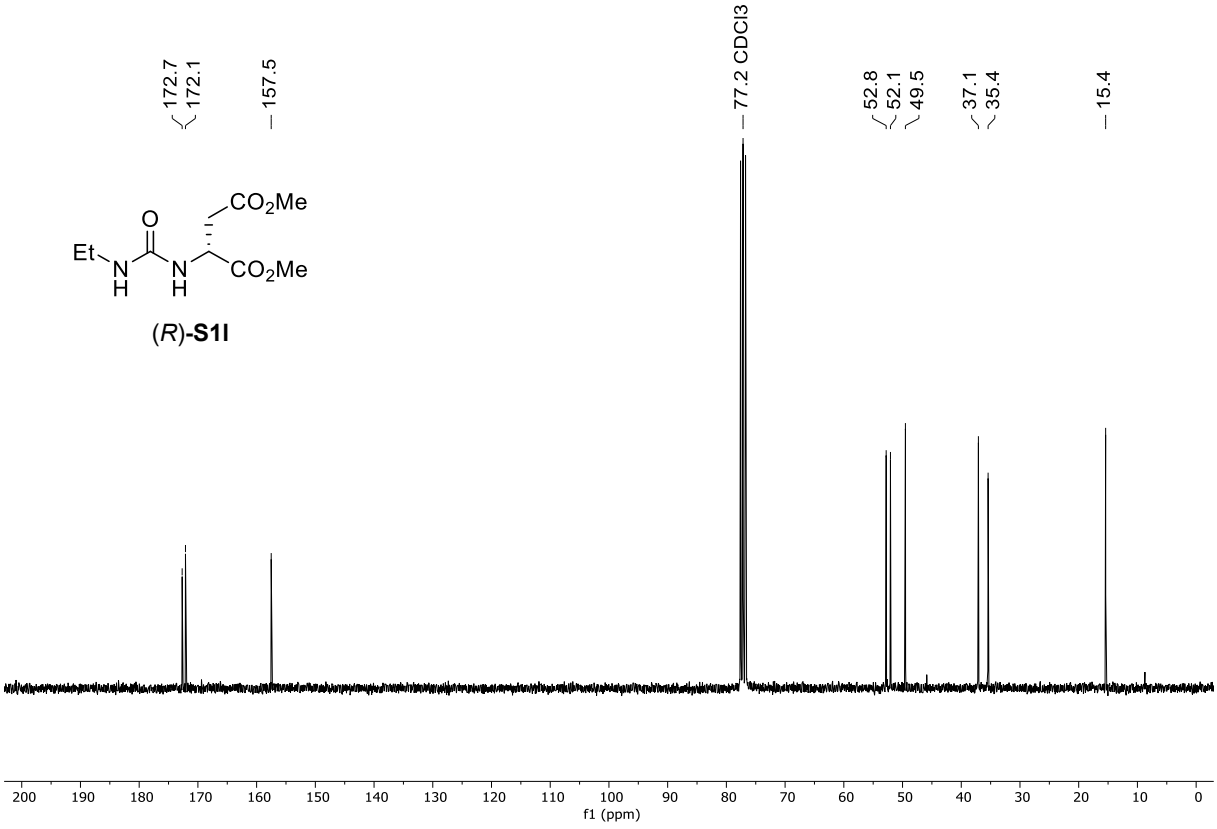
MS (EI): *m/z* (%) = 233, (6, $[M+H]^+$), 232 (8, $[M]^+$), 201 (19), 173 (100), 161 (66), 128 (35), 102 (69), 88 (83), 70 (99), 60 (97).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_9H_{16}N_2O_5Na]^+$: 255.0951; found: 255.0955 (Error PPM: 1.6).

IR (ATR, neat, $\tilde{\nu}$) = 3366 (w), 3342 (w), 2965 (w), 2932 (vw), 1743 (s), 1725 (vs), 1698 (w), 1631 (vs), 1574 (vs), 1511 (m), 1452 (w), 1433 (vs), 1380 (w), 1360 (s), 1297 (vs), 1264 (s), 1242 (vs), 1225 (vs), 1187 (vs), 1154 (vs), 1107 (s), 1087 (m), 1060 (m), 1017 (m), 987 (s), 973 (m), 954 (w), 916 (w), 889 (m), 846 (m), 824 (w), 777 (w), 699 (w), 620 (s), 589 (s), 551 (s), 512 (m), 428 (m) cm^{-1}.

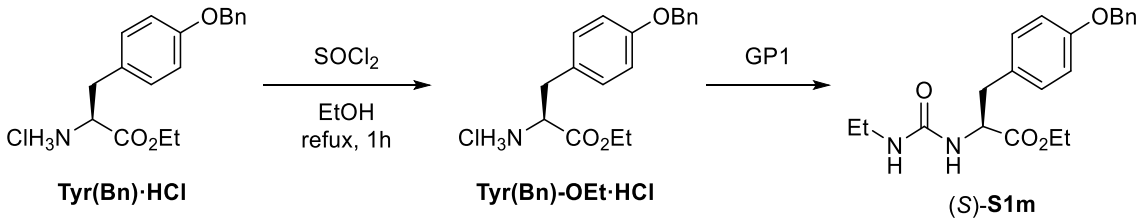


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

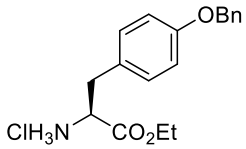
Synthesis of ethyl (*S*)-3-(4-(benzyloxy)phenyl)-2-(3-ethylureido)propanoate ((*S*)-S1m)



Tyr(Bn)·HCl **Tyr(Bn)-OEt·HCl** (*S*)-**S1m**

The title compound was synthesized in two steps starting from (*S*)-2-amino-3-(4-(benzyloxy)phenyl)propanoic acid ((*S*)-**Tyr(Bn)·HCl**).

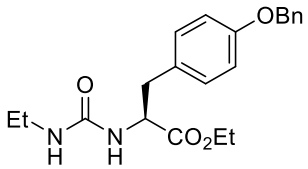
Step 1: Synthesis of ethyl (*S*)-2-amino-3-(4-(benzyloxy)phenyl)propanoate hydrochlorid salt ((*S*)-Tyr(Bn)-OEt·HCl)



(*S*)-Tyr(Bn)-OEt·HCl

(*S*)-2-amino-3-(4-(benzyloxy)phenyl)propanoic acid (2.19 g, 8.06 mmol, 1.00 equiv.) was dissolved in ethanol (40 mL) and slowly triturated with thionyl dichloride (2.75 g, 23.1 mmol, 2.87 equiv.). Subsequently, the reaction mixture was refluxed for 1h. Volatile compounds were removed under reduced pressure, affording the crude compound (*S*)-**Tyr(Bn)-OEt·HCl** which was used as is in the following step.

Step 2: Synthesis of: ethyl (*S*)-3-(4-(benzyloxy)phenyl)-2-(3-ethylureido)propanoate ((*S*)-S1m)



(*S*)-**S1m**

Following the general procedure GP1, using the crude ethyl (*S*)-2-amino-3-(4-(benzyloxy)phenyl)propanoate hydrochloride salt ((*S*)-**Tyr(Bn)-OEt·HCl**) (8.06 mmol), afforded the title compound (*S*)-**S1m** (1.52 g, 4.11 mmol, 51% yield) as a off-white crystalline solid.

R_f = 0.27 (*n*-pentane:ethyl acetate = 1:1, Vanilin stain)

m.p. = 77-84 °C

$[\alpha]_D^{24}$ = 18.4 (c = 0.5 g/100 ml, CH_2Cl_2)

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.48 – 7.28 (m, 5H), 7.10 – 7.01 (m, 2H), 6.94 – 6.83 (m, 2H), 5.01 (s, 2H), 4.91 (d, *J* = 8.0 Hz, 1H), 4.71 (dt, *J* = 8.0, 5.8 Hz, 1H), 4.53 (t, *J* = 5.5 Hz,

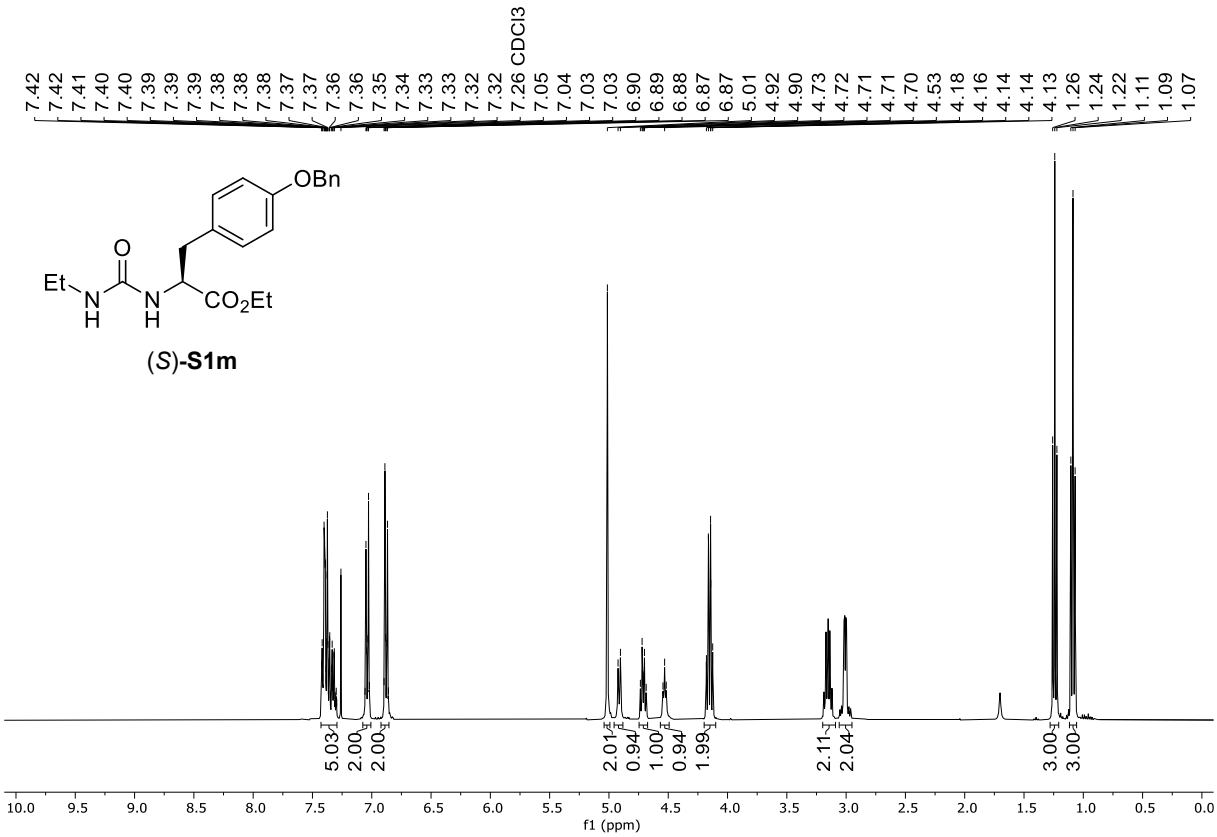
1H), 4.15 (q, *J* = 6.9 Hz, 2H), 3.15 (qd, *J* = 7.2, 5.4 Hz, 2H), 3.06 – 2.94 (m, 2H), 1.24 (t, *J* = 7.2 Hz, 3H), 1.09 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 173.1, 157.9, 157.3, 137.1, 130.6, 128.7, 128.7, 128.1, 127.6, 114.9, 70.1, 61.4, 54.2, 37.9, 35.4, 15.5, 14.3.

MS (EI): *m*/*z* (%) = 370 (2, $[M]^+$), 325 (4), 297 (7), 282 (100), 226 (41), 197 (79), 135 (33), 92 (76), 65 (46).

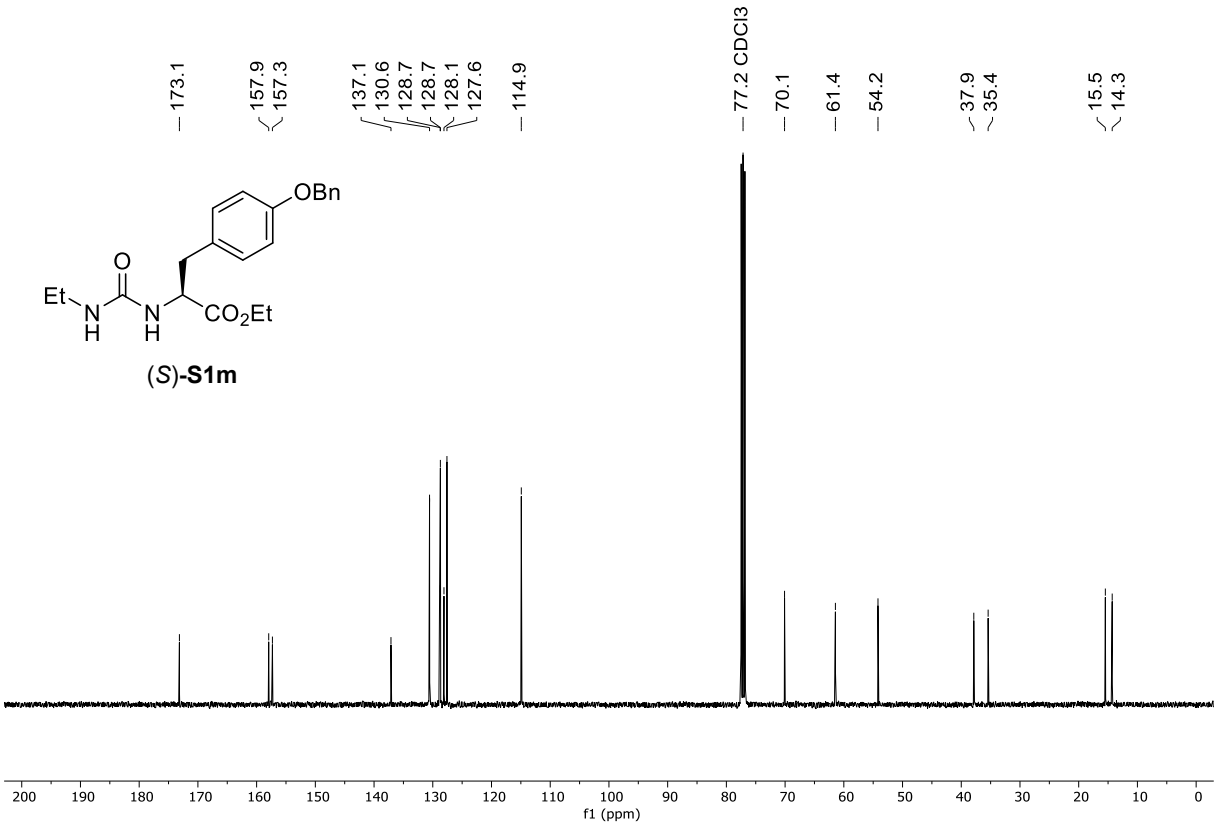
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{21}H_{26}N_2O_4Na]^+$: 393.1785; found: 393.1789 (Error PPM: 1.0).

IR (ATR, neat, $\tilde{\nu}$) = 3375 (w), 3277 (w), 2975 (w), 2938 (w), 2926 (w), 2873 (w), 1731 (vs), 1672 (m), 1621 (vs), 1568 (vs), 1511 (vs), 1497 (m), 1478 (m), 1452 (s), 1372 (w), 1346 (w), 1317 (w), 1254 (s), 1238 (vs), 1225 (vs), 1213 (vs), 1193 (vs), 1179 (vs), 1130 (m), 1091 (m), 1046 (m), 1015 (vs), 922 (w), 907 (w), 887 (w), 850 (w), 807 (m), 795 (m), 777 (w), 752 (w), 738 (s), 695 (vs), 669 (vs), 620 (s), 569 (w), 540 (vs), 514 (s), 475 (m), 436 (m), 420 (m) cm^{-1}.



(*S*)-**S1m**

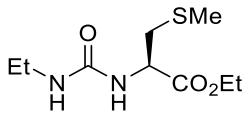
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



(*S*)-**S1m**

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl (ethylcarbamoyl)-*L*-methioninate ((*S*)-S1o)



(*S*)-**S1o**

Following the general procedure GP1, using ethyl *S*-methyl-*L*-cysteinate hydrochloride ((*S*)-**Met(Me)-OEt·HCl**), (7.00 mmol), afforded the title compound (*S*)-**S1o** (1.51 g, 6.06 mmol, 87% yield) as a colorless crystalline solid.

R_f = 0.38 (*n*-pentane:ethyl acetate = 1:1, Vanilin stain)

m.p. = 91-92°C

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{25}}$ = 14.6 (c = 0.5 g/100 ml, CH_2Cl_2)

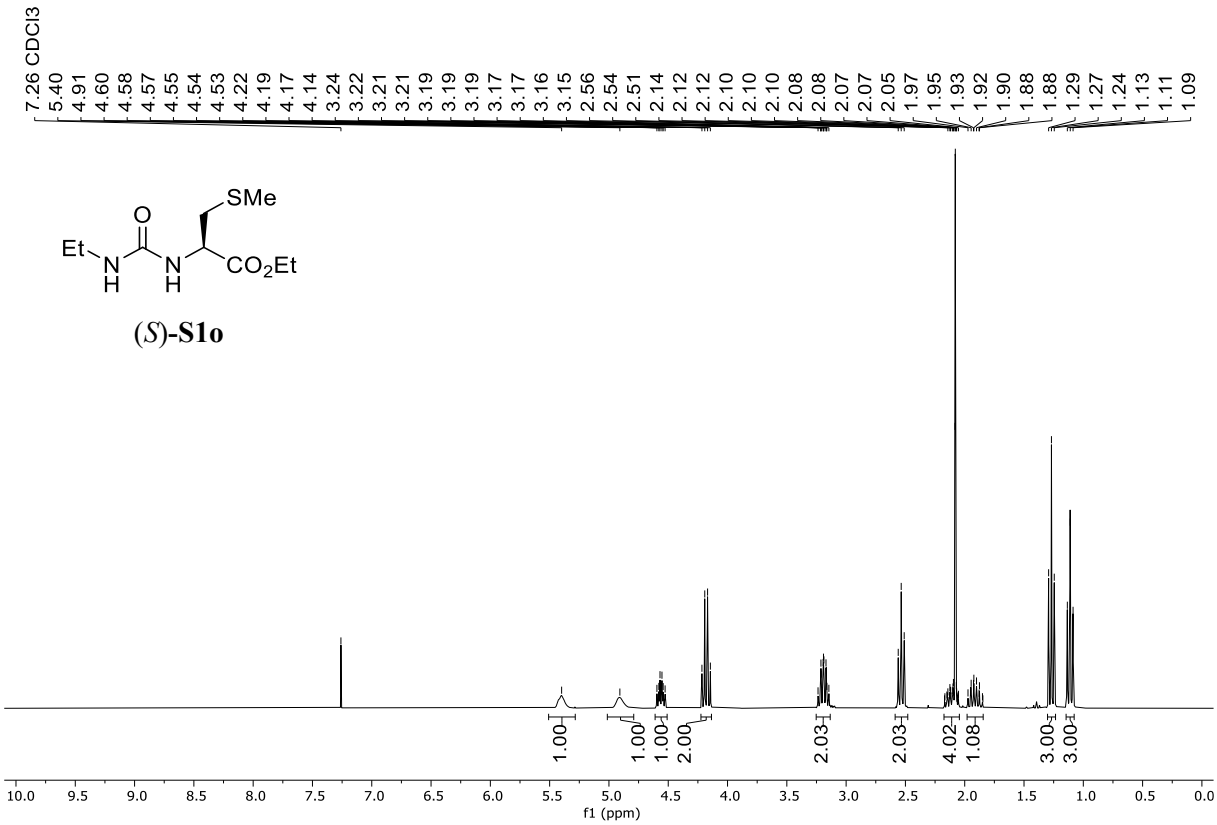
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.40 (bs, 1H), 4.91 (bs, 1H), 4.56 (td, *J* = 7.9, 4.9 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.25 – 3.09 (m, 2H), 2.59 – 2.49 (m, 2H), 2.08 (d, *J* = 0.7 Hz, 4H), 1.99 – 1.84 (m, 1H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.11 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 173.7, 157.8, 61.6, 52.5, 35.4, 32.6, 30.2, 15.6, 15.5, 14.3.

MS (EI): *m/z* (%) = 248 (79, $[M]^+$), 202 (13), 187 (59), 174 (100), 104 (98), 88 (32), 61 (94).

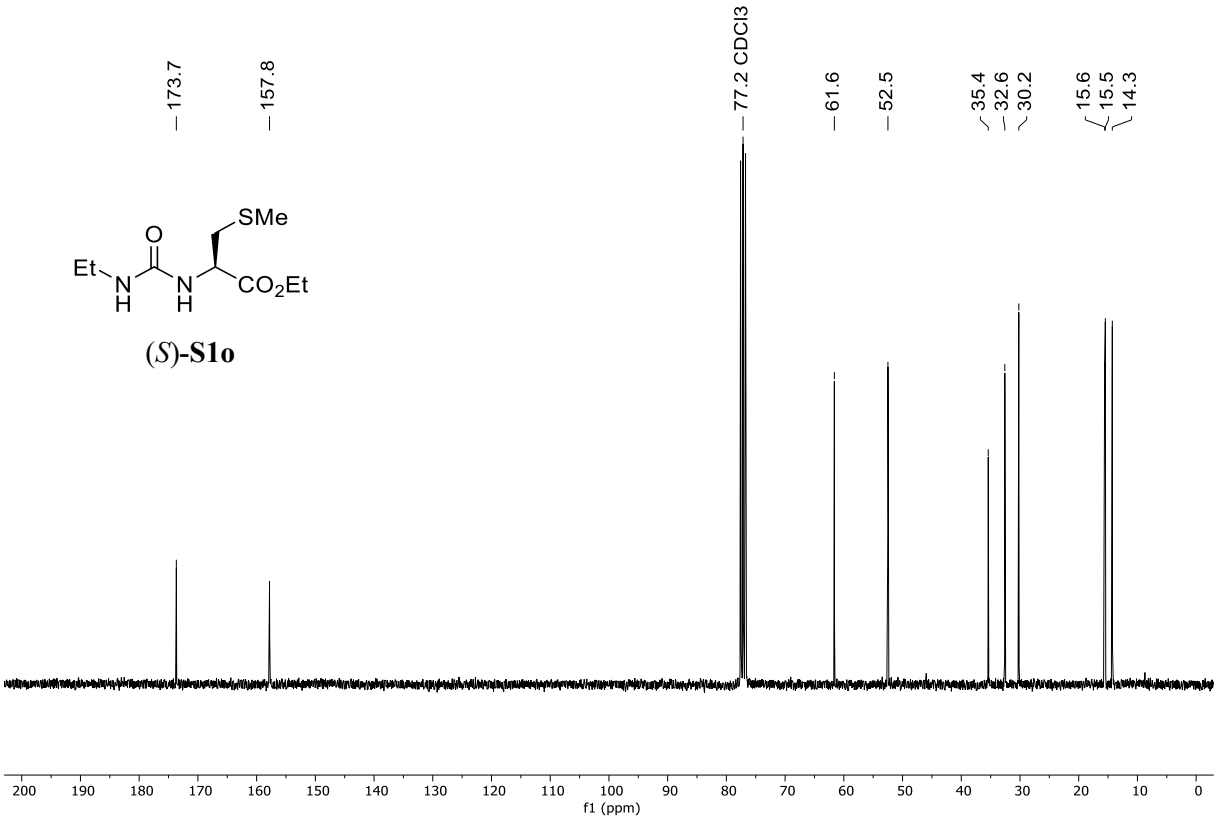
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{10}H_{20}N_2O_4SNa]^+$: 287.1036; found: 287.1035 (Error PPM: -0.3).

IR (ATR, neat, $\tilde{\nu}$) = 3338 (m), 2987 (w), 2969 (w), 2928 (w), 1741 (vs), 1629 (vs), 1562 (vs), 1519 (m), 1482 (w), 1474 (w), 1452 (m), 1433 (w), 1427 (w), 1382 (w), 1368 (m), 1350 (w), 1331 (w), 1299 (w), 1276 (s), 1250 (m), 1209 (vs), 1179 (vs), 1160 (vs), 1134 (m), 1115 (m), 1089 (s), 1062 (w), 1024 (vs), 979 (w), 956 (w), 940 (w), 920 (w), 873 (w), 799 (w), 771 (w), 742 (w), 703 (w), 683 (m), 618 (vs), 569 (vs), 520 (w), 440 (m), 426 (s) cm^{-1}.



(*S*)-**S1o**

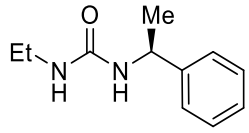
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



(*S*)-**S1o**

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of (*S*)-1-ethyl-3-(1-phenylethyl)urea ((*S*)-S1p)



(*S*)-**S1p**

Following the general procedure GP1 without the addition of NEt_3, using (*S*)-1-phenylethan-1-amine (11.20 mmol), afforded the title compound (*S*)-**S1p** (1845 mg, 9.60 mmol, 96% yield) as a colorless crystalline solid.

$\mathbf{R}_f$ = 0.15 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 108-110 °C

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{26}}$ = -21.5 (c = 1.0 g/100 ml, DCM)

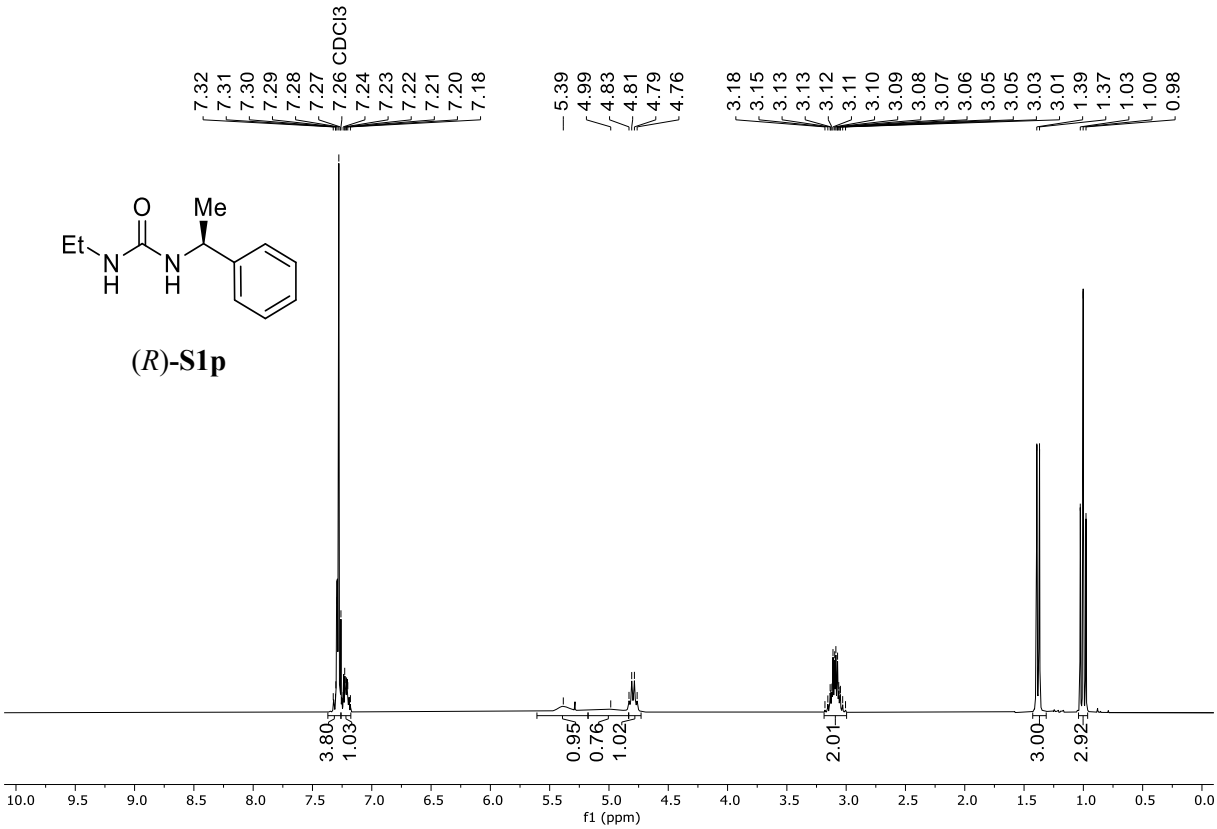
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.37 – 7.26 (m, 4H), 7.26 – 7.18 (m, 1H), 5.39 (bs, 1H), 4.99 (bs, 1H), 4.80 (q, *J* = 6.9 Hz, 1H), 3.19 – 3.00 (m, 2H), 1.38 (d, *J* = 6.9 Hz, 3H), 1.00 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 158.2, 144.7, 128.7, 127.1, 126.0, 49.9, 35.1, 23.4, 15.5.

MS (EI): *m/z* (%) = 192 (90 ,$[M]^+$), 177 (92), 120 (93) 105 (100), 77 (95).

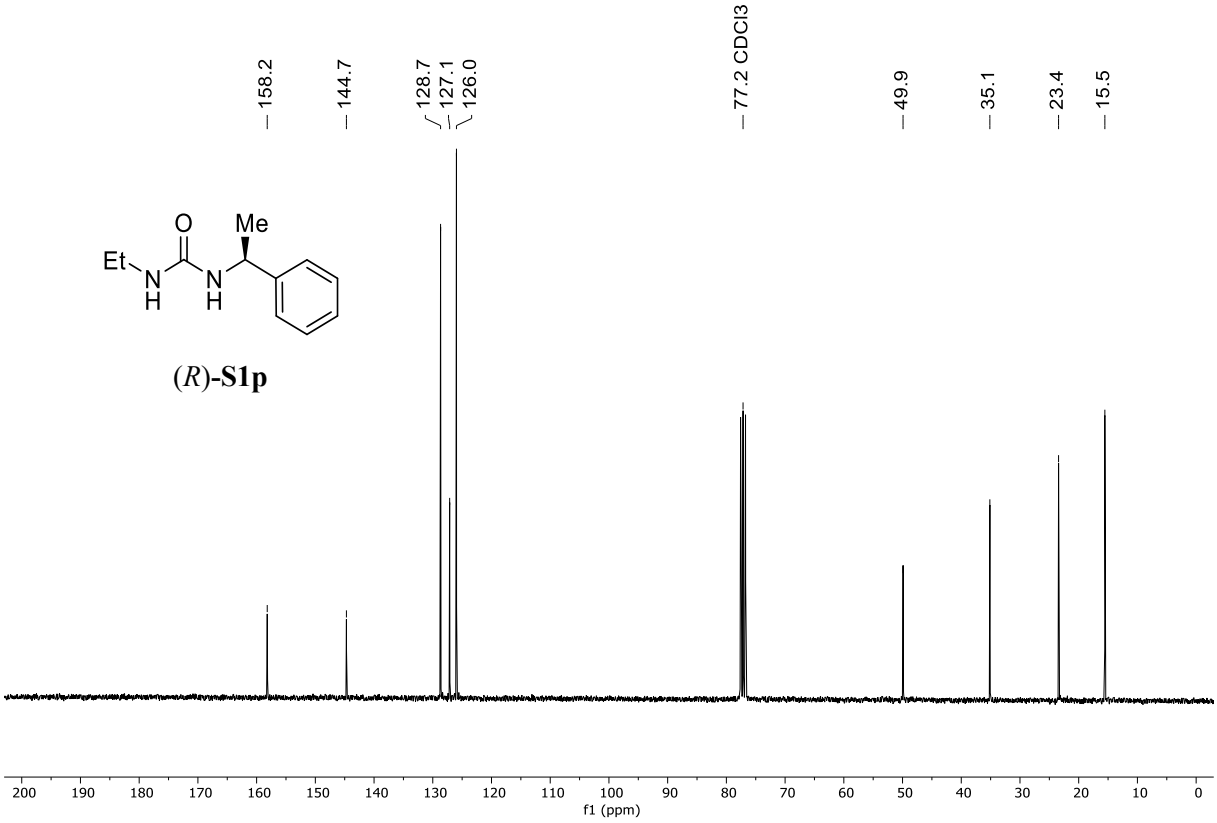
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{11}H_{16}N_2ONa]^+$: 215.1155; found: 215.1153 (Error PPM: -0.9).

IR (ATR, neat, $\tilde{\nu}$) = 3338 (w), 3311 (w), 2967 (w), 2922 (w), 2867 (w), 1625 (vs), 1586 (vs), 1566 (vs), 1493 (m), 1446 (m), 1370 (w), 1327 (w), 1285 (w), 1250 (s), 1209 (s), 1152 (w), 1119 (m), 1070 (m), 1026 (w), 1001 (w), 924 (w), 907 (w), 746 (s), 697 (vs), 665 (vs), 591 (s), 557 (s), 520 (m), 430 (w), 414 (w) cm^{-1}.



(*R*)-**S1p**

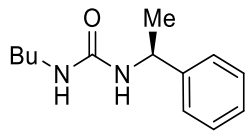
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



(*R*)-**S1p**

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of (*S*)-1-butyl-3-(1-phenylethyl)urea ((*S*)-S1q)



(**S**)-**S1q**

Butyl isocyanate (1.70 ml, 15.0 mmol, 1.0 equiv.) was added dropwise to a solution of a (*S*)-α-methyl benzylamine (1.97 ml, 15.0 mmol, 1.0 equiv.) in CH_2Cl_2 at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. Subsequently, the solvent was removed under reduced pressure to afford the title compound (*S*)-**S1q** (3.30 g, 15.0 mmol, 99% yield) as a colorless crystalline solid.

R_f = 0.47 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 33-34 °C

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{26}}$ = -4.2 (c = 0.19 g/100 ml, MeCN)

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.37 – 7.22 (m, 5H), 4.76 (dq, *J* = 6.7, 6.7 Hz, 2H), 4.70 (bs, 1H), 4.29 (bs, 1H), 3.19 – 3.01 (m, 2H), 1.44 (d, *J* = 6.6 Hz, 3H), 1.41 – 1.13 (m, 4H), 0.85 (t, *J* = 7.3 Hz, 2H).

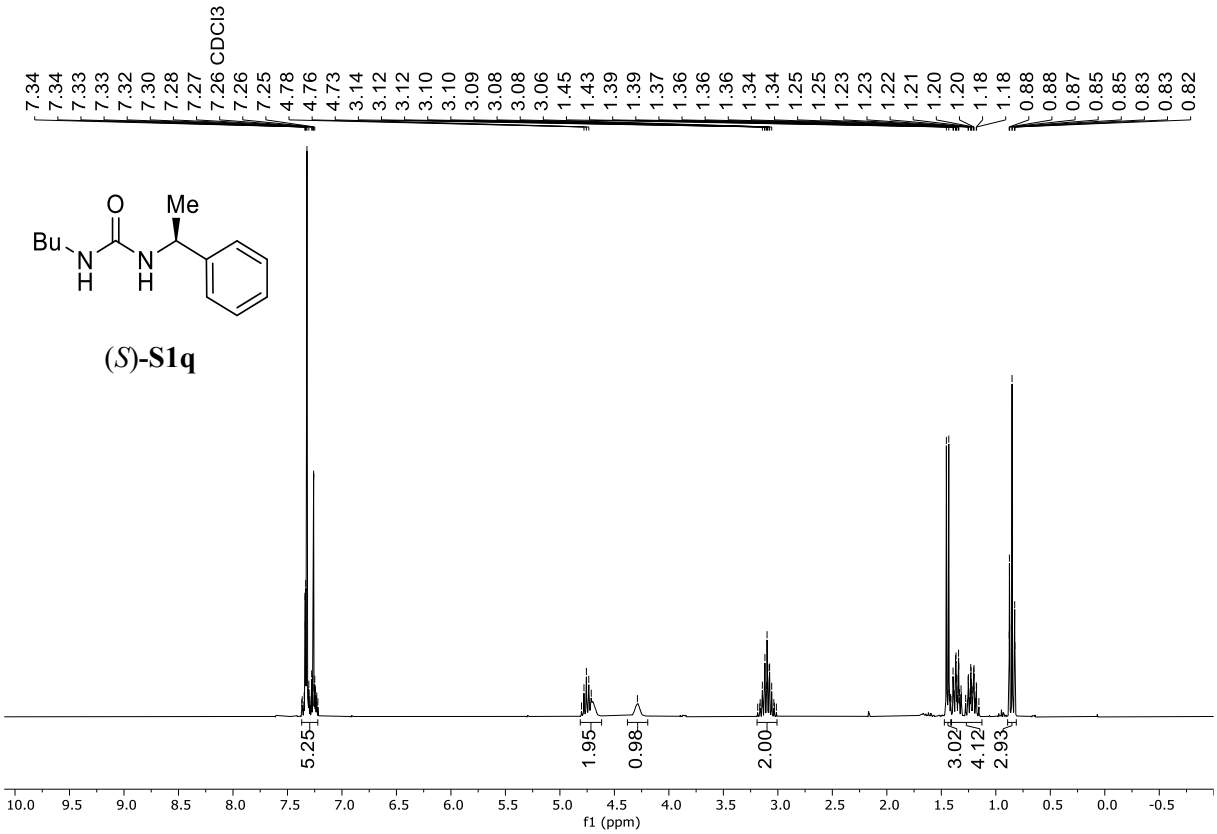
^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 157.8, 144.3, 128.9, 127.5, 126.0, 50.6, 40.3, 32.3, 23.6, 20.0, 13.9.

MS (EI): *m/z* (%) = 220 (70), 205 (8), 132 (12), 120 (65), 106 (100), 77 (27).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{13}H_{20}N_2ONa]^+$: 243.1468; found: 243.1474 (Error PPM: 2.7).

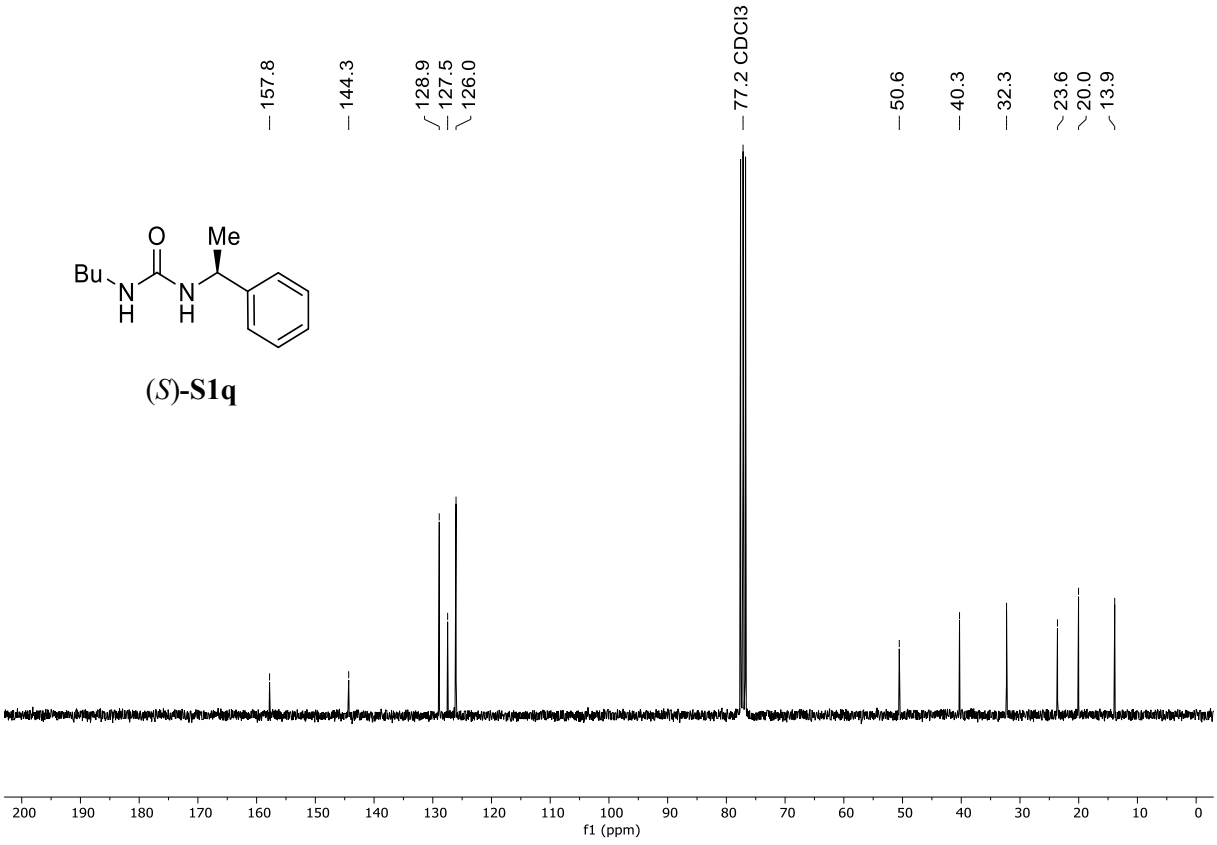
IR (ATR, neat, $\tilde{\nu}$) = 3317 (w), 3118 (w), 3087 (w), 3062 (w), 3029 (w), 2960 (m), 2929 (m), 2863 (w), 1691 (w), 1623 (s), 1563 (s), 1493 (m), 1446 (m), 1370 (w), 1341 (w), 1289 (m), 1239 (s), 1183 (w), 1157 (w), 1117 (w), 1103 (w), 1090 (w), 1074 (w), 1024 (w), 965 (w), 909 (w), 747 (m), 695 (s), 654 (s), 579 (m), 525 (m), 482 (m), 437 (w).

The resulting spectra matches the described literature values.[4]



(*S*)-**S1q**

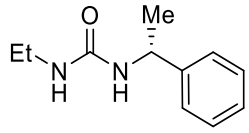
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



(*S*)-**S1q**

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of (*R*)-1-ethyl-3-(1-phenylethyl)urea ((*R*)-S1r)



(*R*)-S1r

Following the general procedure GP1 without the addition of NEt_3, using (*R*)-1-phenylethan-1-amine (10.23 mmol), afforded the title compound (*R*)-**S1r** (1.96 g, 10.22 mmol, 100%) as a colorless crystalline solid.

$\mathbf{R}_f$ = 0.15 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 109-110 °C

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{26}}$ = +19.3 (c = 1.0 g/100 ml, DCM)

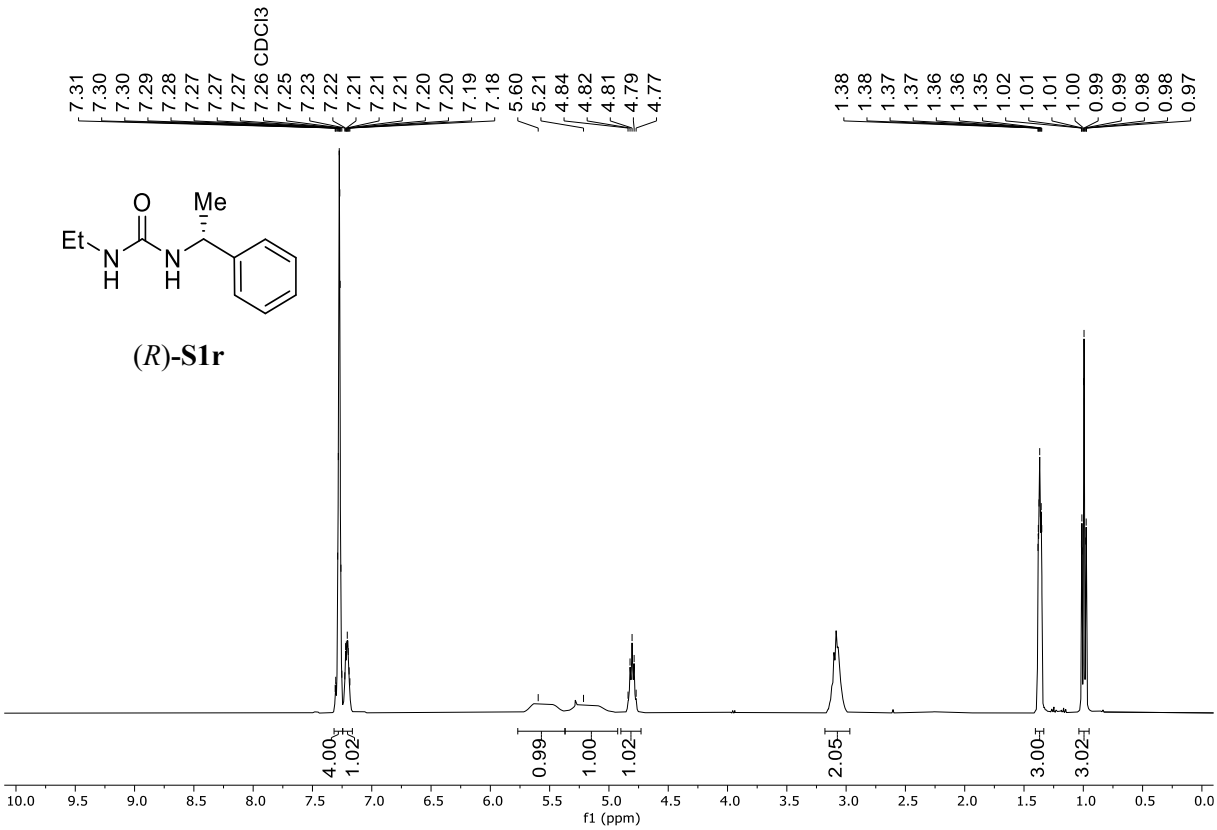
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.32 – 7.25 (m, 4H), 7.25 – 7.16 (m, 1H), 5.60 (bs, 1H), 5.21 (bs, 1H), 4.90 – 4.73 (m, 1H), 3.18 – 2.97 (m, 2H), 1.40 – 1.33 (m, 3H), 1.04 – 0.95 (m, 3H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 158.4, 144.8, 128.6, 127.0, 125.9, 49.8, 35.0, 23.4, 15.6.

MS (EI): *m/z* (%) = 192 (100, $[M]^+$), 177 (89), 120 (88) 105 (89), 77 (85).

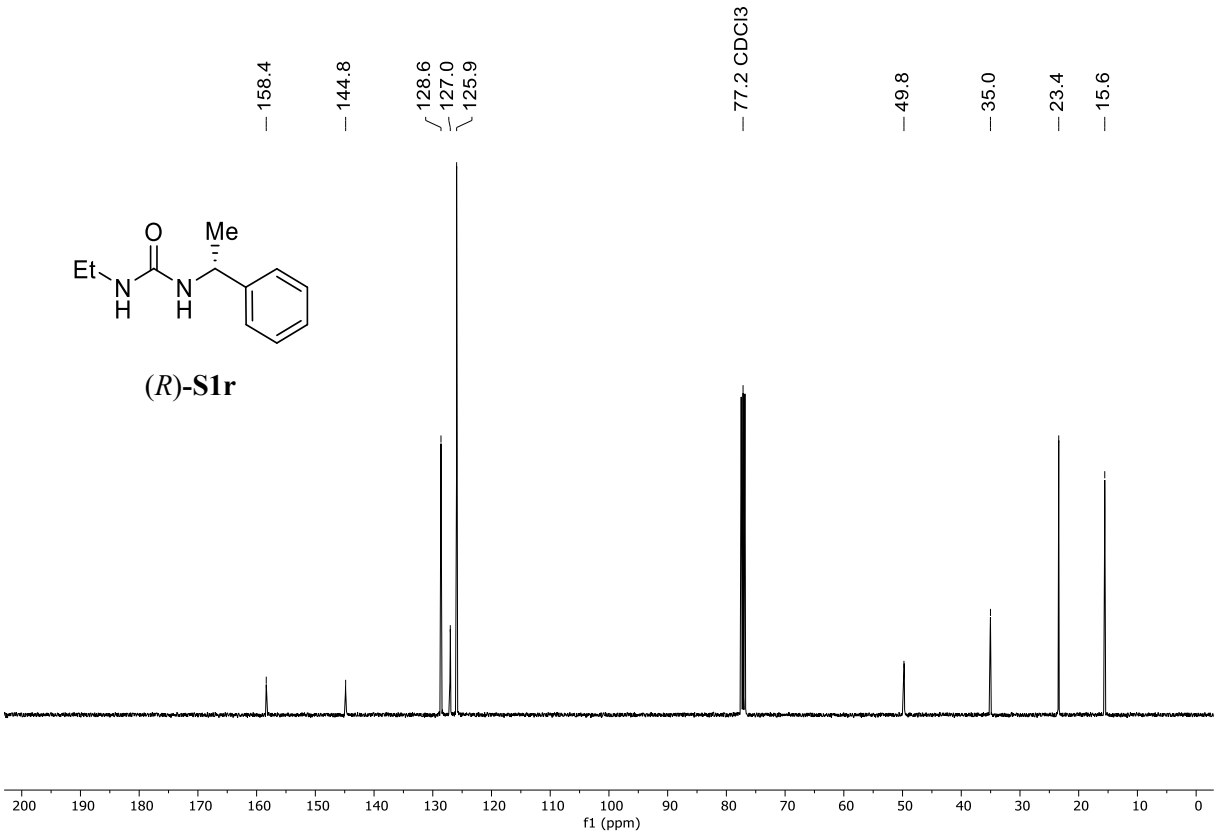
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{11}H_{16}N_2ONa]^+$: 215.1155 ;found: 215.1161 (Error PPM: 2.8).

IR (ATR, neat, $\tilde{\nu}$) = 3338 (w), 3311 (w), 2965 (w), 2924 (w), 2867 (w), 1625 (vs), 1586 (vs), 1566 (vs), 1493 (m), 1444 (m), 1370 (w), 1327 (w), 1285 (m), 1248 (vs), 1209 (s), 1152 (w), 1119 (m), 1070 (m), 1026 (w), 1001 (w), 924 (w), 907 (w), 746 (s), 697 (vs), 663 (vs), 591 (s), 557 (s), 520 (m), 416 (w) cm^{-1}.



(*R*)-**S1r**

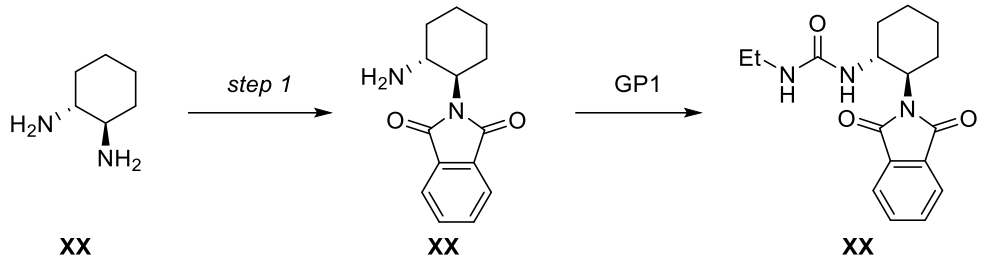
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



(*R*)-**S1r**

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of 1-((1*R*,2*R*)-2-(1,3-dioxois oindolin-2-yl)cyclohexyl)-3-ethylurea ((1*R*,2*R*)-S1s)



The title compound (1*R*,2*R*)-**S1s** was synthesized in two steps from (1*R*,2*R*)-cyclohexane-1,2-diamine ((1*R*,2*R*)-**DACH**).

Step 1: Synthesis of 2-((1*R*,2*R*)-2-aminocyclohexyl)isoindoline-1,3-dione ((1*R*,2*R*)-PhtDACH)

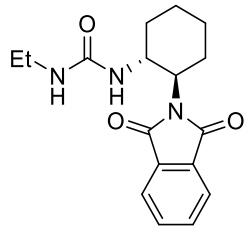
Following a modified literature known procedure (1*R*,2*R*)-cyclohexane-1,2-diamine ((1*R*,2*R*)-**DACH**) (1.84 g, 16.2 mmol, 1.0 equiv.), phthalic anhydride (2.40 g, 16.2 mmol, 1.0 equiv.), *p*-toluenesulfonic acid (1.33 g, 7.01 mmol, 0.4 equiv.) and *p*-xylene (70 mL) were added to a 250 mL round bottom flask.[5] The temperature was raised to 160°C for 4 h. During the reaction process, a milky white solid was continuously generated. After the 4h, the solution was allowed to cool to room temperature, filtered it, and washed the filter cake several times with *n*-pentane. The obtained solid was dissolved in dichloromethane (10 mL) and triturated with saturated sodium bicarbonate solution (100 mL). The dichloromethane was separated, and the aqueous phase was extracted with dichloromethane. The organic layer were combined and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The title product (1*R*,2*R*)-**PhtDACH** (1.13 g, 4.27 mmol. 29% yield) was obtained as a white solid.

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.86 – 7.77 (m, 1H), 7.75 – 7.63 (m, 1H), 3.84 – 3.73 (m, 1H), 3.40 (td, *J* = 10.5, 4.1 Hz, 1H), 2.18 (qd, *J* = 12.6, 3.6 Hz, 1H), 2.10 – 1.98 (m, 1H), 1.87 – 1.70 (m, 2H), 1.51 – 1.12 (m, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 168.9, 134.0, 123.3, 58.7, 51.0, 36.8, 29.5, 25.8, 25.2.

The resulting spectra matches the described literature values.[5]

Step 2: Synthesis of 1-((1*R*,2*R*)-2-(1,3-dioxois oindolin-2-yl)cyclohexyl)-3-ethylurea ((1*R*,2*R*)-S1s)



(1*R*,2*R*)-**S1s**

Following the general procedure GP1 without the addition of NEt_3, using (1*R*,2*R*)-**PhtDACH** (4.6 mmol), afforded the title compound (1*R*,2*R*)-**S1s** (1.35 g, 4.27 mmol, 93% yield) as a colorless crystalline solid.

R_f = 0.20 (*n*-pentane:ethyl acetate = 1:3, Vanillin stain)

m.p. = 203-204°C

$[\alpha]_D^{26}$ = -50.0 (c = 0.5 g/100 ml, DCM)

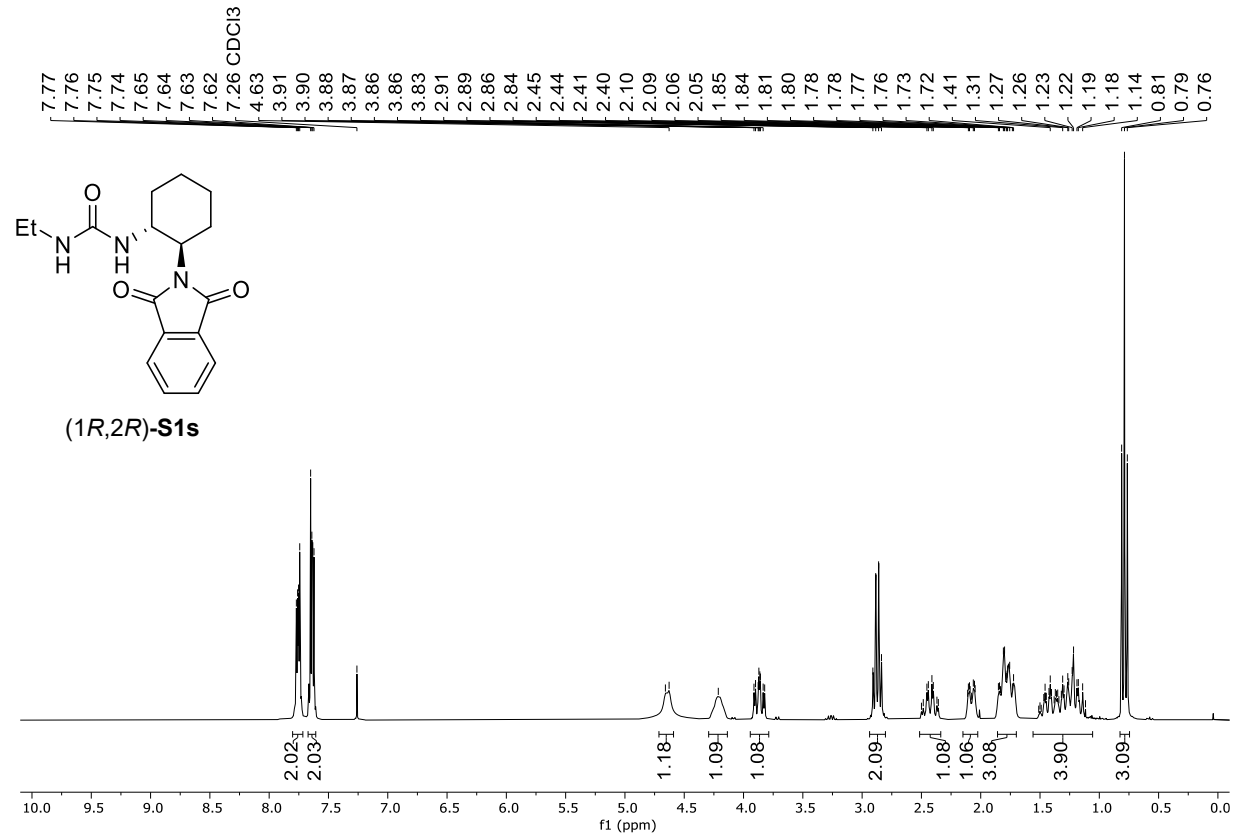
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.85 – 7.70 (m, 1H), 7.69 – 7.57 (m, 1H), 4.64 (d, *J* = 9.3 Hz, 1H), 4.21 (bs, 1H), 3.87 (ddd, *J* = 12.3, 10.7, 4.0 Hz, 1H), 2.87 (q, *J* = 7.2 Hz, 1H), 2.43 (ddd, *J* = 13.2, 12.6, 4.0 Hz, 1H), 2.15 – 1.98 (m, 1H), 1.89 – 1.68 (m, 2H), 1.54 – 1.10 (m, 2H), 0.79 (t, *J* = 7.2 Hz, 2H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 168.9, 157.7, 133.8, 131.9, 123.1, 55.8, 50.4, 35.0, 34.0, 29.0, 25.5, 25.0, 15.2.

MS (EI): *m/z* (%) = 315 (30, $[M]^+$), 243 (82), 168 (100), 148 (77), 130 (76), 97 (95), 76 (39).

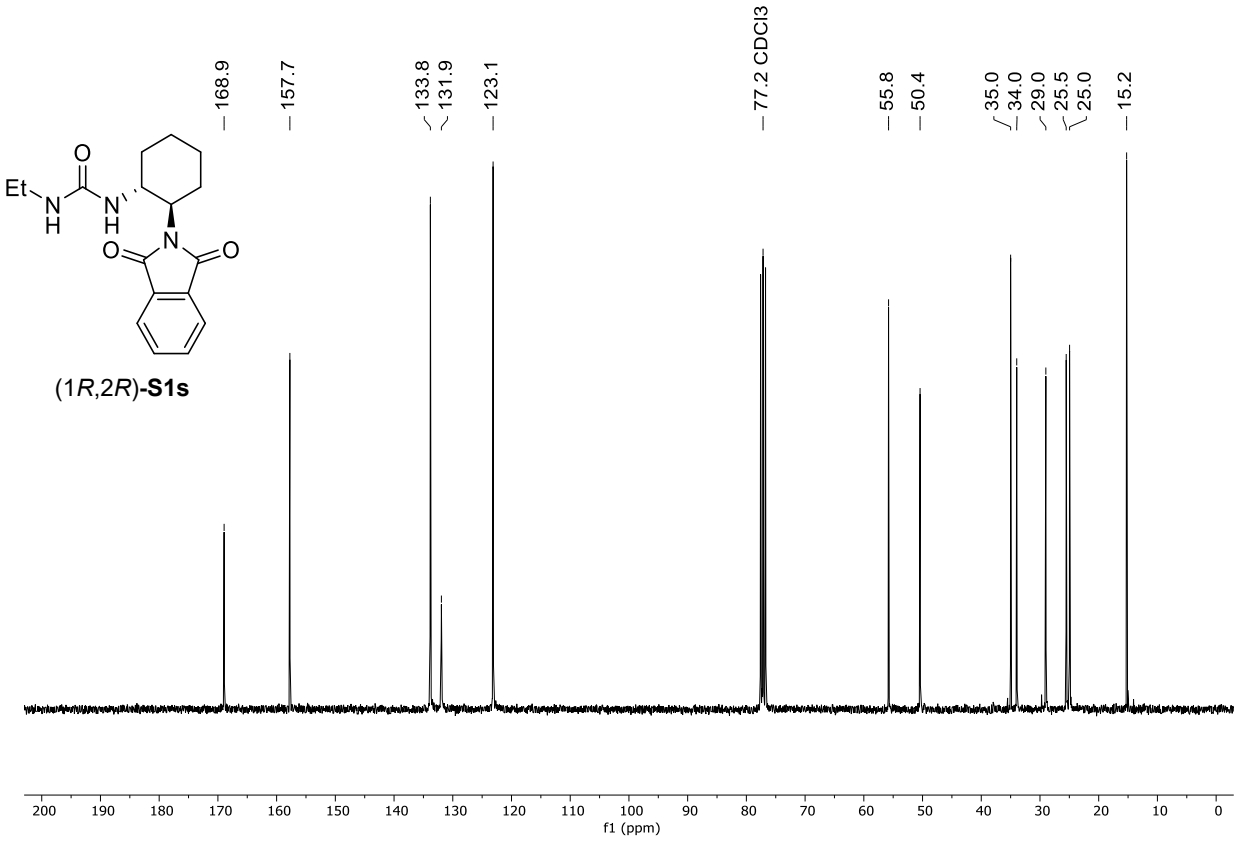
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{17}H_{21}N_3O_3Na]^+$: 338.1475; found: 338.1475 (Error PPM: 0.0).

IR (ATR, neat, $\tilde{\nu}$) = 3330 (w), 3297 (w), 2930 (w), 2857 (w), 1770 (w), 1705 (vs), 1627 (vs), 1566 (vs), 1466 (w), 1448 (m), 1389 (s), 1372 (vs), 1360 (vs), 1329 (s), 1315 (s), 1262 (s), 1254 (s), 1238 (m), 1209 (m), 1189 (m), 1170 (m), 1158 (s), 1095 (m), 1079 (m), 1064 (s), 1048 (s), 1017 (m), 1007 (m), 954 (w), 907 (w), 871 (w), 846 (w), 799 (w), 771 (w), 718 (vs), 667 (s), 636 (vs), 599 (s), 565 (m), 528 (vs), 493 (m), 434 (s), 404 (m) cm^{-1}.



(1*R*,2*R*)-**S1s**

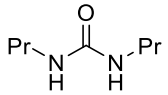
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



(1*R*,2*R*)-**S1s**

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of1,3-dipropylurea (S1v)



S1v

The title compound was synthesized following a literature known procedure.[6] Propyl amine (452 µL, 5.5 mmol, 5.5 equiv.) was dissolved in THF (4.0 ml) and the reaction mixture was cooled by an ice bath. Subsequently, propyl isocyanat (468 µL, 5.0 mmol, 5 equiv.) was added to the cooled solution and the mixture was stirred for 1 h, gradually warming to ambient temperature. Subsequently, the solvent was removed under reduced pressure using a rotary evaporator, yielding the title compound **S1v** (636.0 mg, 4.4 mmol, 88% yield) as a colorless crystalline solid.

R_f = 0.13 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 103-105 °C

^{1}H NMR (300 MHz, DMSO-d_6 [2.50 ppm], ppm) δ = 5.75 (t, *J* = 5.8 Hz, 2H), 2.97 – 2.87 (m, 4H), 1.44 – 1.26 (m, 4H), 0.82 (t, *J* = 7.4 Hz, 6H).

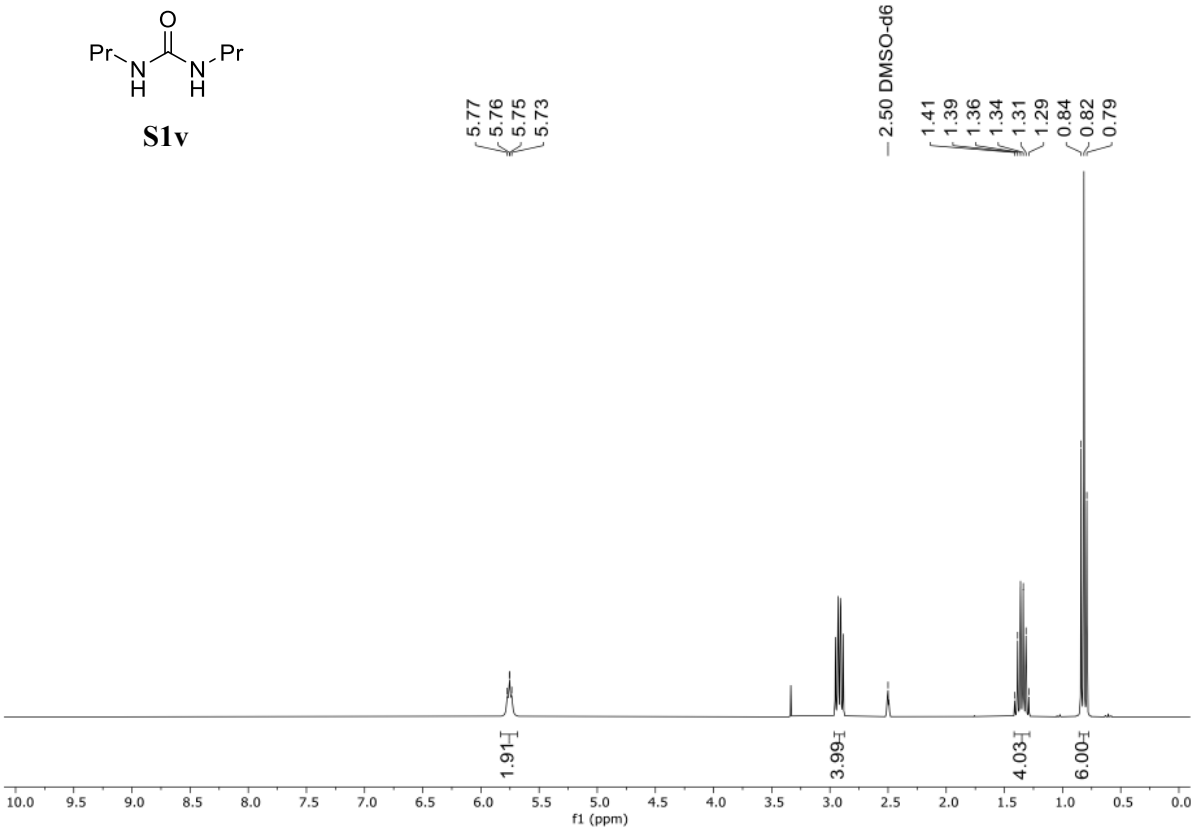
^{13}C NMR (75 MHz, DMSO-d_6 [39.52 ppm], ppm) *δ* = 158.1, 41.1, 23.3, 11.3.

MS (GC-EI): *m/z* (%) = 144 (48 [M^+]), 56 (23), 43 (24), 30 (100).

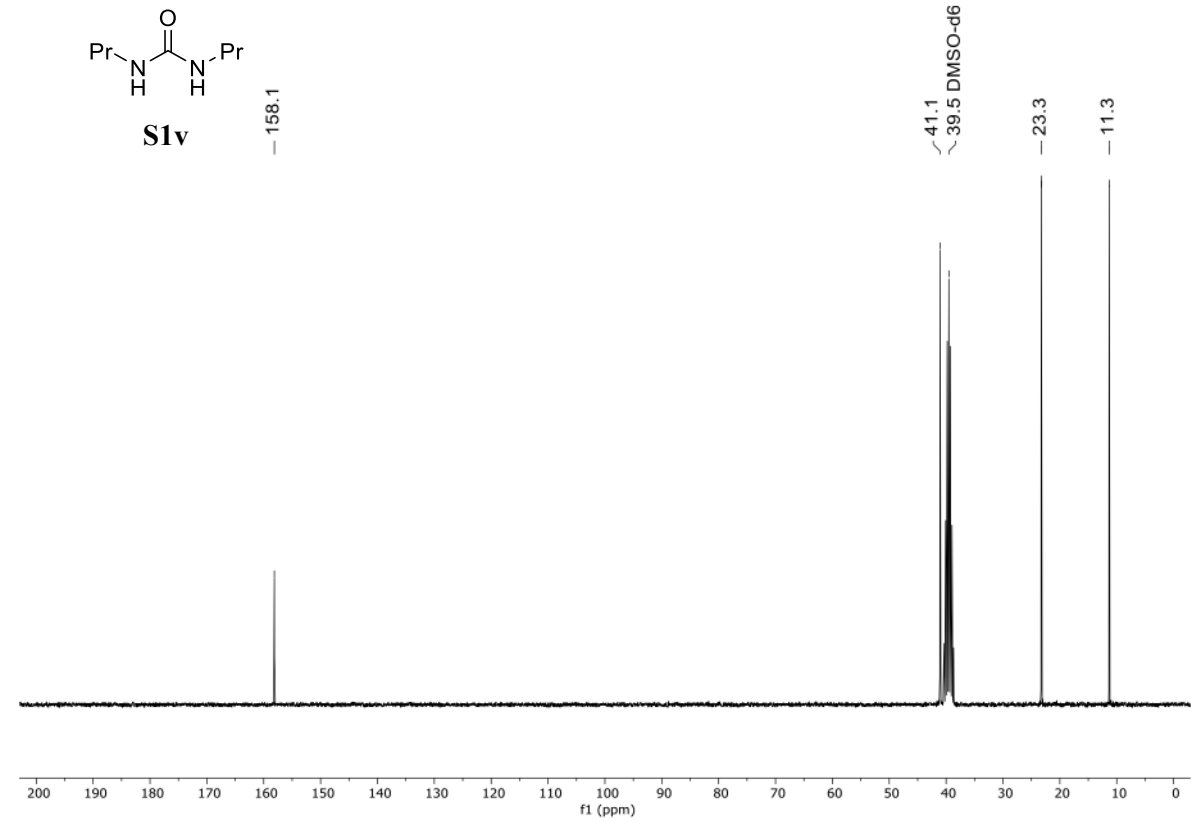
HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd for $[C_7H_{17}N_2O]^+$: 145.1335; found: 145.1334 (Error PPM: -0.7).

IR (ATR, neat, cm-1): 3327 (w), 2960 (m), 2931 (w), 2870 (m), 1691 (w), 1622 (s), 1578 (s), 1460 (m), 1374 (w), 1313 (w), 1236 (s), 1157 (m), 1072 (w), 891 (w), 756 (w), 592 (s), 436 (w).

The analytic data agrees with those reported in literature.[6]

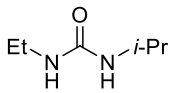


^{1}H NMR (300 MHz, DMSO-d_6 [2.50 ppm], ppm)



^{13}C NMR (75 MHz, DMSO-d_6 [39.52 ppm], ppm)

Synthesis of 1-ethyl-3-isopropylurea (S1w)



S1w

Following the general procedure GP1 without the addition of NEt_3, using isopropylamine (5.0 mmol), afforded the title compound **S1w** (485 mg, 3.73 mmol, 74% yield) as a colorless crystalline solid.

R_f = 0.29 (*n*-pentane:ethyl acetate = 1:2, Vanillin stain)

m.p. = 155-157 °C

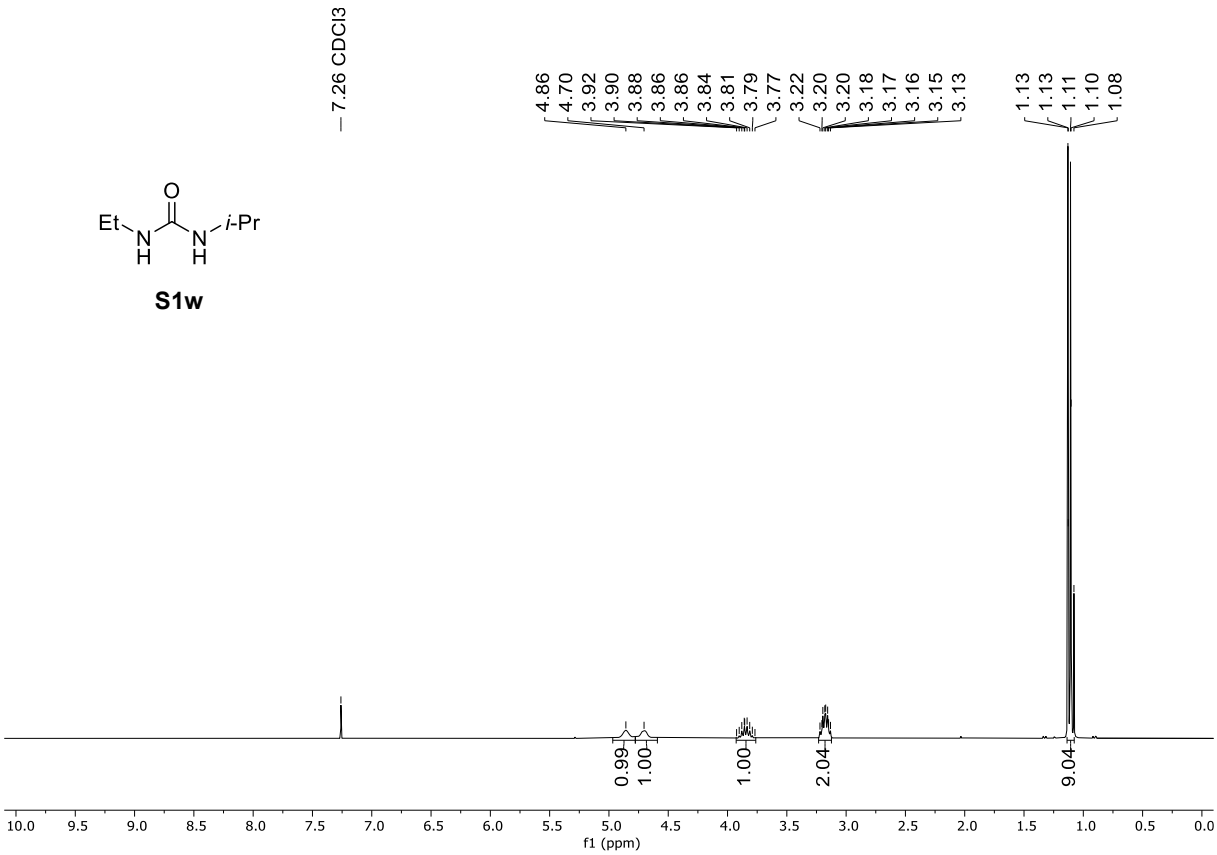
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.86 (s, 1H), 4.70 (s, 1H), 3.84 (dq, *J* = 13.3, 6.4 Hz, 1H), 3.18 (qd, *J* = 7.2, 4.8 Hz, 2H), 1.19 – 1.01 (m, 9H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 158.1, 42.1, 35.2, 23.6, 15.7.

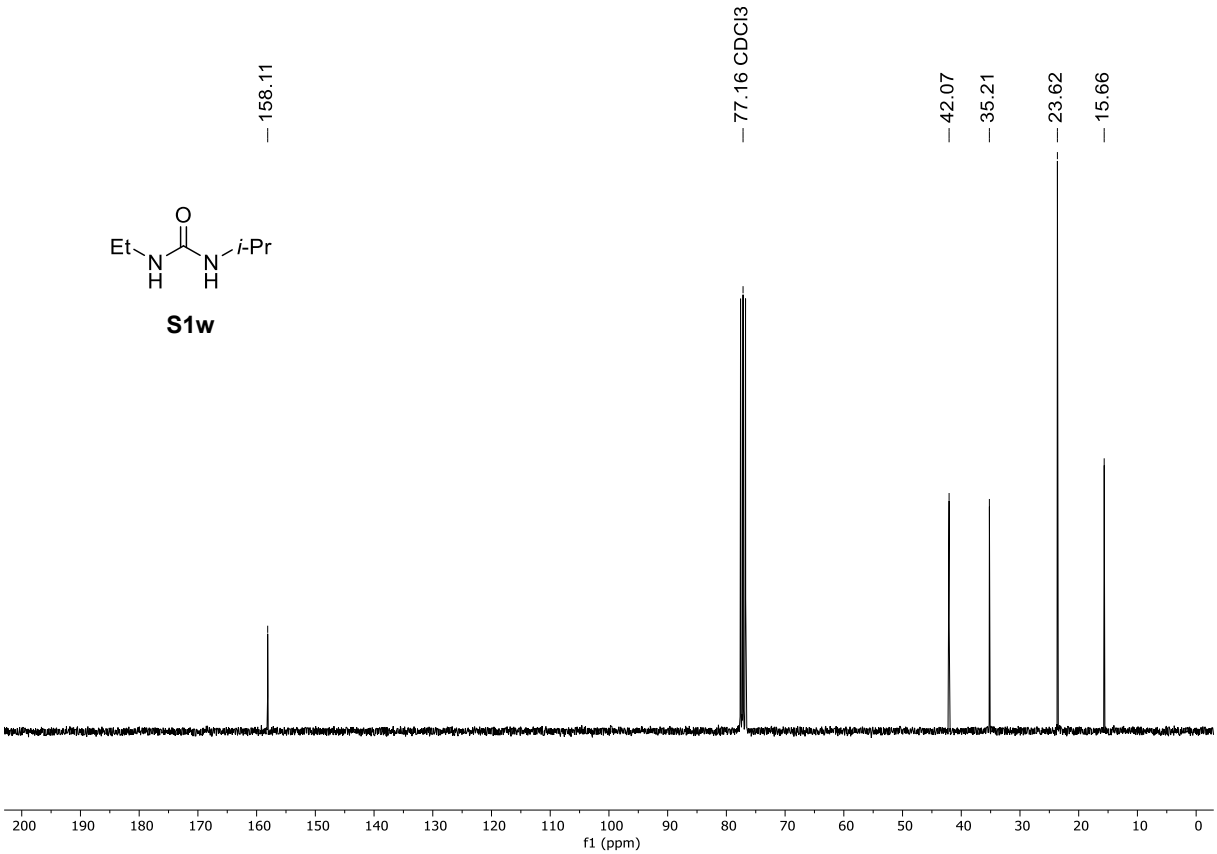
MS (EI): *m/z* (%) = 130 (100, $[M]^+$), 115 (86), 73 (9), 58 (57).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_6H_{15}N_2O]^+$: 131.1179; found: 131.1182 (Error PPM: 2.3).

IR (ATR, neat, $\tilde{\nu}$) = 3330 (w), 2969 (w), 2928 (w), 2871 (w), 1621 (s), 1562 (vs), 1456 (m), 1384 (w), 1364 (w), 1319 (w), 1297 (w), 1250 (vs), 1199 (m), 1164 (m), 1142 (m), 1128 (m), 1083 (w), 1020 (w), 771 (w), 644 (vs), 524 (m), 459 (w), 414 (w) cm^{-1}.

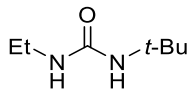


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of 1-(*tert*-butyl)-3-ethylurea (S1x)



S1x

Tert-butylamine (146 mg, 210 µL, 2.00 mmol, 1.00 equiv) was dissolved in dichloromethane (4.00 mL) cooled to 0 °C and ethyl isocyanat (157 mg, 172 µL, 2.20 mmol, 1.10 equiv) was added. The mixture was stirred for 4 h, gradually warming to ambient temperature. After completion, the reaction was quenched by adding water, and the mixture was extracted with CH_2Cl_2 (3 × 10 ml). The combined organic layer were washed with brine, dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure using a rotary evaporator, which afforded the title compound **S1x** (218 mg, 1.51 mmol, 76% yield) as a colorless crystalline solid.

R_f = 0.38 (*n*-pentane:ethyl acetate = 1:2, Vanillin stain)

m.p. = 144-146°C

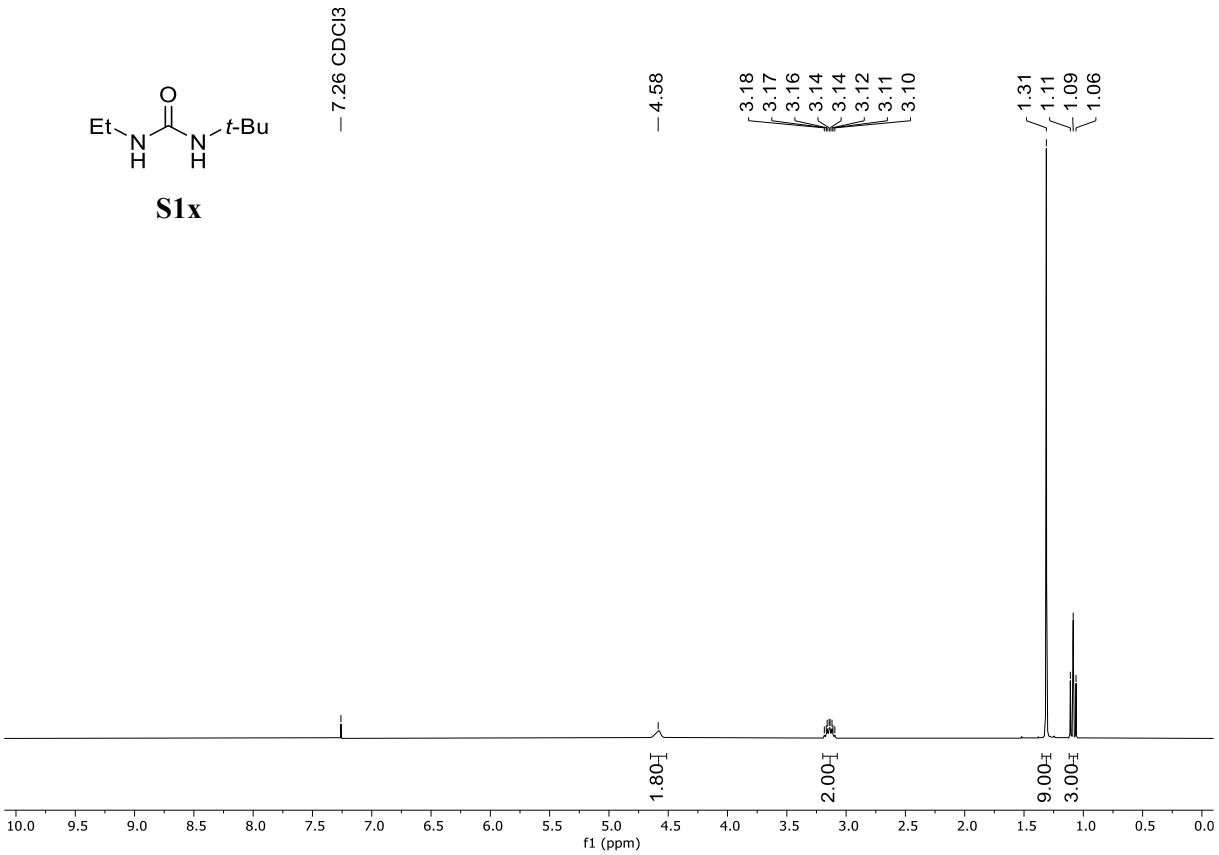
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.58 (s, 2H), 3.14 (qd, *J* = 7.2, 4.8 Hz, 2H), 1.31 (s, 9H), 1.09 (t, *J* = 7.2 Hz, 3H)

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 158.0, 50.3, 35.1, 29.7, 15.6.

MS (EI): *m/z* (%) = 144 (9 [M^+]), 129 (34), 58 (100), 57 (14), 44 (10).

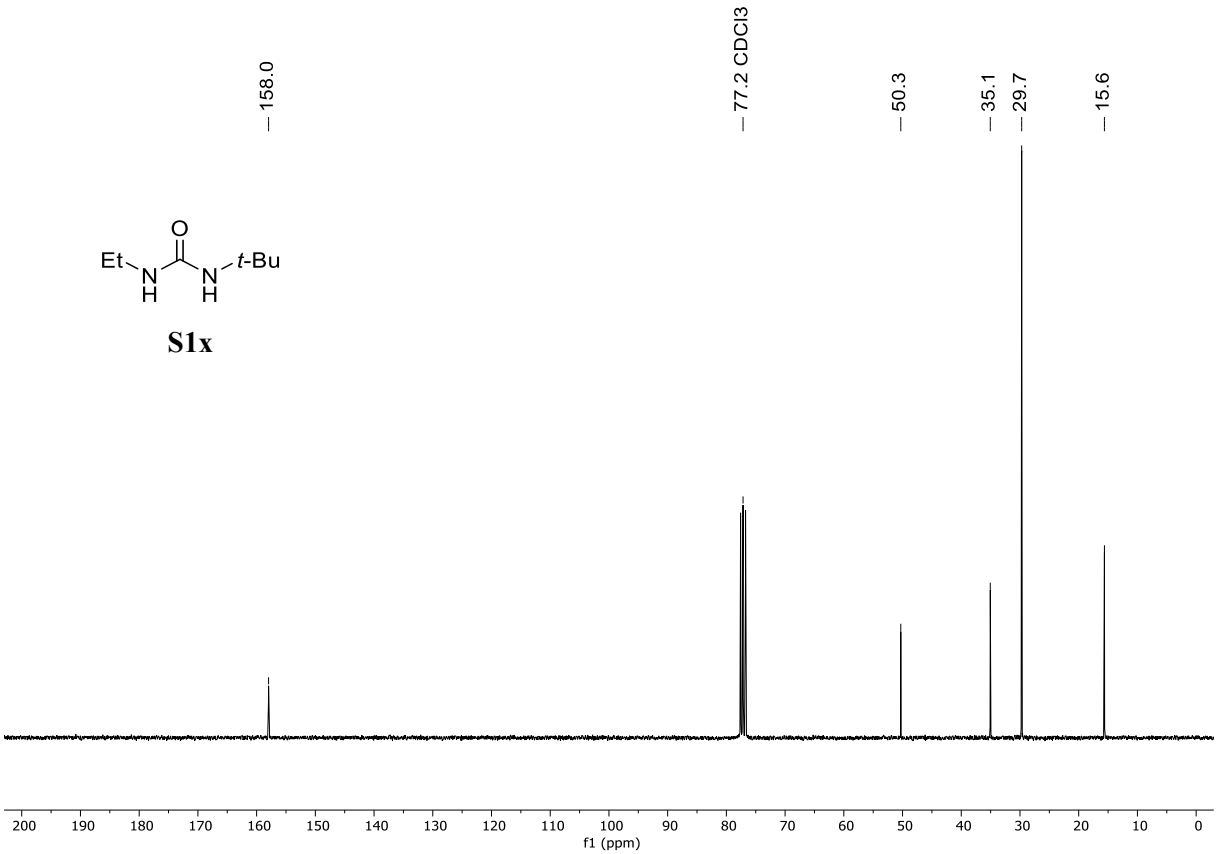
HRMS (ESI-TOF): *m/z* [M+H]$^+$ calcd. for $[C_7H_{17}N_2O]^+$: 145.1335; found: 145.1337 (Error PPM: 1.4).

IR (ATR, $\tilde{\nu}$) = 3330 (w), 2965 (m), 2930 (w), 2869 (w), 1631 (vs), 1558 (vs), 1476 (w), 1450 (s), 1389 (m), 1378 (w), 1358 (m), 1297 (m), 1272 (vs), 1246 (m), 1217 (vs), 1160 (vs), 1083 (w), 1048 (w), 944 (w), 773 (w), 642 (vs), 530 (m), 463 (w), 434 (w) cm^{-1}.



S1x

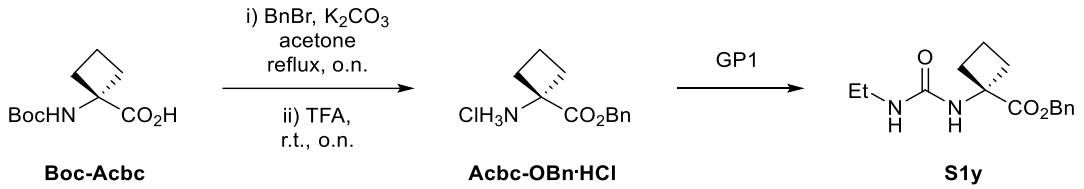
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



S1x

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of benzyl 1-(3-ethylureido)cyclobutane-1-carboxylate (S1y)



Boc-Acbc **Acbc-OBn·HCl** **S1y**

The title compound **S1y** was synthesized in two steps starting from boc-1-aminocyclobutane carboxylic acid (**Boc-Acbc**).

Step 1: Synthesis of benzyl 1-aminocyclobutane-1-carboxylate hydrochloride (Acbc-OBn·HCl)

Acbc-OBn·HCl

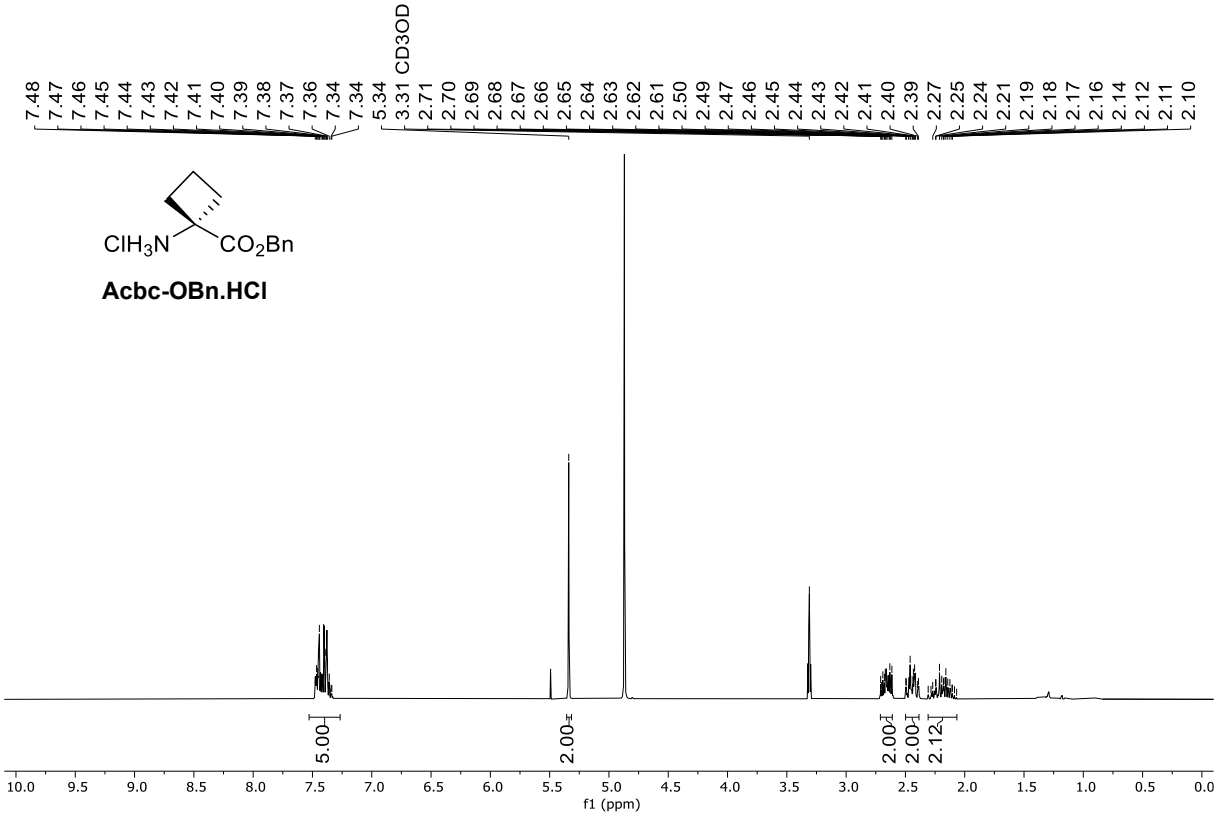
The title compound was synthesized according to an adapted literature procedure.[7] Boc-1-aminocyclobutane carboxylic acid (**Boc-Acbc**) (10.0 g, 46.5 mmol, 1.00 equiv) and potassium carbonate (18.8 g, 186 mmol, 4.00 equiv) were suspended in acetone (500 mL). Subsequently, benzyl bromide (6.00 mL, 50.4 mmol, 1.09 equiv.) was added, and the reaction mixture was stirred at reflux overnight. Afterwards, the reaction mixture was cooled to 0 °C using an ice bath, filtered, and evaporated to dryness *in vacuo*. The crude residue was redissolved in dichloromethane (250 mL), dried over anhydrous sodium sulfate, and filtered. Next, trifluoroacetic acid (21.5 mL, 279 mmol, 6.00 equiv.) was added dropwise, and the solution was stirred overnight at ambient temperature. Afterwards, the reaction mixture was neutralized using a saturated potassium carbonate solution and extracted with 3 × 100 ml EtOAc dichloromethane. The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness *in vacuo*. Benzyl 1-aminocyclobutane-1-carboxylate was precipitated with hydrogen chloride, yielding the title compound **Acbc-OBn·HCl** (11.2 g, 46.5 mmol, 100% yield) as a colorless solid.

^{1}H NMR (300 MHz, Methanol-d4 [3.31 ppm], ppm) δ = 7.53 – 7.27 (m, 5H), 5.34 (s, 2H), 2.71 – 2.61 (m, 2H), 2.50 – 2.39 (m, 2H), 2.31 – 2.07 (m, 2H).

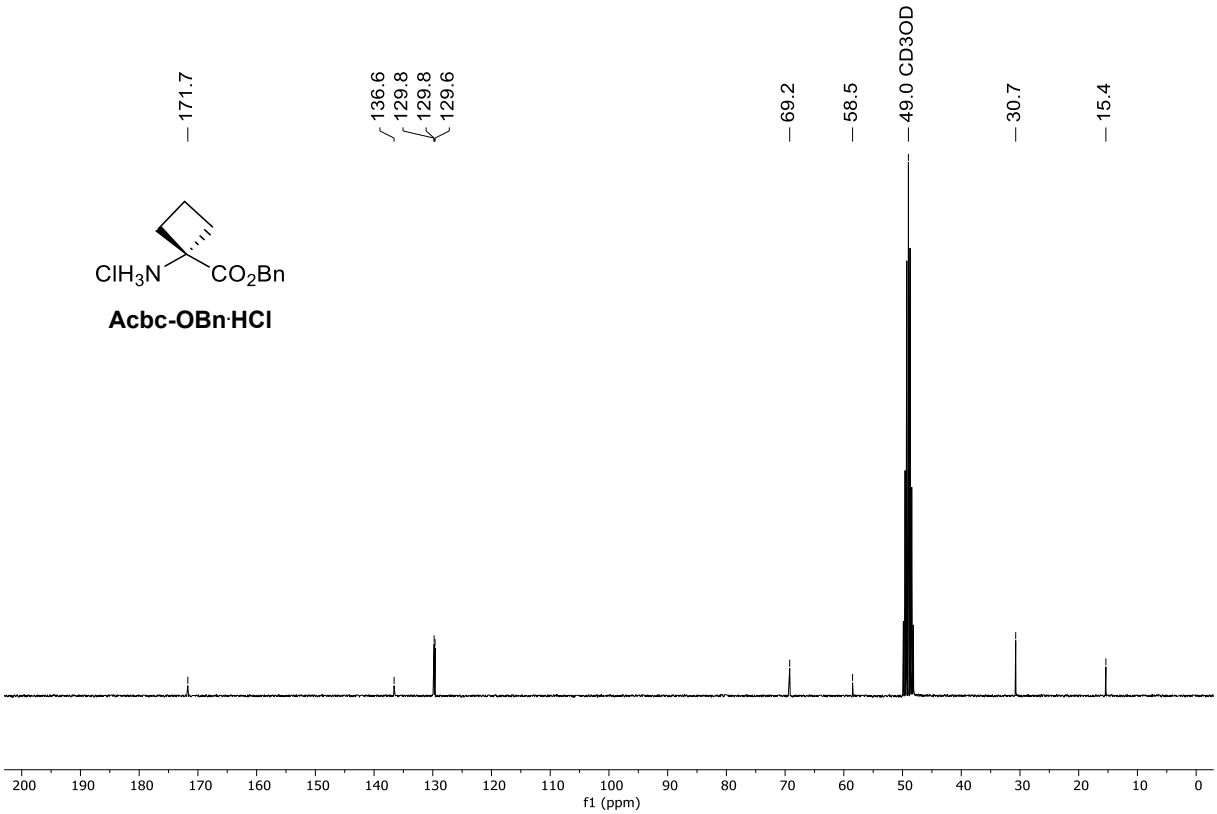
^{13}C NMR (75 MHz, Methanol-d4 [49.00 ppm], ppm) δ = 171.7, 136.6, 129.8, 69.2, 58.5, 30.7, 15.4.

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{12}H_{16}NO_2]^+$: 206.1176; found: 206.1180 (Error PPM: 1.9).

The analytic data agrees with those reported in literature.[7]

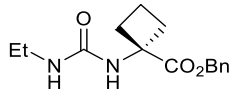


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Step 2: Synthesis of Benzyl 1-(3-ethylureido)cyclobutane-1-carboxylate (S1y)



S1y

Following the general procedure GP1, using benzyl 1-aminocyclobutane-1-carboxylate hydrochloride (**Acbc-OBn·HCl**) (6.04 g, 25.0 mmol, 1.00 equiv) and recrystallization from ethyl acetate afforded the title compound **S1y** (5.03 g, 18.2 mmol, 73% yield) as a colorless crystalline solid.

$\mathbf{R}_f$ = 0.23 (*n*-pentane:ethyl acetate = 1:1, Vanillin stain)

m.p. = 108-110 °C

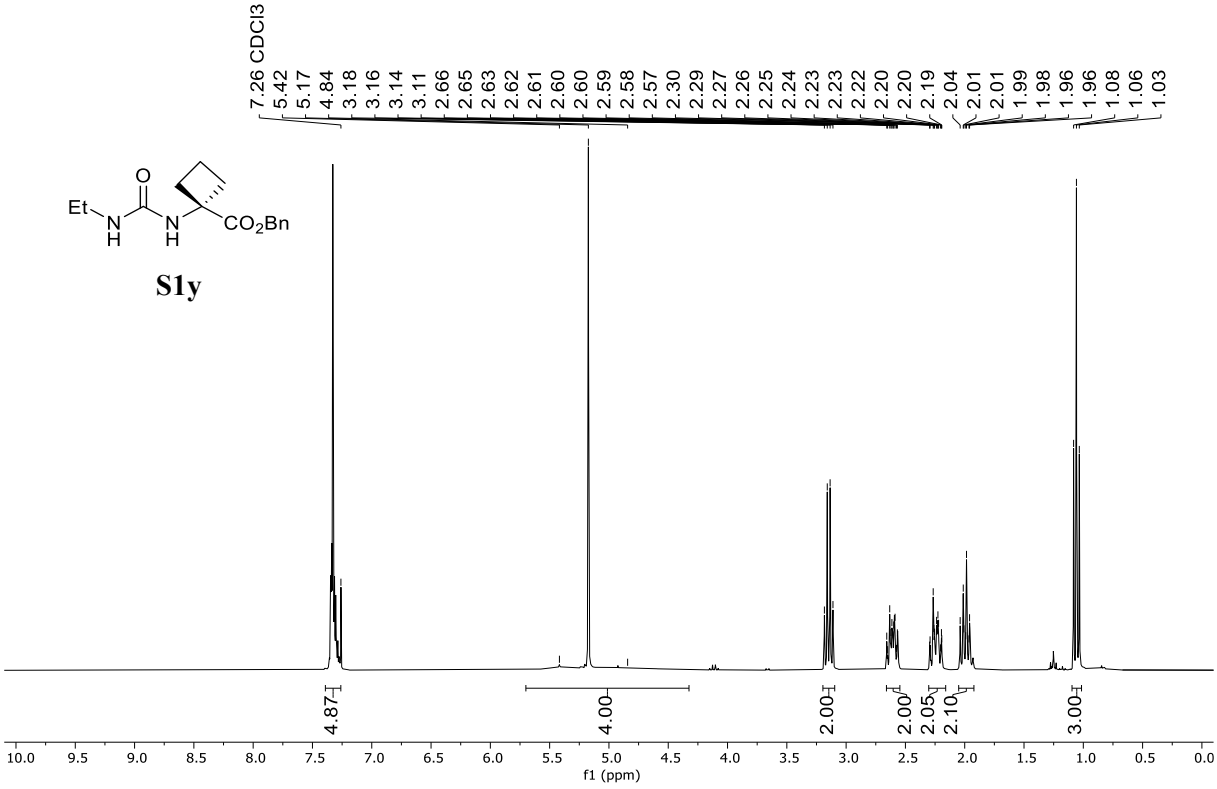
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.39 – 7.26 (m, 5H), 5.42 (bs, 1H) 5.17 (s, 2H), 4.84 (bs, 1H) 3.15 (q, *J* = 7.2 Hz, 2H), 2.66 – 2.55 (m, 2H), 2.30 – 2.16 (m, 2H), 2.05 – 1.92 (m, 2H), 1.06 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.7, 157.7, 136.1, 128.6, 128.2, 128.0, 67.0, 58.5, 35.2, 31.8, 15.5, 15.5.

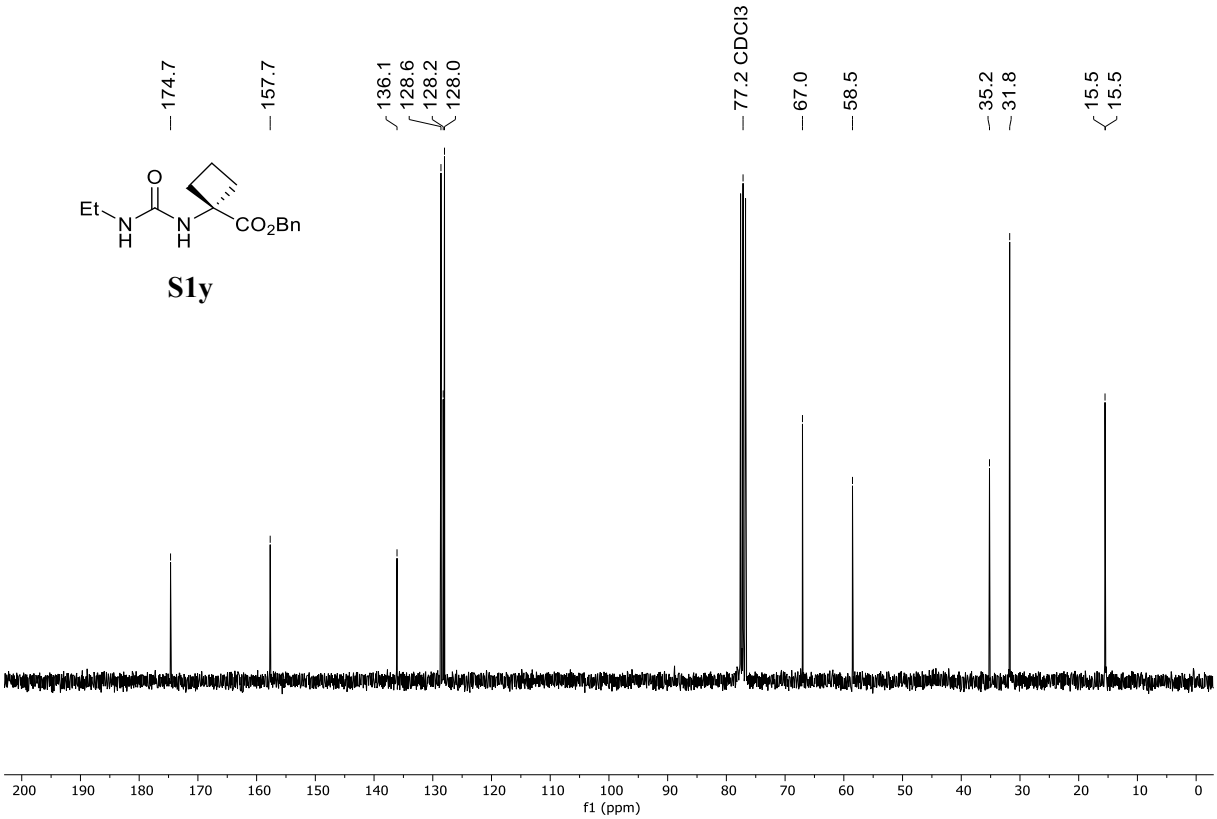
MS (EI): *m/z* (%) = 248 (5), 177 (16), 158 (5), 157 (90), 141 (6), 114 (11), 91 (100), 70 (26).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for: $[C_{15}H_{20}N_2O_3Na]^+$: 299.1366; found: 299.1361 (Δppm: -1.7).

IR (ATR, neat, $\tilde{\nu}$) = 3375 (w), 3277 (w), 3109 (vw), 3008 (w), 2977 (w), 2952 (w), 2879 (w), 1719 (vs), 1666 (m), 1627 (vs), 1562 (vs), 1493 (m), 1482 (s), 1470 (w), 1450 (m), 1431 (w), 1382 (w), 1309 (m), 1270 (m), 1234 (m), 1217 (vs), 1181 (s), 1150 (s), 1142 (m), 1119 (vs), 1101 (s), 1081 (m), 1032 (w), 1013 (w), 995 (w), 958 (s), 920 (m), 909 (m), 883 (w), 814 (w), 795 (w), 775 (w), 754 (vs), 728 (w), 697 (vs), 671 (s), 585 (s), 546 (w), 512 (s), 465 (w), 418 (w) cm^{-1}.



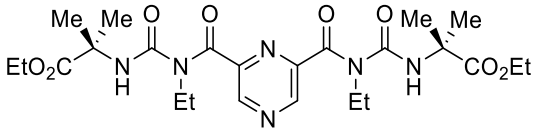
[1]H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



[13]C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

4.2 Synthesis of carbamoyl-substituted pyrazine carboxamides according to GP2

Synthesis of diethyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))-bis(azanediyl))bis(2-methylpropanoate) (1a)



1a

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (1.01 g, 6.00 mmol, 1.00 equiv.) and the corresponding urea **S1a** (3.03 g, 15.0 mmol, 2.50 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 1:1), afforded the title compound **1a** (2.60 g, 4.85 mmol, 81% yield) as a yellow resin.

$\mathbf{R}_f$ = 0.29 (*n*-heptane/EtOAc = 1:1)

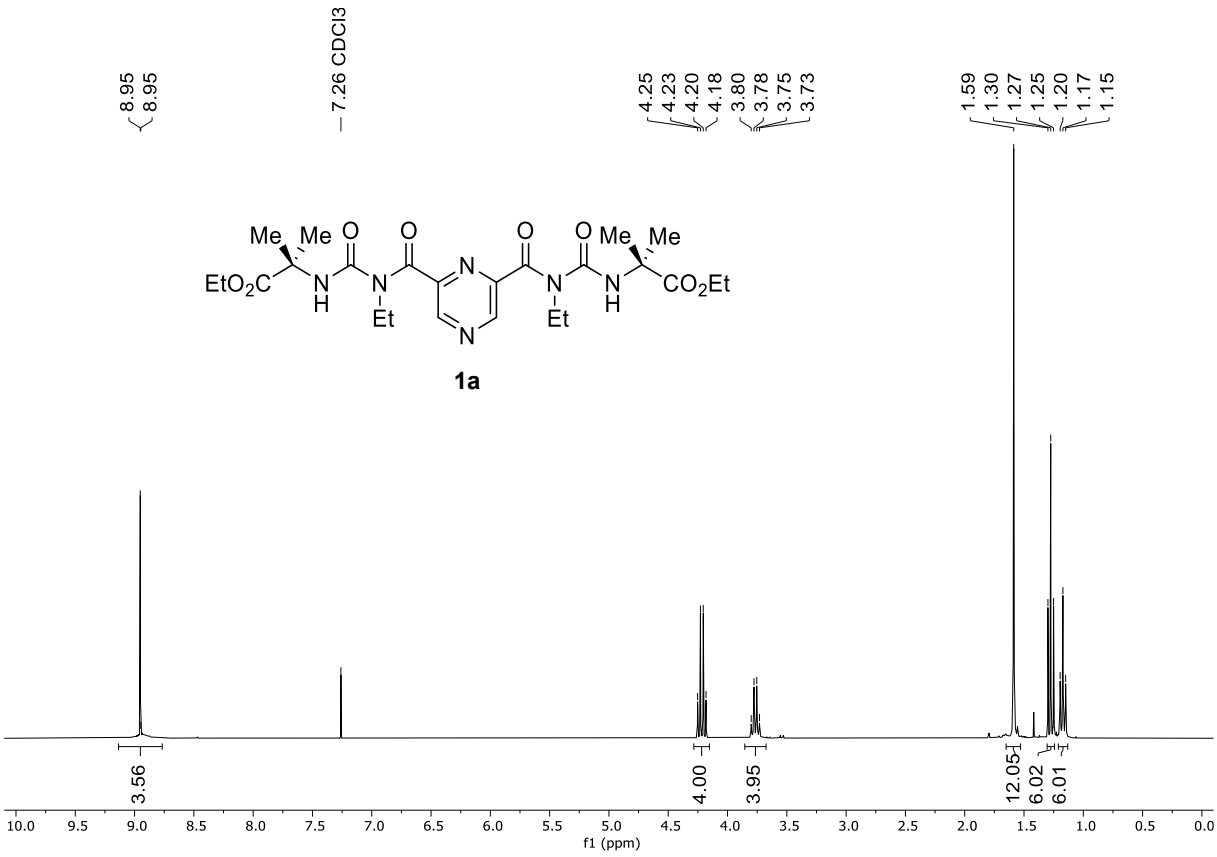
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.95 (s, 2H), 8.95 (bs, 2H), 4.22 (q, *J* = 7.1 Hz, 4H), 3.77 (q, *J* = 7.0 Hz, 4H), 1.59 (s, 12H), 1.27 (t, *J* = 7.1 Hz, 6H), 1.17 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.2 ppm], ppm) δ = 174.2, 168.8, 152.5, 147.4, 144.8, 61.7, 57.2, 41.4, 25.0, 15.0, 14.3.

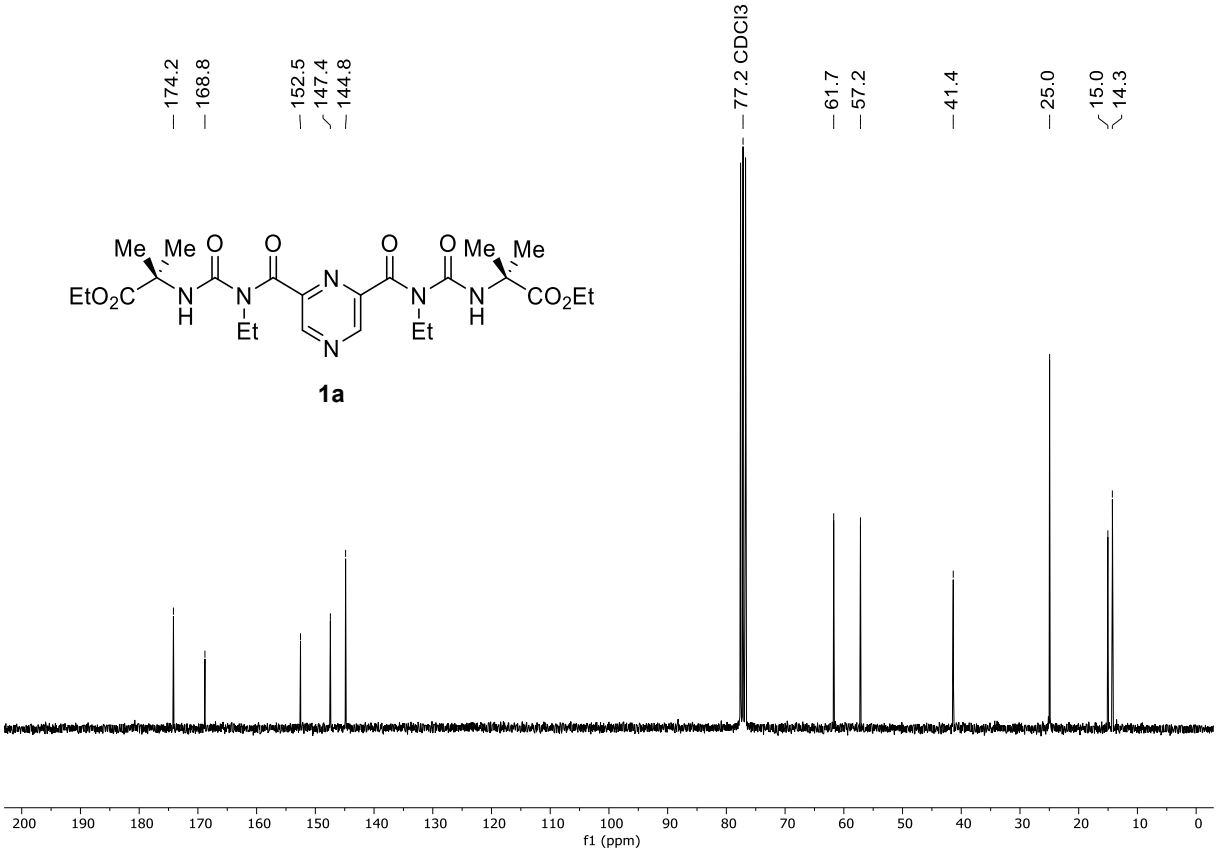
MS (ESI): *m/z* (%) = 559 (100, [M+Na$^+$]), 380 (25), 288 (10), 223 (22).

HRMS (ESI-TOF): *m/z* [M+Na]$^+$ calcd. for [$C_{24}H_{36}N_6O_8Na$]$^+$: 559.2487; found: 559.2493 (Error PPM: 1.1).

IR (ATR, neat, $\tilde{\nu}$) = 3305 (w), 2983 (w), 2938 (w), 1737 (s), 1705 (vs), 1654 (s), 1513 (vs), 1450 (m), 1380 (s), 1366 (s), 1283 (vs), 1256 (s), 1205 (s), 1170 (vs), 1146 (vs), 1095 (vs), 1068 (vs), 1017 (vs), 966 (w), 940 (w), 911 (w), 860 (w), 814 (w), 793 (w), 769 (s), 705 (w), 626 (m), 599 (m), 571 (m), 546 (m), 506 (w), 459 (w), 422 (m) cm^{-1}.

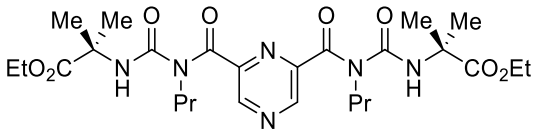


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(propylazanediyl))bis(carbonyl))-bis(azanediyl))bis(2-methylpropanoate) (1b)



1b

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (168 mg, 1.0 mmol, 1 equiv.) and the corresponding urea **S1b** (541 mg, 2.50 mmol, 2.50 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1), afforded the title compound **1b** (456 mg, 0.8 mmol, 81%) as a yellow resin.

$\mathbf{R}_f$ = 0.70 (*n*-pentane:ethyl acetate = 1:1, UV)

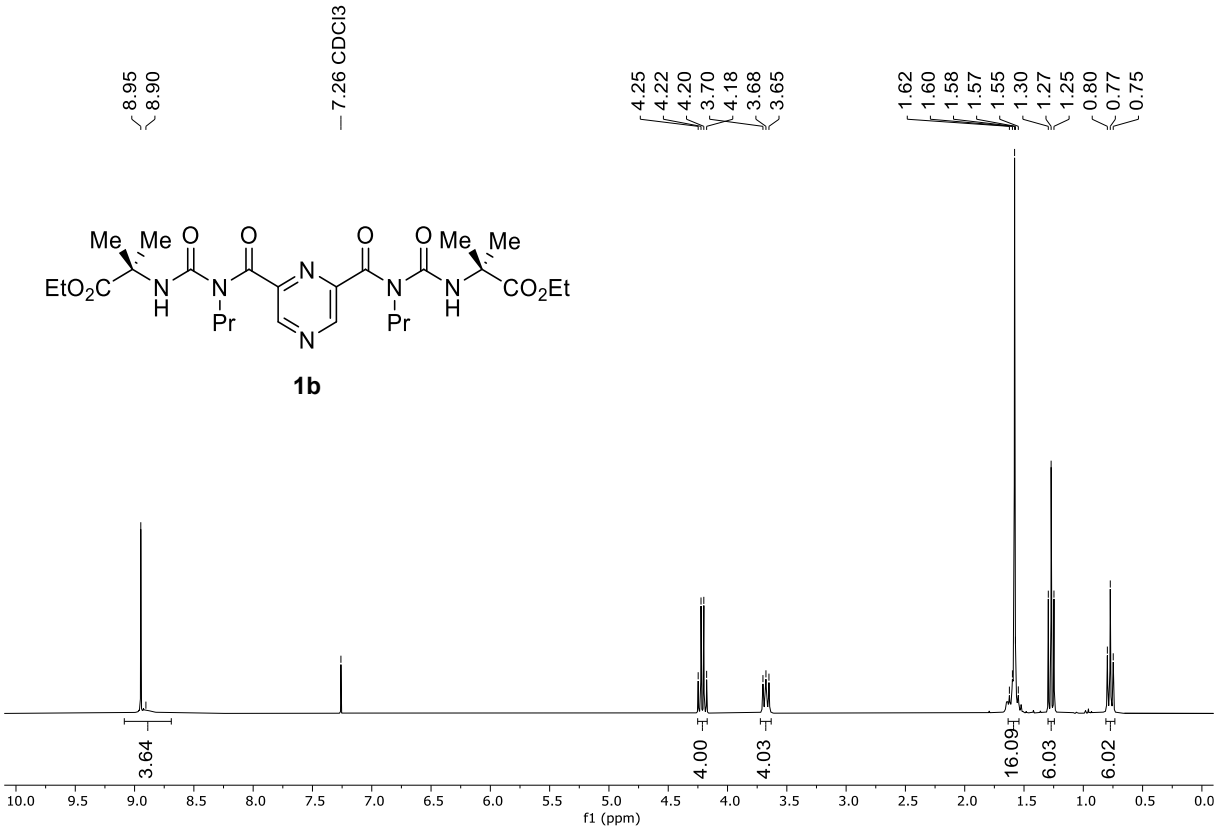
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.95 (s, 2H), 8.90 (bs, 2H) 4.21 (q, *J* = 7.1 Hz, 4H), 3.72 – 3.63 (m, 4H), 1.63 – 1.53 (m, 16H), 1.27 (t, *J* = 7.1 Hz, 6H), 0.77 (t, *J* = 7.4 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.2 ppm], ppm) δ = 174.2, 168.8, 152.7, 147.4, 145.0, 61.7, 57.1, 47.5, 24.9, 23.1, 14.2, 11.1.

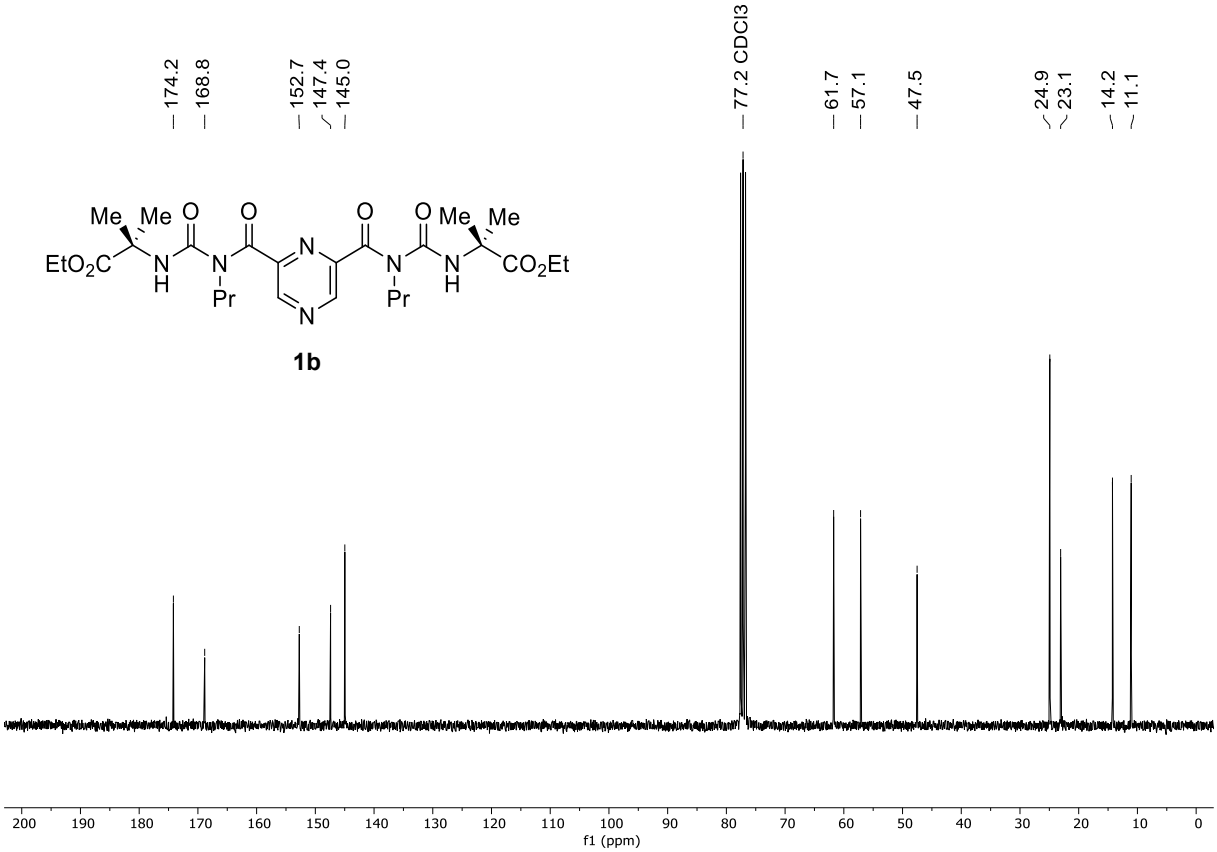
MS (ESI): *m/z* (%) = 587 (100, [M+Na$^+$]) ,434 (7), 408 (33), 251 (20).

HRMS (ESI-TOF): *m/z* [M+Na]+ calcd for $[C_{26}H_{40}N_6O_8Na]^+$: 587.2800; found: 587.2813 (Error PPM: 2.2).

IR (ATR, neat, cm^{-1}): 3306(w), 2966(w), 2876(w), 1707(s), 1655(m), 1514(s), 1455(m), 1382(m), 1364(m), 1282(m), 1241(m), 1201(m), 1147(s), 1080(s), 1017(s), 862(w), 767(m), 712(w), 546(m), 420(m).

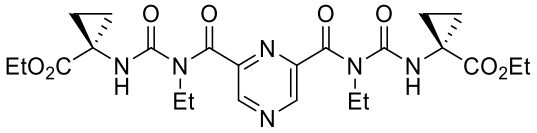


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)

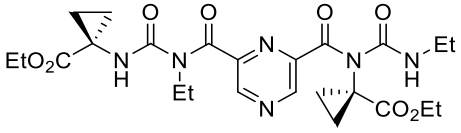


^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 1,1'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis(azanediyl))bis(cyclopropane-1-carboxylate) (1c) and ethyl 1-(3-(6-((1-(ethoxycarbonyl)cyclopropyl)(ethylcarbamoyl)carbamoyl)pyrazine-2-carbonyl)-3-ethylureido)cyclopropane-1-carboxylate (1c')



1c (major isomer)



1c'(minor isomer)

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (338 mg, 2.01 mmol, 1.00 equiv.) and the corresponding urea **S1c** (1.00 g, 4.99 mmol, 2.48 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1), afforded the title compounds **1c:1c'** as an inseparable mixture (802 mg, 1.51 mmol, 75% yield, **1c:1c'** = 2:1) as a yellow solid.

$\mathbf{R}_f$ = 0.32 (*n*-pentane:ethyl acetate = 1:2, UV)

m.p. = 63-65°C

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.02 (s, 1H), 8.94 (s, 2H), 8.91 – 8.73 (m, 2H), 4.20 – 4.12 (m, 6H), 3.85 – 3.70 (m, 5H), 3.35 – 3.28 (m, 1H), 1.63 – 1.57 (m, 5H), 1.25 – 1.11 (m, 25H).Collection of peaks of both isomers.

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 172.1, 168.9, 154.7, 147.4, 144.8, 61.6, 41.6, 34.6, 17.7, 14.9, 14.3. Collection of peaks of the major isomere

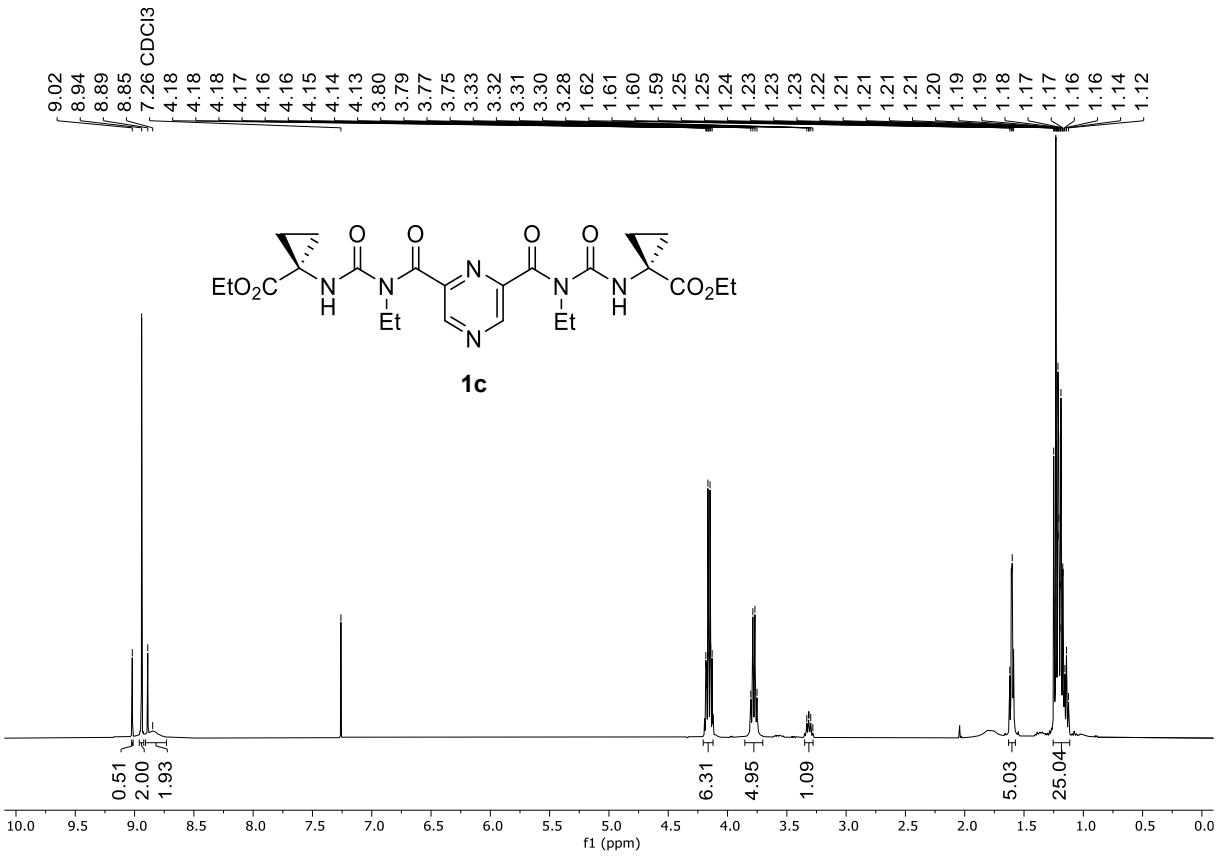
HPLC (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI)**: 1c** tR = 18.7, **1c'** tR = 18.9.

1c MS (ESI): *m/z* (%) = 555 (100, $[M+Na]^+$), 404 (59), 378 (52), 294 (24), 286 (26), 223 (25).

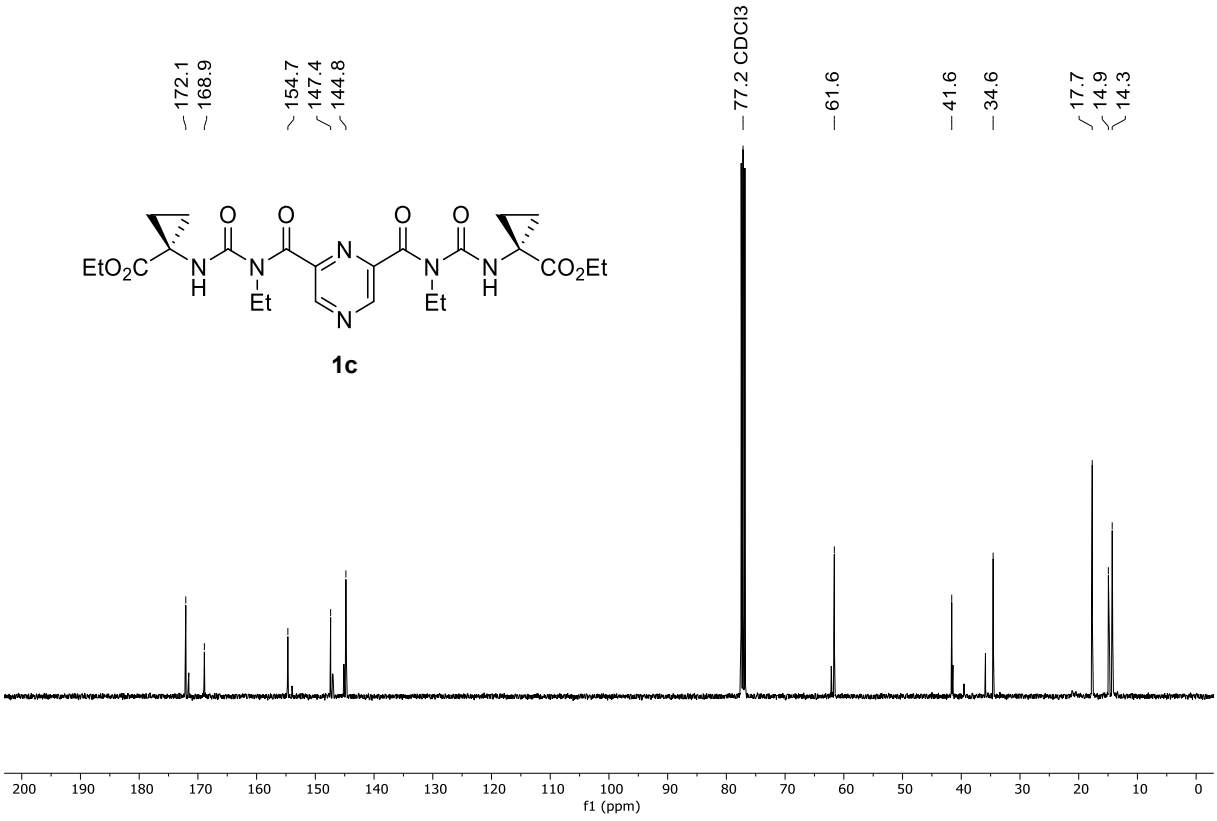
1c' MS (ESI): m/z (%) = 555 (100, $[M+Na]^+$), 488 (59), 404 (67), 378 (52), 307 (14), 286 (15), 223 (25).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{24}H_{32}N_6O_8Na]^+$: 555.2173; found: 555.2181 (Error PPM: 1.4).

IR (ATR, neat, $\tilde{\nu}$) = 3307 (w), 2981 (w), 2940 (vw), 1694 (vs), 1656 (vs), 1509 (s), 1446 (m), 1421 (m), 1393 (s), 1370 (vs), 1329 (s), 1295 (s), 1252 (vs), 1177 (vs), 1156 (vs), 1093 (s), 1068 (vs), 1017 (vs), 956 (w), 938 (w), 909 (m), 863 (w), 840 (w), 812 (w), 771 (s), 750 (s), 705 (m), 624 (m), 606 (m), 563 (m), 502 (m), 410 (s) cm^{-1}.

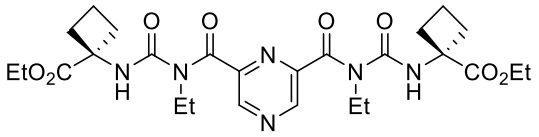


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of iethyl 1,1'-((((pyrazine-2,6 dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis(azanediyl))bis(cyclobutane-1-carboxylate) (1d)



1d

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (180 mg, 1.07 mmol, 1.00 equiv.) and the corresponding urea **S1d** (578 mg, 2.70 mmol, 2.51 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 1:1), afforded the title compound **1d** (418 mg, 745 µmol, 69% yield) as a yellow resin.

R_f = 0.23 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = N/A

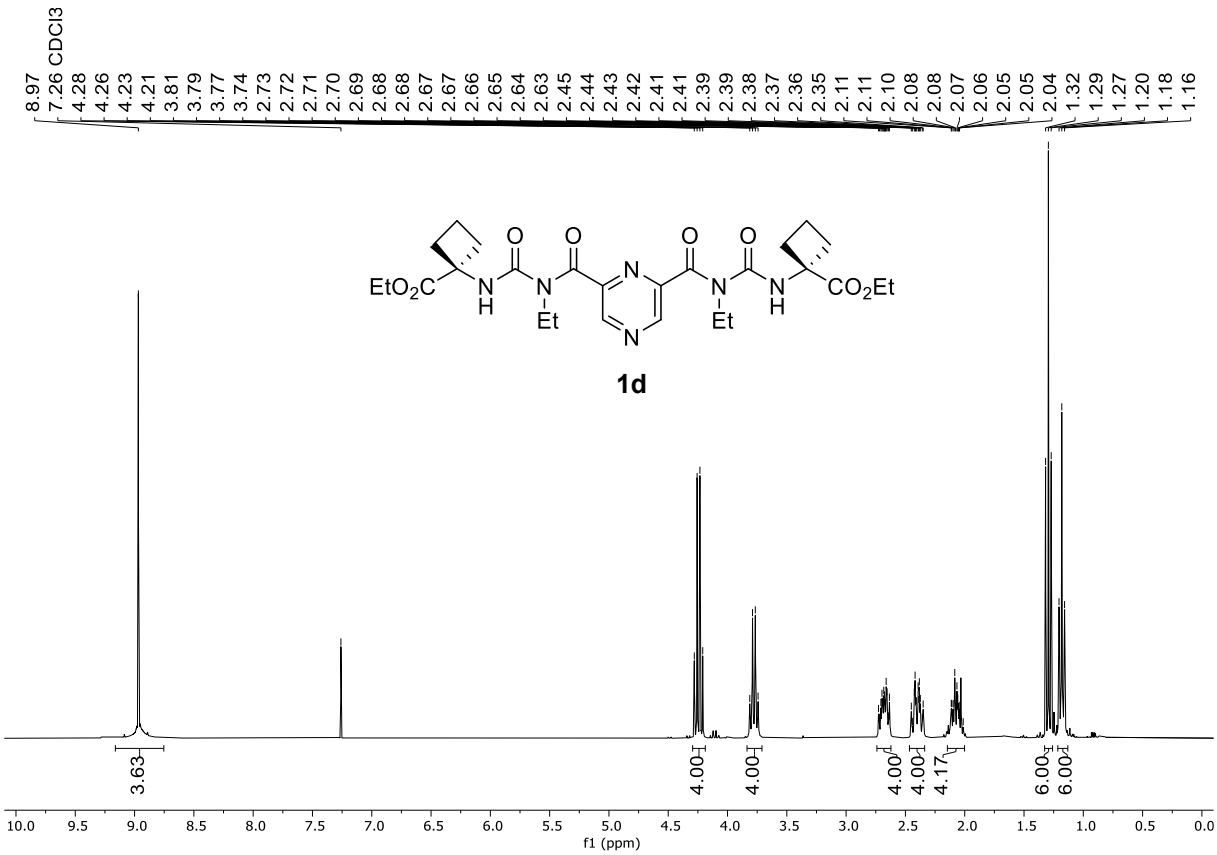
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.97 (s, 2H), 8.97 (bs, 2H), 4.24 (q, *J* = 7.1 Hz, 4H), 3.78 (q, *J* = 7.0 Hz, 4H), 2.74 – 2.62 (m, 4H), 2.47 – 2.34 (m, 4H), 2.15 – 2.00 (m, 4H), 1.29 (t, *J* = 7.1 Hz, 6H), 1.18 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 173.2, 168.8, 152.9, 147.4, 144.9, 61.6, 59.0, 41.4, 31.6, 15.4, 15.0, 14.3.

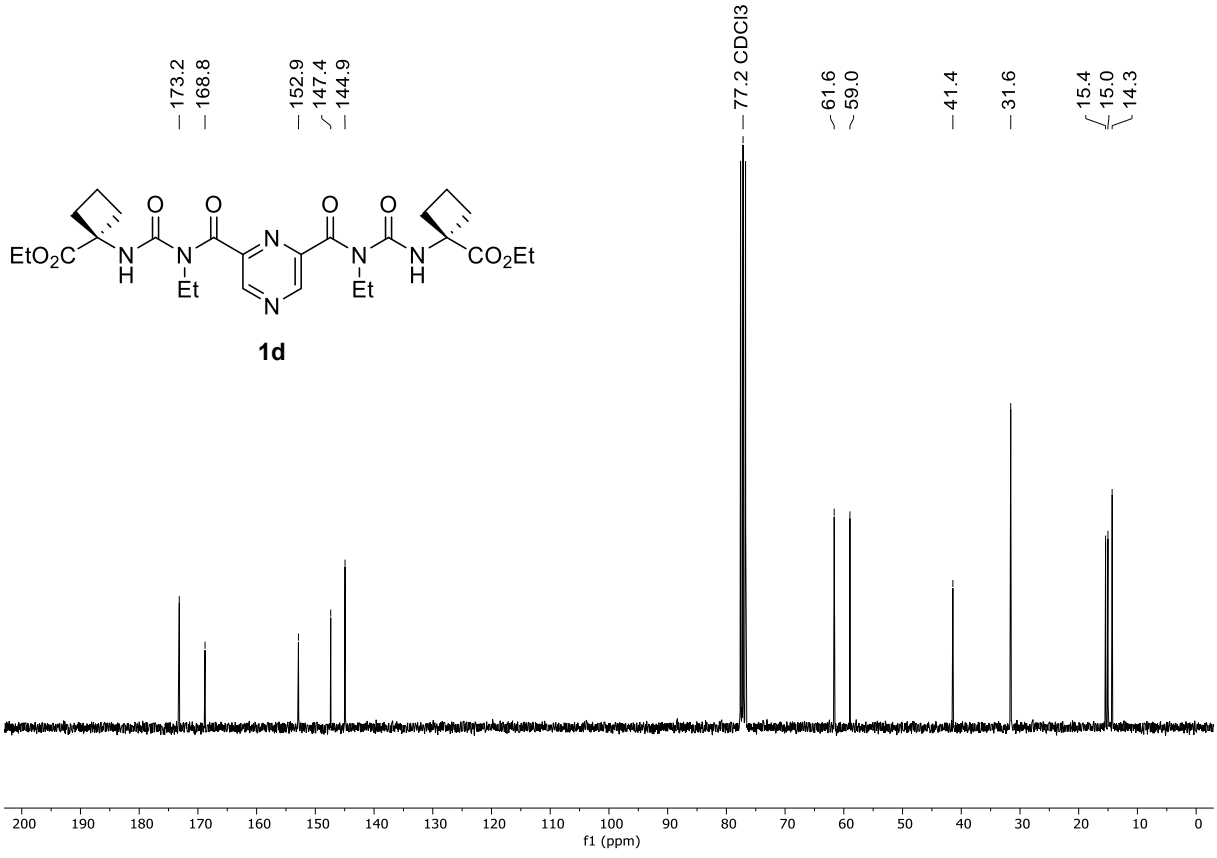
MS (ESI): *m/z* (%) = 583 (100, $[M+Na]^+$), 392 (22), 300 (12), 223 (13).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{26}H_{36}N_6O_8Na]^+$: 583.2487; found: 583.2498 (Error PPM: 1.9)

IR (ATR, neat, ṽ) = 3320 (w), 2979 (w), 2959 (w), 1733 (s), 1701 (vs), 1654 (vs), 1507 (vs), 1460 (m), 1446 (m), 1427 (m), 1393 (s), 1368 (vs), 1309 (vs), 1256 (vs), 1199 (vs), 1099 (vs), 1079 (vs), 1017 (vs), 975 (w), 956 (w), 940 (w), 911 (w), 860 (w), 795 (m), 773 (s), 734 (w), 697 (w), 671 (m), 593 (m), 577 (m), 546 (m), 514 (m), 493 (m), 483 (m), 420 (s) cm^{-1}.

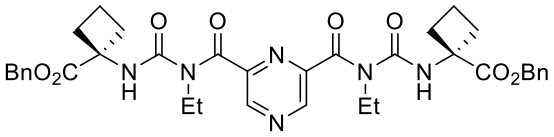


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dibenzyl 1,1'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis(azanediyl))bis(cyclobutane-1-carboxylate) (1y) JM10



1y

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (1.01 g, 6.00 mmol, 1.00 equiv.) and the corresponding urea **S1y** (4.14 g, 15.0 mmol, 2.50 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1 to 1:1), afforded the title compound **1y** (3.02 g, 4.42 mmol, 74% yield) as a yellow solid.

$\mathbf{R}_f$ = 0. (*n*-pentane:ethyl acetate = 3:2, Detection)

m.p. = 125-126 °C

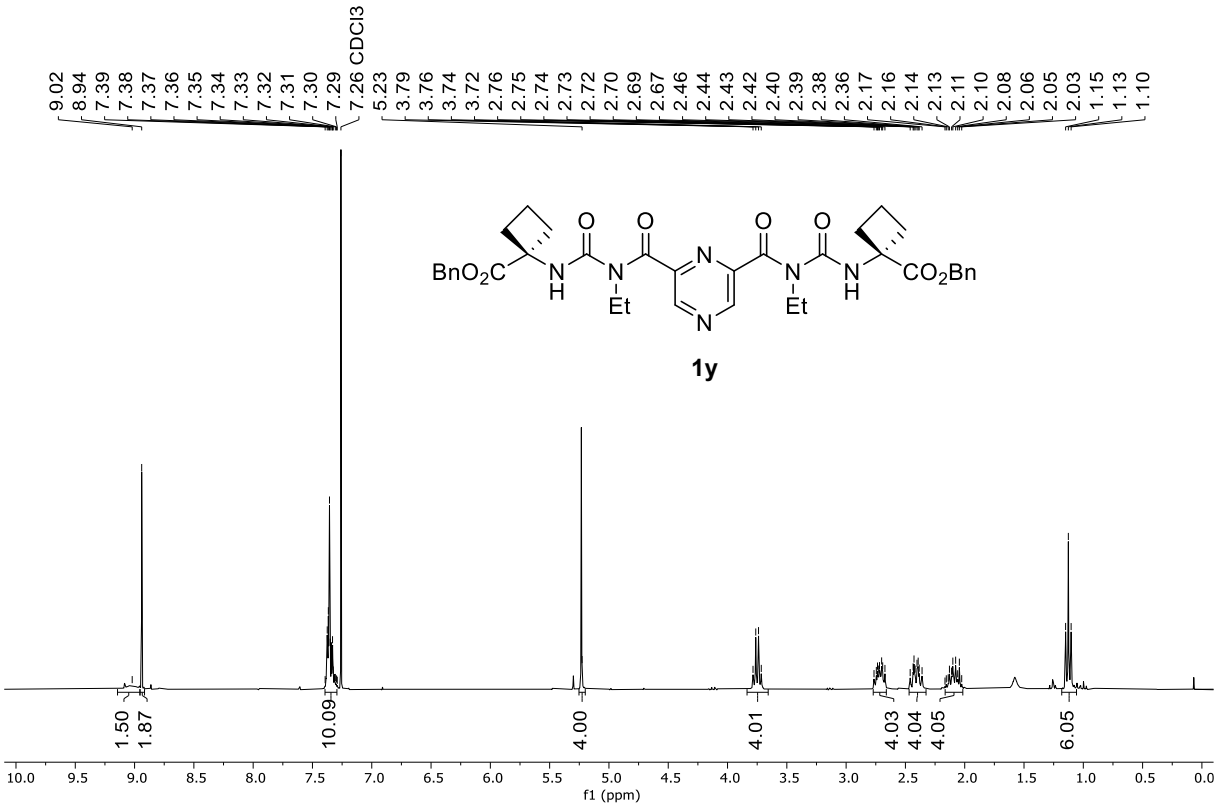
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.02 (bs, 2H), 8.94 (s, 2H), 7.44 – 7.26 (m, 10H), 5.23 (s, 4H), 3.75 (q, *J* = 6.9 Hz, 4H), 2.78 – 2.66 (m, 4H), 2.49 – 2.33 (m, 4H), 2.22 – 1.98 (m, 4H), 1.13 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 173.0, 168.8, 152.9, 147.4, 144.9, 135.9, 128.7, 128.4, 128.1, 67.3, 59.0, 41.4, 31.6, 15.5, 15.0.

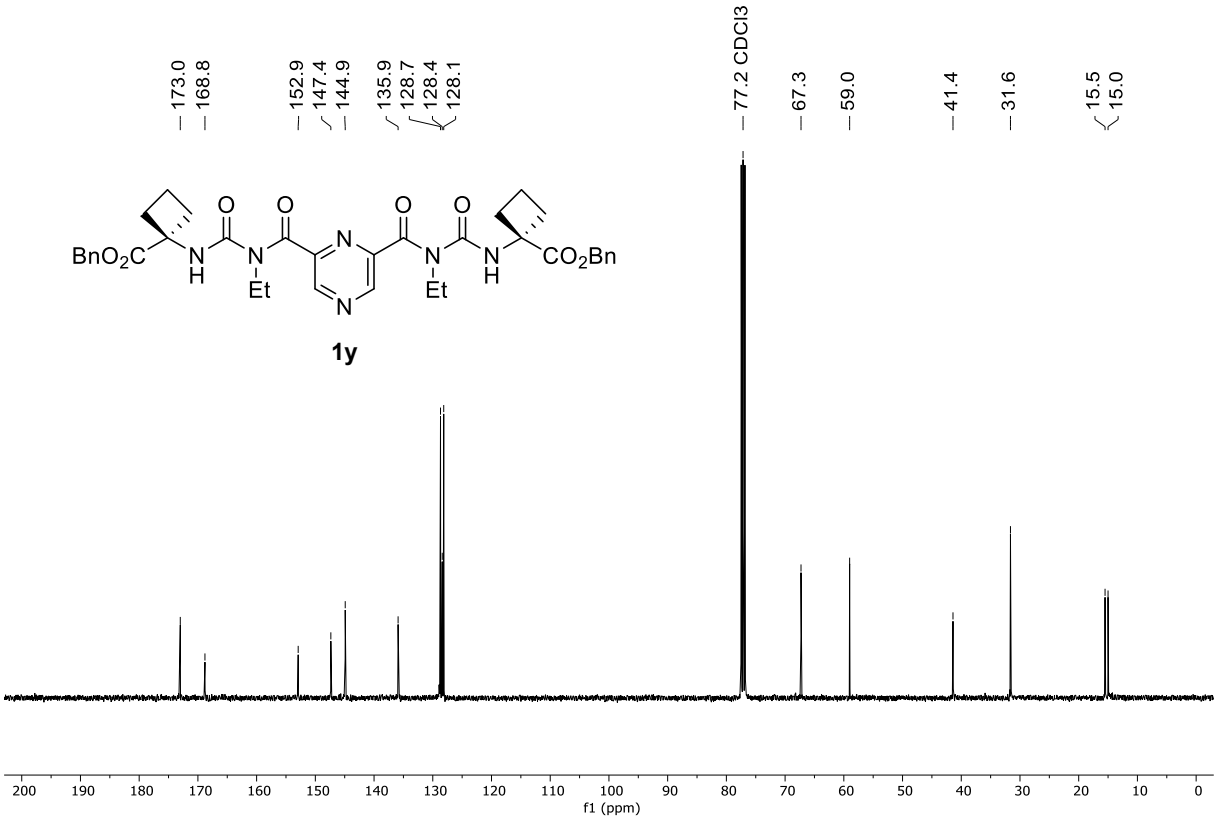
MS (ESI): *m/z* (%) = 707 (33, $[M+Na]^+$), 480 (35), 454 (100), 223 (29).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{36}H_{40}N_6O_8Na]^+$: 707.2800; found: 707.2804 (Error PPM: 0.6).

IR (ATR, neat, $\tilde{\nu}$) = 3305 (w), 2975 (w), 2955 (w), 1733 (s), 1701 (vs), 1654 (vs), 1588 (w), 1499 (vs), 1456 (s), 1427 (s), 1395 (s), 1376 (vs), 1356 (s), 1305 (vs), 1256 (vs), 1195 (vs), 1101 (vs), 1079 (vs), 1030 (m), 1017 (s), 1003 (m), 964 (s), 909 (m), 863 (w), 822 (w), 791 (m), 775 (s), 738 (vs), 695 (vs), 677 (s), 581 (s), 504 (s), 459 (s), 418 (s), 402 (s).

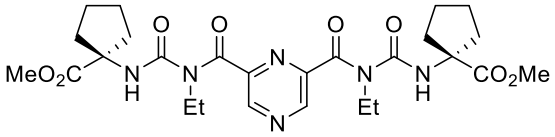


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dimethyl 1,1'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis-(azanediyl))bis(cyclopentane-1-carboxylate) (1e)



2e

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (169 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea **S1e** (535 mg, 2.50 mmol, 2.49 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 1:1)., afforded the title compound **1e** (411 mg, 734 µmol, 73% yield) as an off-white solid.

R_f = 0.21 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = broad melting point starting at 45 °C

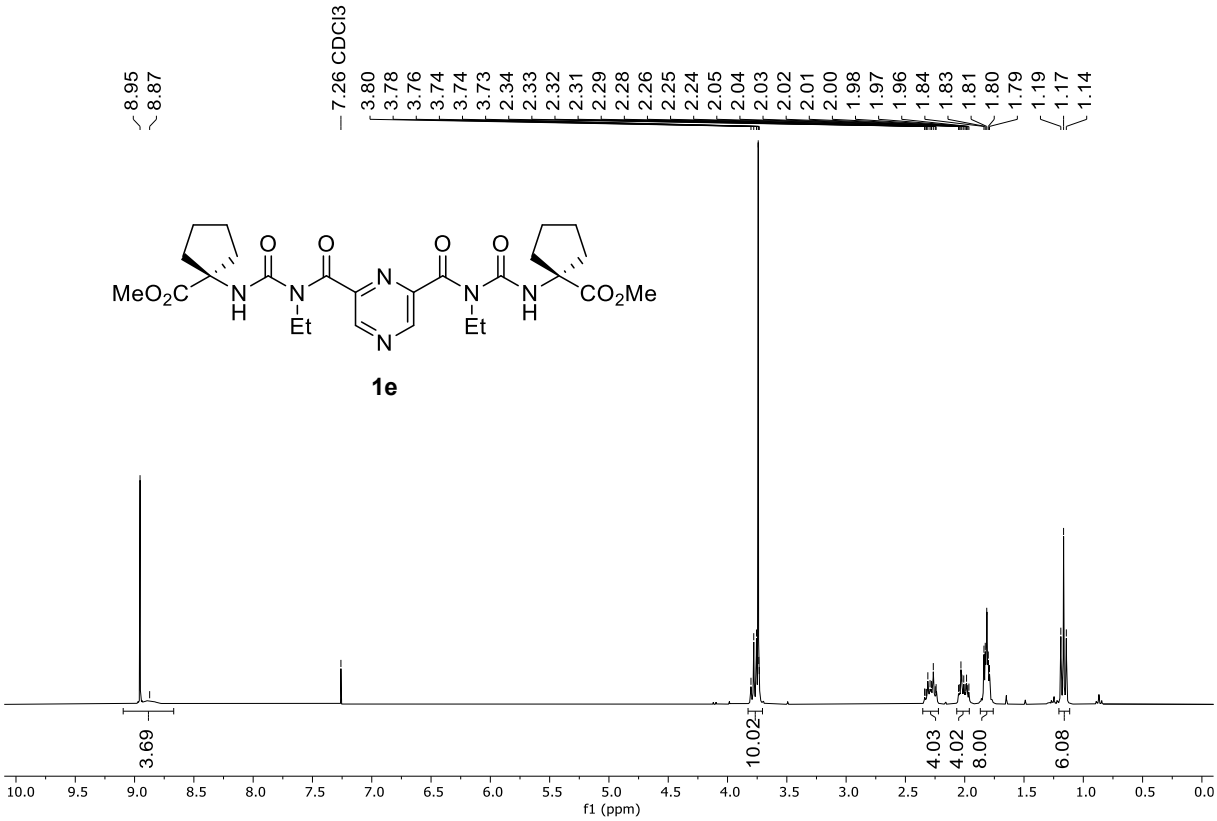
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.95 (s, 2H), 8.87 (bs, 2H), 3.83 – 3.71 (m, 10H), 2.35 – 2.22 (m, 4H), 2.07 – 1.96 (m, 4H), 1.87 – 1.76 (m, 8H), 1.17 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.4, 168.9, 153.1, 147.4, 144.8, 66.7, 52.7, 41.5, 37.5, 24.8, 15.1.

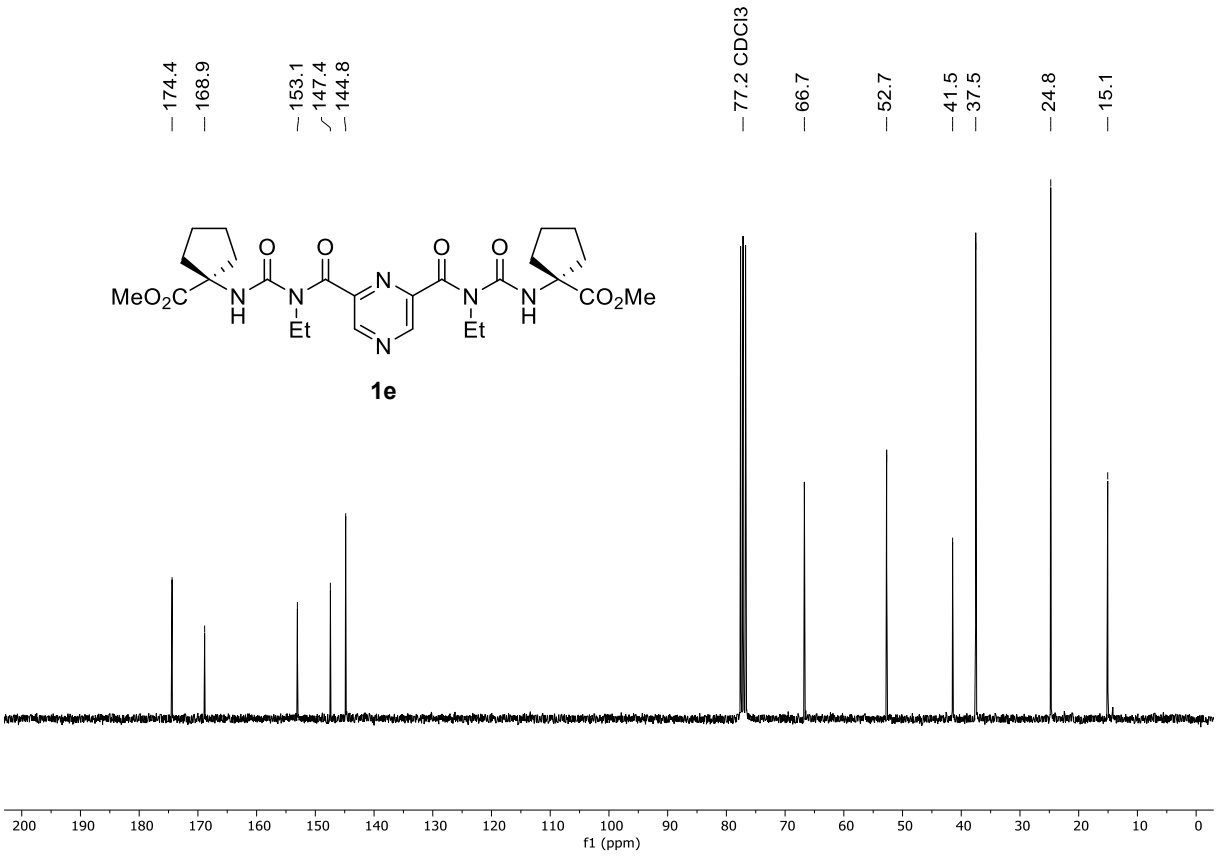
MS (ESI): *m/z* (%) = 583 (100, $[M+Na]^+$), 392 (38), 223 (37).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{26}H_{36}N_6O_8Na]^+$: 583.2487; found: 583.2491 (Error PPM: 0.7).

IR (ATR, neat, ṽ) = 3317 (w), 2955 (w), 2873 (w), 1737 (vs), 1703 (vs), 1654 (vs), 1509 (vs), 1435 (vs), 1395 (s), 1370 (vs), 1317 (s), 1250 (vs), 1195 (vs), 1166 (vs), 1079 (vs), 1032 (s), 1015 (vs), 962 (m), 936 (m), 907 (m), 824 (w), 795 (s), 773 (s), 746 (m), 705 (m), 571 (s), 504 (m), 459 (m), 420 (s) cm^{-1}.

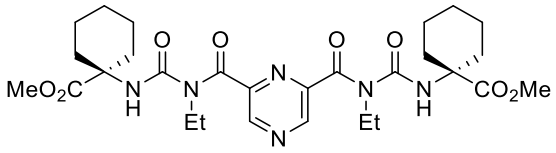


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dimethyl 1,1'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis-(azanediyl))bis(cyclohexane-1-carboxylate) (1f)



1f

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (168 mg, 1.00 mmol, 1.00 equiv) and the corresponding urea **S1f** (571 mg, 2.50 mmol, 2.50 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 1:1), afforded the title compound **1f** (378 mg, 642 µmol, 64% yield) as an off-white solid.

R_f = 0.31 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = broad melting point starting at 50 °C

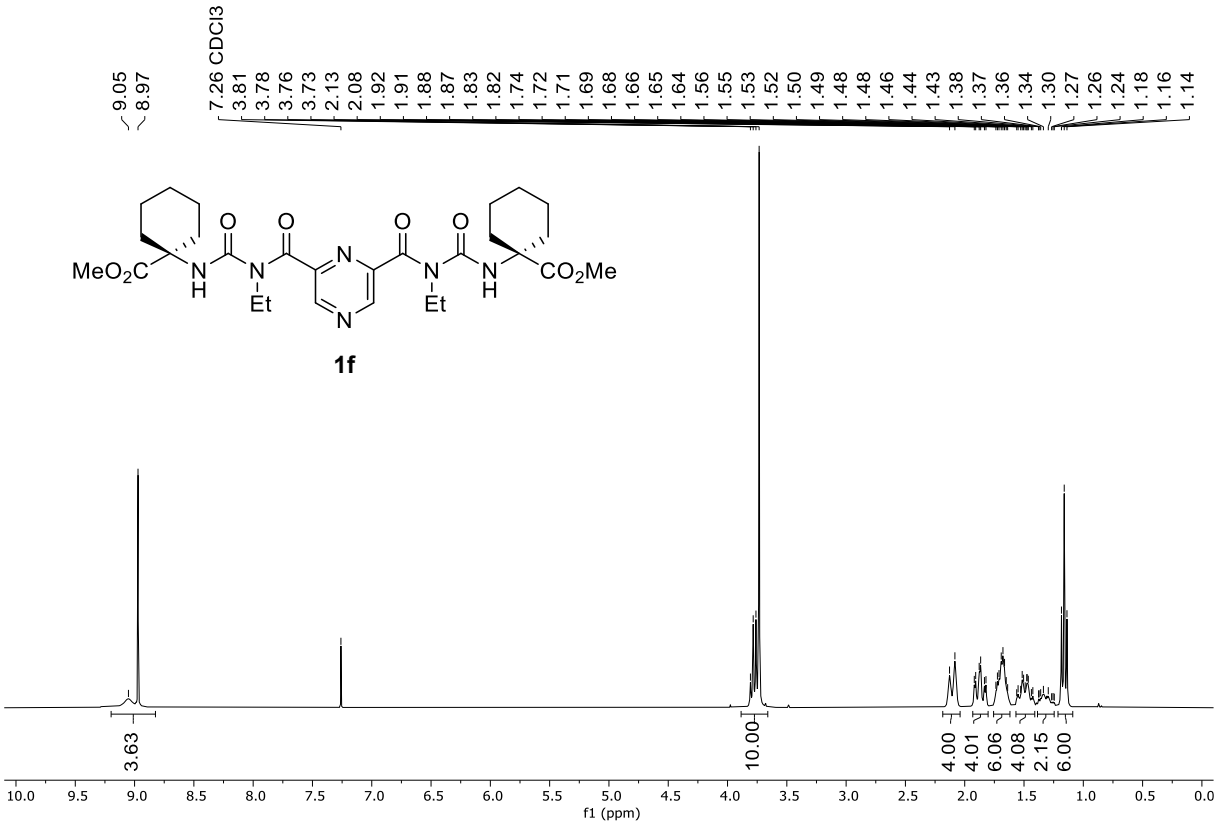
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.05 (bs, 2H), 8.97 (s, 2H), 3.89 – 3.66 (m, 10H), 2.19 – 2.04 (m, 4H), 1.87 (td, *J* = 12.9, 3.7 Hz, 4H), 1.76 – 1.62 (m, 6H), 1.57 – 1.41 (m, 4H), 1.39 – 1.25 (m, 2H), 1.16 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.4, 169.1, 152.5, 147.6, 144.8, 59.7, 52.5, 41.5, 32.5, 25.3, 21.6, 15.2.

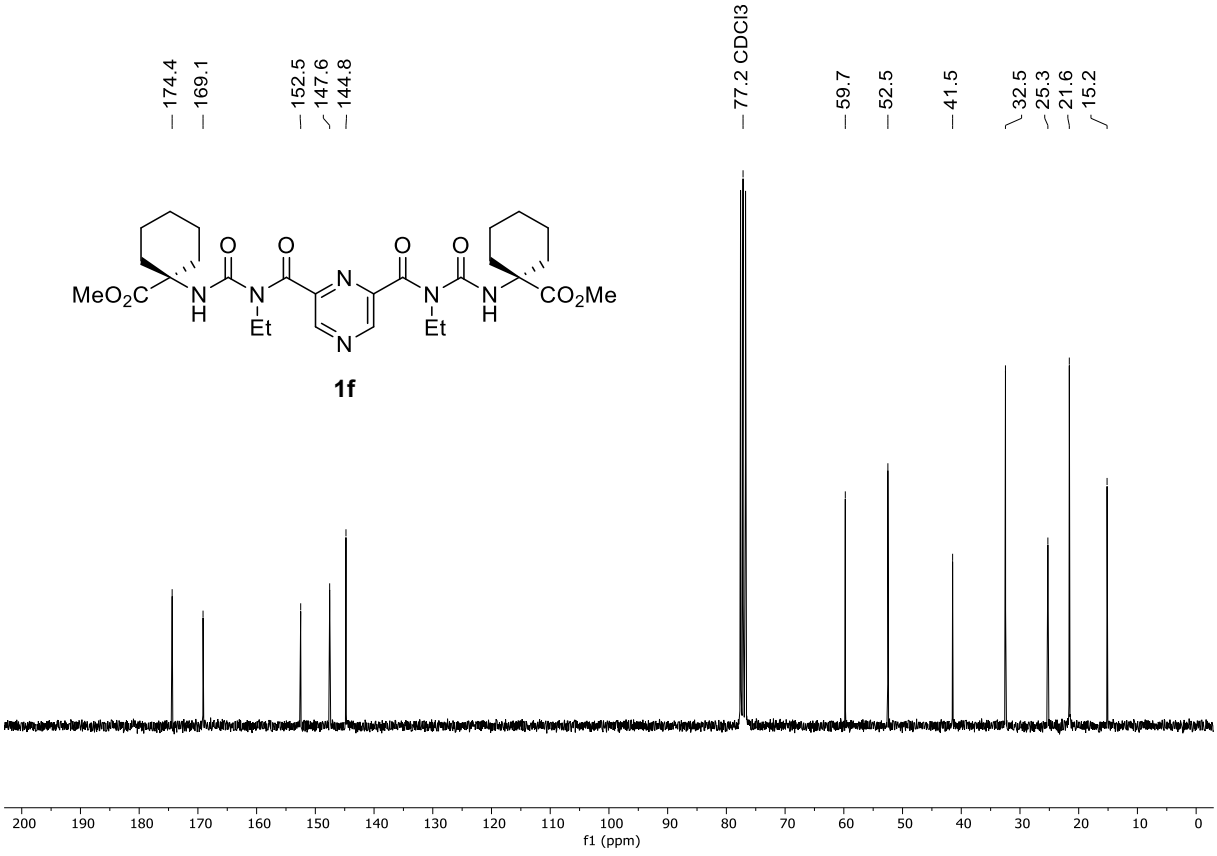
MS (ESI): *m/z* (%) = 611 (100, $[M+Na]^+$), 406 (52), 223 (33).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{28}H_{40}N_6O_8Na]^+$: 611.2800; found: 611.2817 (Error PPM: 2.8).

IR (ATR, neat, $\tilde{\nu}$) = 3293 (w), 2936 (w), 2859 (w), 1739 (s), 1707 (vs), 1652 (s), 1515 (vs), 1446 (s), 1433 (s), 1376 (s), 1354 (s), 1315 (w), 1276 (vs), 1232 (vs), 1211 (vs), 1195 (vs), 1160 (vs), 1099 (s), 1070 (vs), 1015 (s), 983 (m), 960 (w), 934 (w), 903 (w), 850 (w), 826 (w), 793 (m), 775 (s), 738 (w), 695 (m), 612 (m), 587 (w), 542 (m), 512 (w), 475 (w), 416 (m) cm^{-1}.

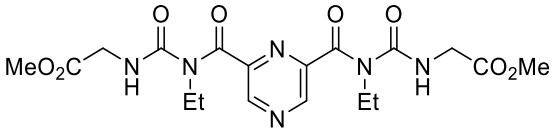


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)

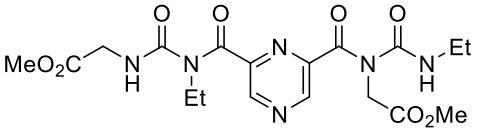


^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dimethyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis-(azanediyl))diacetate (1g) and methyl *N*-(6-(ethyl((2-methoxy-2-oxoethyl)carbamoyl)-carbamoyl)pyrazine-2-carbonyl)-*N*-(ethylcarbamoyl)glycinate (1g')



1g (major isomer)



1g' (minor isomer)

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (168 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea **S1g** (430 mg, 2.47 mmol, 2.47 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 1:2), afforded the title compounds **1g** and **1g**'as an inseparable mixture (132 mg, 292 µmol, 29% yield, **1g:1g'** = 6.9:1) as a yellow resin.

$\mathbf{R}_f$ = 0.23 (*n*-pentane:ethyl acetate = 1:2, UV)

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.97 (s, 2H), 8.84 (s, 2H), 4.13 (d, *J* = 5.3 Hz, 4H), 3.91 – 3.74 (m, 10H), 1.19 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.2 ppm], ppm) δ = 169.9, 168.8, 154.2, 147.1, 145.0, 52.5, 42.4, 41.6, 14.9.

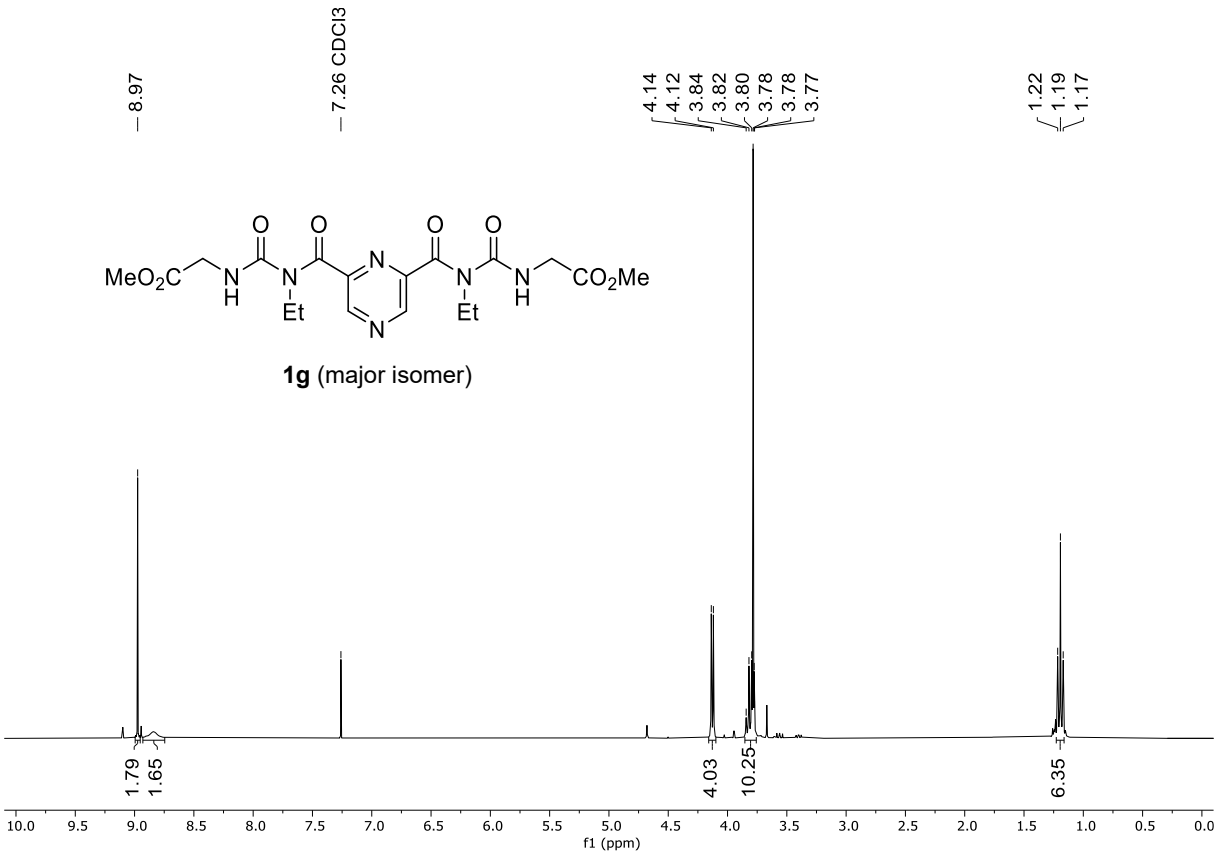
HPLC (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI)**:** **1g** t_R = 13.5, **1g'** t_R = 14.1.

1g MS (ESI): *m/z* (%) = 475 (83, [M+Na$^+$]), 364 (100), 338 (15).

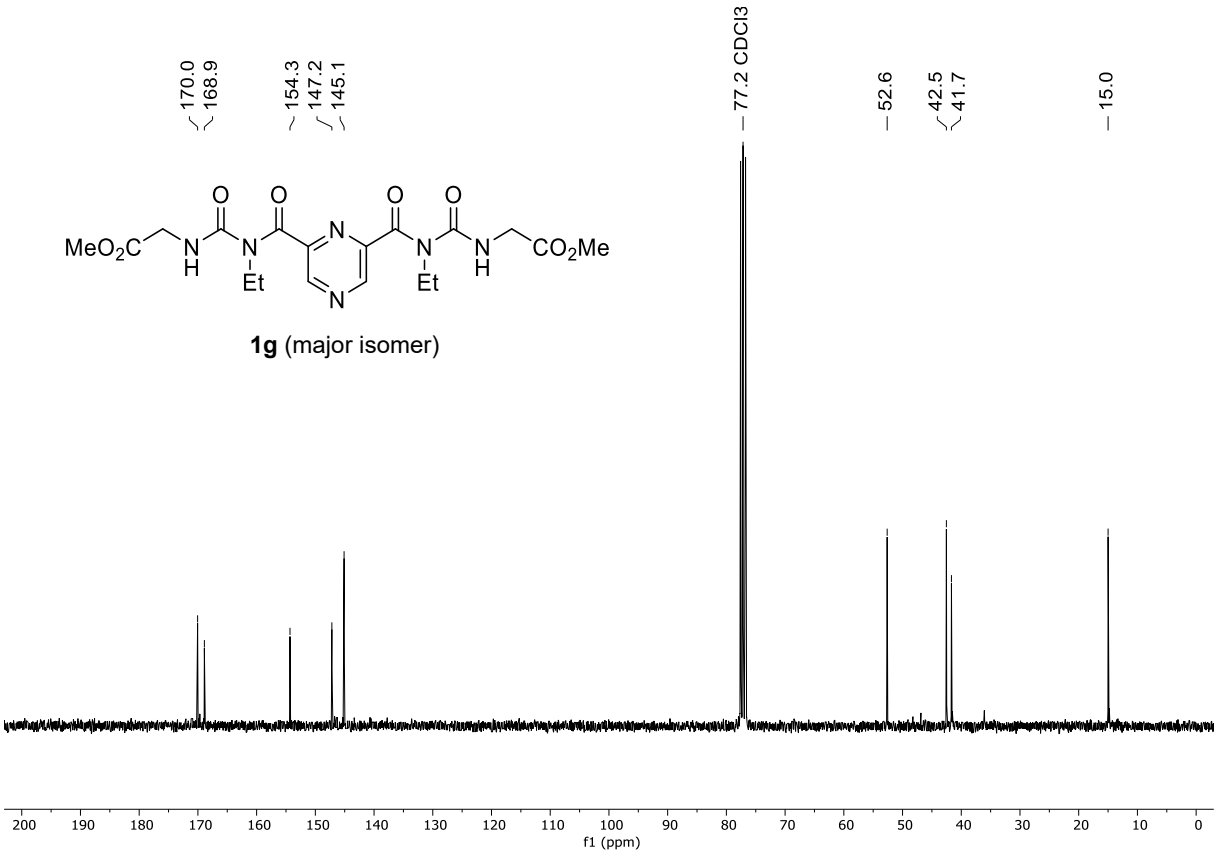
1g' MS (ESI): m/z (%) = 475 (100, [M+Na$^+$]), 408 (63), 246 (10).

HRMS (ESI-TOF): *m/z* [M+Na]$^+$ calcd. for [$C_{18}H_{24}N_6O_8Na$]$^+$: 475.1548 found: 475.1561 (Error PPM: 2.7).

IR (ATR, neat, $\tilde{\nu}$) = 3315 (w), 2981 (w), 2955 (w), 1747 (s), 1692 (vs), 1654 (vs), 1517 (vs), 1433 (s), 1368 (vs), 1268 (s), 1193 (vs), 1099 (vs), 1060 (vs), 1015 (vs), 985 (s), 928 (w), 911 (w), 877 (w), 848 (w), 793 (m), 773 (s), 703 (m), 567 (s), 534 (s), 414 (s) cm^{-1}.

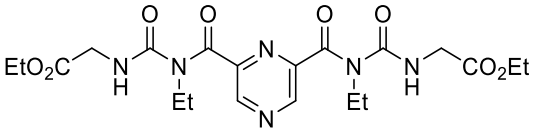


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)

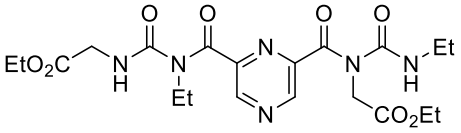


^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))-bis(azanediyl))diacetate (1h) and ethyl ((6-((2-ethoxy-2-oxoethyl)(ethylcarbamoyl)carbamoyl)-pyrazine-2-carbonyl)(ethyl)carbamoyl)glycinate (1h')



1h (major isomer)



1h' (minor isomer)

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (168 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea **S1h** (435 mg, 2.50 mmol, 2.50 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 1:1 to 1:2), afforded the title compounds **1h** and **1h**'as an inseparable mixture (203 mg, 423 µmol, 42% yield, **1h:1h'** = 8.7:1) as a yellow resin.

$\mathbf{R}_f$ = 0.15 (*n*-pentane:ethyl acetate = 1:1, UV)

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.97 (s, 2H), 8.81 (s, 2H), 4.23 (q, *J* = 7.2 Hz, 4H), 4.10 (d, *J* = 5.3 Hz, 4H), 3.80 (q, *J* = 7.0 Hz, 4H), 1.29 (t, *J* = 7.2 Hz, 6H), 1.19 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 169.6, 168.9, 154.3, 147.2, 145.1, 61.8, 42.7, 41.6, 15.0, 14.3.

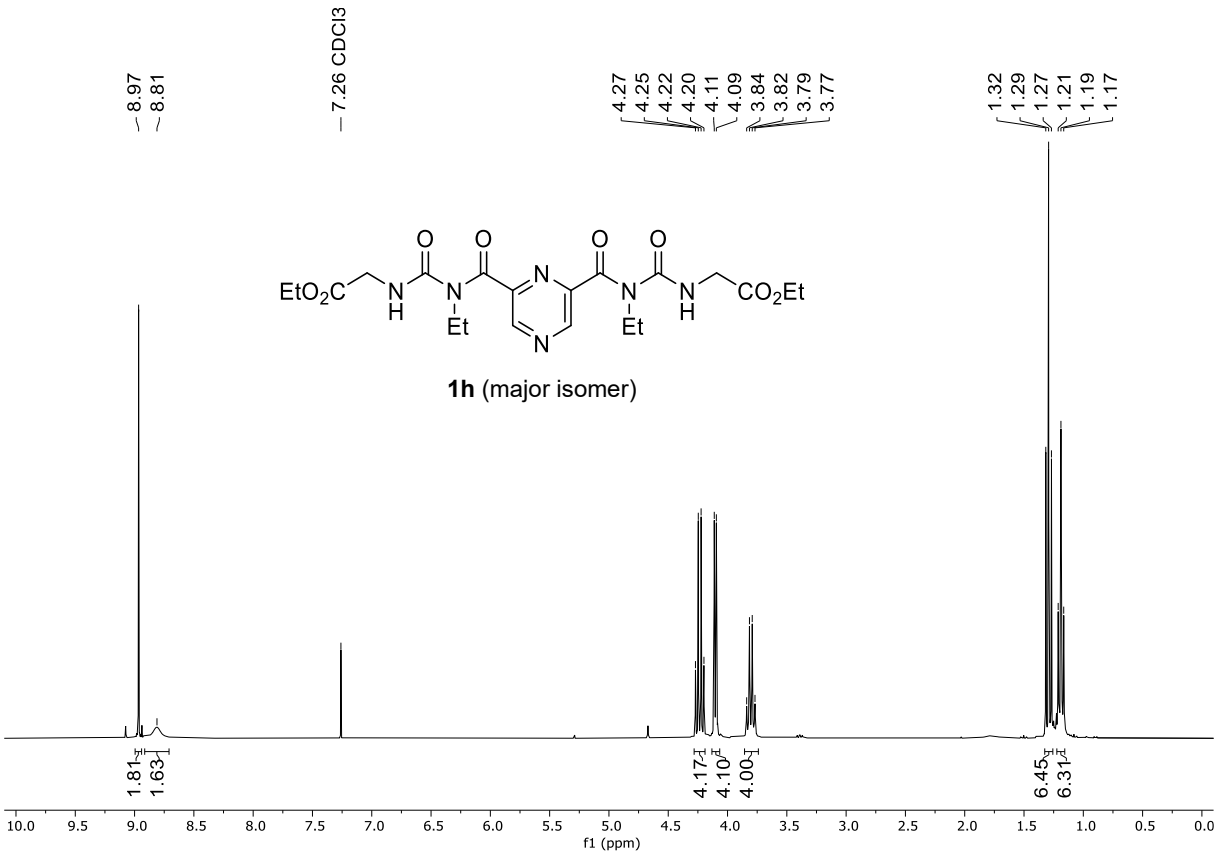
HPLC (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI): **1h** tR = 16.7, **1h'** tR = 17.3.

1h MS (ESI): *m/z* (%) = 503 (100, $[M+Na]^+$), 378 (68), 268 (39), 260 (25).

1h' MS (ESI): m/z (%) = 503 (100, $[M+Na]^+$), 436 (25), 268 (13), 260 (13).

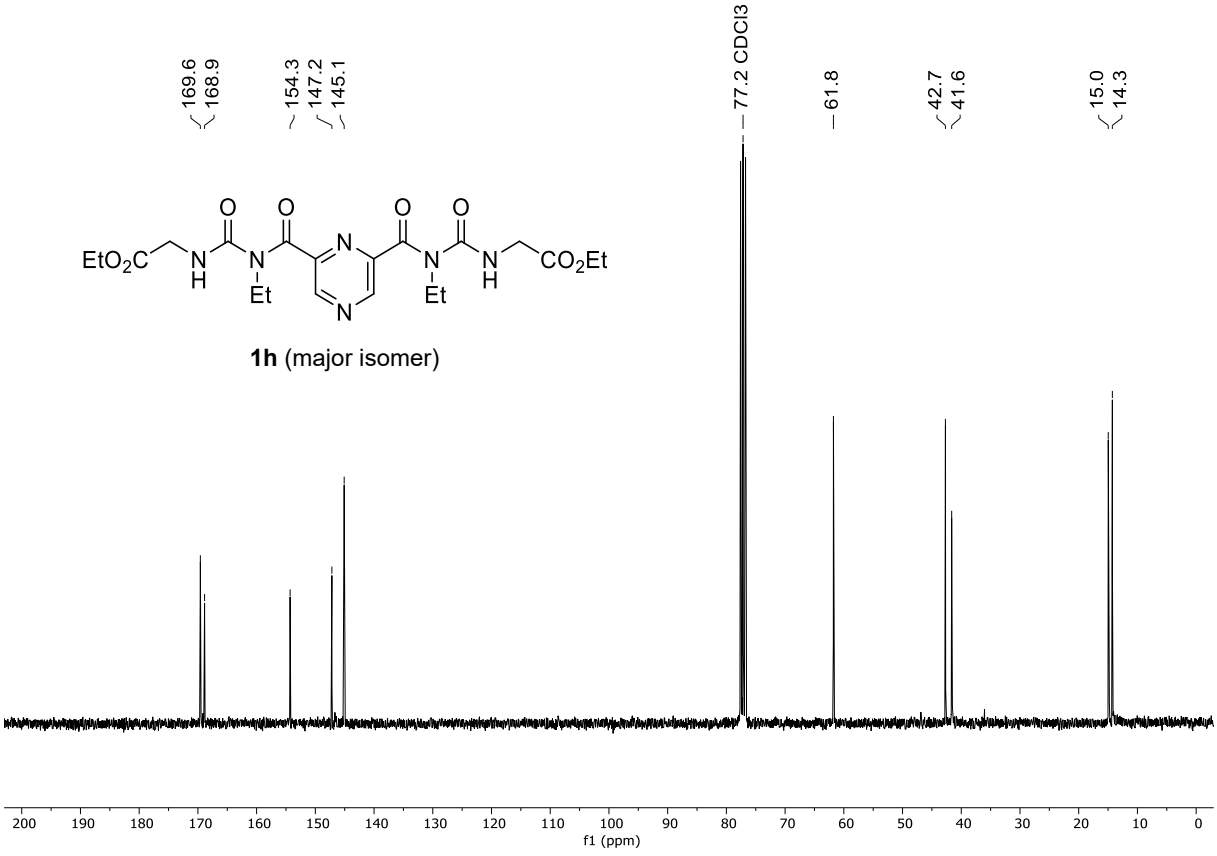
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{20}H_{28}N_6O_8Na]^+$: 503.1861; found: 503.1862 (Error PPM: 0.2).

IR (ATR, neat, $\tilde{\nu}$) = 3315 (w), 2983 (w), 2938 (vw), 2910 (vw), 1743 (m), 1690 (vs), 1656 (vs), 1517 (vs), 1462 (m), 1431 (m), 1393 (s), 1374 (vs), 1354 (s), 1289 (m), 1268 (m), 1189 (vs), 1095 (vs), 1060 (vs), 1015 (vs), 954 (w), 932 (w), 911 (w), 863 (w), 812 (w), 793 (w), 773 (s), 732 (w), 703 (w), 632 (m), 593 (m), 567 (m), 536 (m), 414 (m) cm^{-1}.



1h (major isomer)

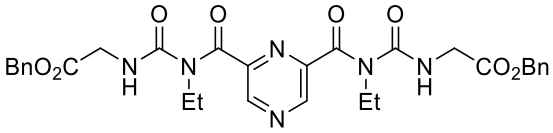
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



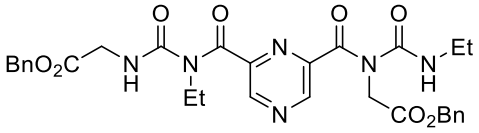
1h (major isomer)

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dibenzyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))-bis(azanediyl))diacetate (1i) and benzyl ((6-((2-(benzyloxy)-2-oxoethyl) (ethylcarbamoyl)carbamoyl)pyrazine-2-carbonyl)(ethyl)carbamoyl) glycinate (1i')



1i (major isomer)



1i' (minor isomer)

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (83.9 mg, 0.5 mmol, 1.00 equiv.) and the corresponding urea **S1i** (292 mg, 1.24 mmol, 2.48 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 1:1), afforded the title compounds **1i** and **1i**'as an inseparable mixture (59 mg, 262 µmol, 53% yield**, 1i:1i'** = 8.1:1) as a yellow resin.

$\mathbf{R}_f$ = 0.27 (*n*-pentane:ethyl acetate = 1:1, UV)

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.97 (s, 2H), 8.88 (s, 2H), 7.41 – 7.28 (m, 10H), 5.21 (s, 4H), 4.17 (d, *J* = 5.3 Hz, 4H), 3.80 (q, *J* = 7.0 Hz, 4H), 1.18 (t, *J* = 7.0 Hz, 6H).

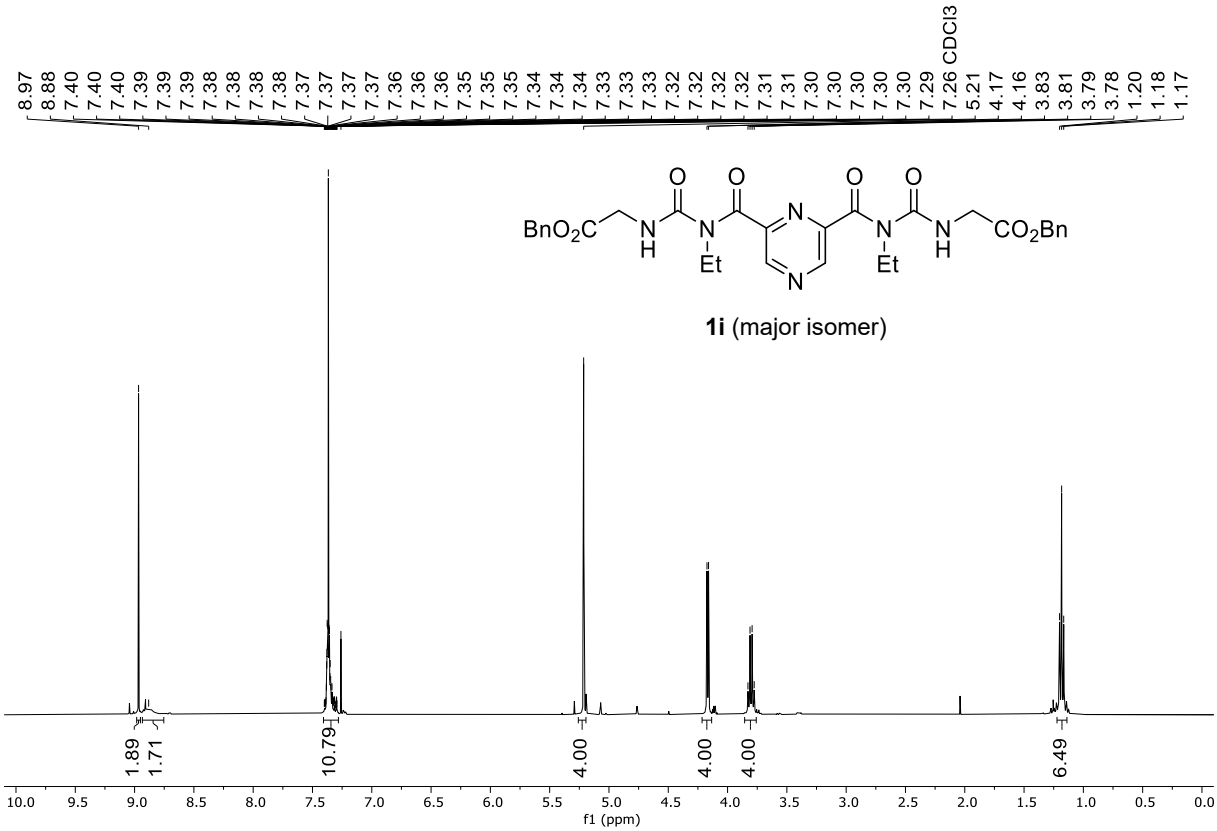
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 169.5, 168.9, 154.3, 147.2, 145.1, 135.3, 128.8, 128.7, 128.7, 128.6, 67.4, 42.7, 41.6, 15.0.

HPLC (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI): **1i** t_R = 23.0, **1i'** t_R = 23.4

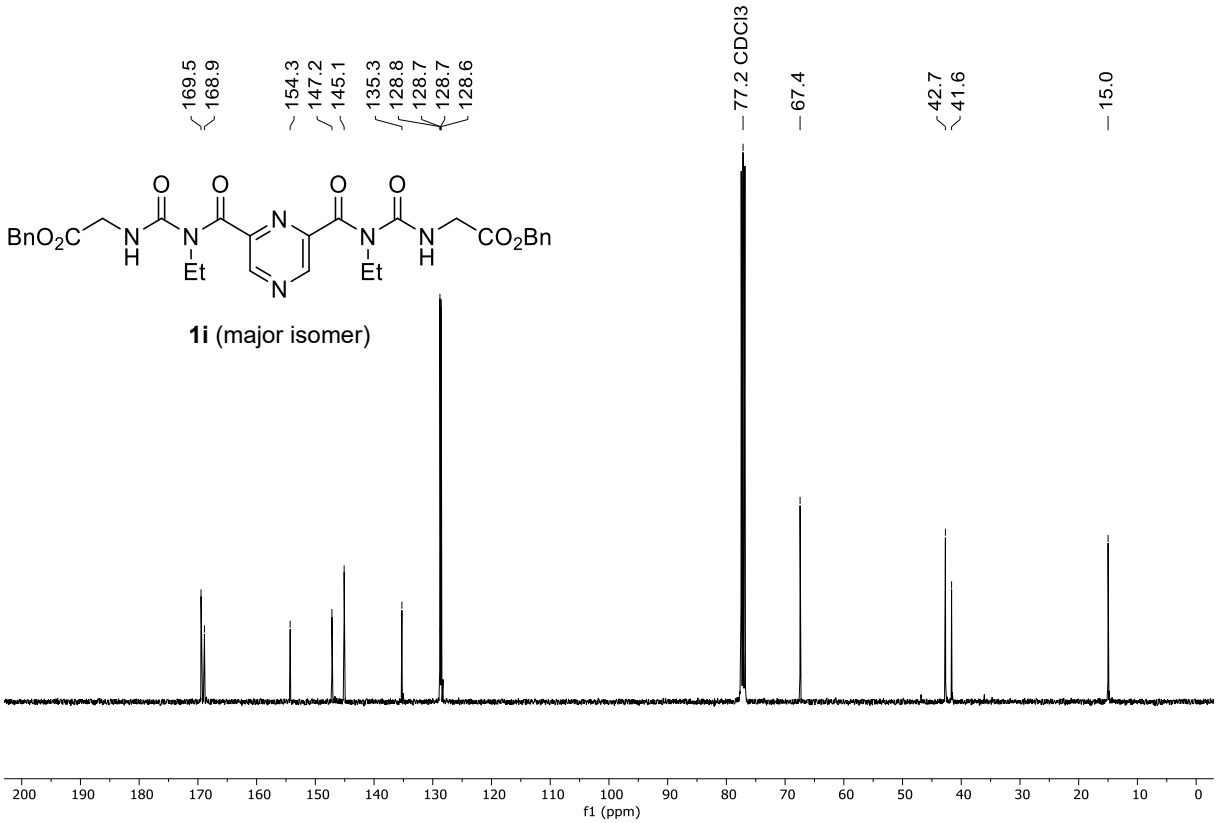
1i MS (ESI): *m/z* (%) = 627 (69, $[M+Na]^+$), 440 (100), 322 (50).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{30}H_{33}N_6O_8]^+$: 605.2355; found: 605.2368 (Error PPM: 2.1).

IR (ATR, neat, $\tilde{\nu}$) = 3309 (w), 2977 (vw), 2938 (vw), 1745 (m), 1701 (vs), 1656 (s), 1513 (vs), 1454 (m), 1431 (m), 1380 (vs), 1354 (vs), 1317 (w), 1258 (m), 1185 (vs), 1099 (vs), 1060 (vs), 1015 (s), 954 (m), 928 (m), 909 (m), 791 (m), 775 (m), 750 (s), 738 (s), 695 (vs), 597 (s), 577 (s), 495 (m), 471 (s), 408 (s) cm^{-1}.

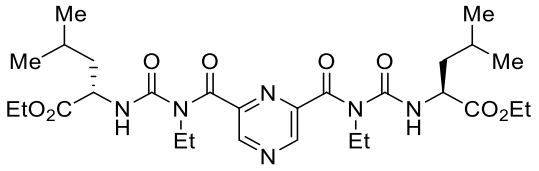


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl)) bis(azanediyl))(2*S*,2'*S*)-bis(4-methylpentanoate) ((*S*)-S1j)



(*S*)-**1j**

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (168 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea (*S*)-**S1j** (553 mg, 2.40 mmol, 2.40 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 5:2), afforded the title compound (*S*)-**1j** (450 mg, 759 µmol, 76% yield) as a yellow resin.

$\mathbf{R}_f$ = 0.24 (*n*-pentane:ethyl acetate = 5:2, UV)

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23}}$ = -22.4 (c = 0.5 g/100 ml, DCM)

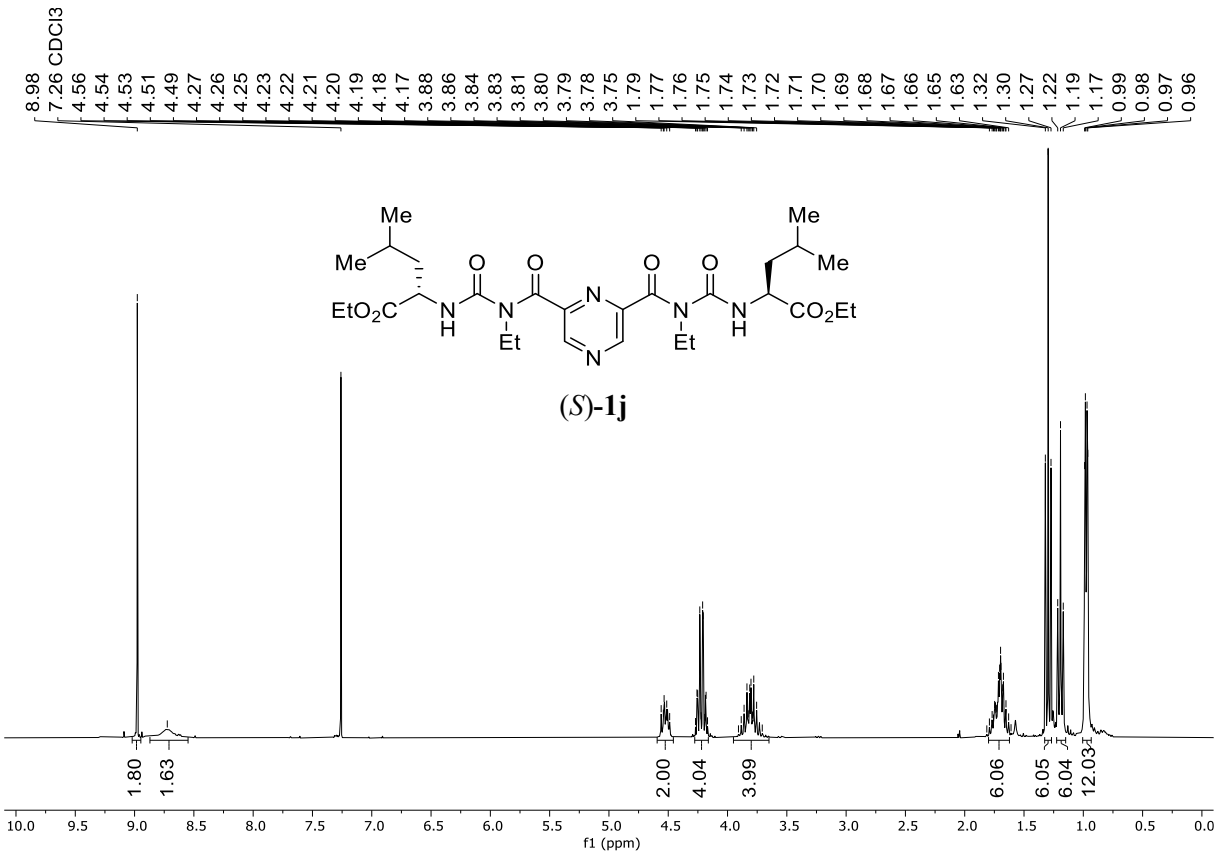
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.98 (s, 2H), 8.72 (bs, 2H), 4.61 – 4.43 (m, 2H), 4.34 – 4.08 (m, 4H), 3.95 – 3.68 (m, 4H), 1.83 – 1.60 (m, 6H), 1.30 (t, *J* = 7.1 Hz, 6H), 1.19 (t, *J* = 7.0 Hz, 6H), 1.02 – 0.93 (m, 12H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 172.8, 168.9, 154.0, 147.2, 145.1, 61.6, 52.7, 41.7, 41.5, 25.1, 23.0, 22.1, 15.0, 14.3.

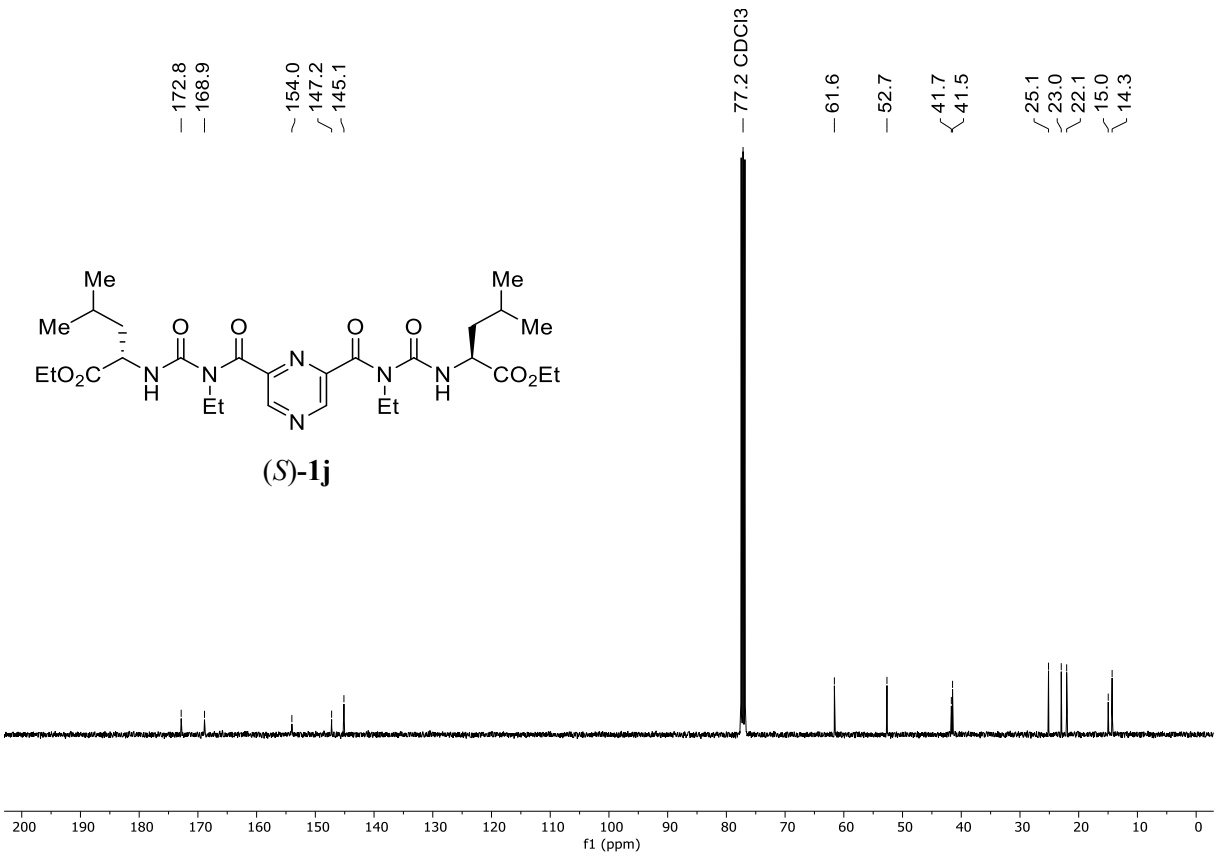
MS (ESI): *m/z* (%) = 615 (100, $[M+Na]^+$), 434 (22), 408 (18), 223 (13).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{28}H_{44}N_6O_8Na]^+$: 615.3113; found: 615.3124 (Error PPM: 1.8).

IR (ATR, neat, $\tilde{\nu}$) = 3305 (w), 2959 (w), 2938 (w), 2912 (w), 2873 (w), 1739 (s), 1703 (vs), 1656 (vs), 1515 (vs), 1464 (m), 1448 (m), 1370 (vs), 1354 (s), 1313 (w), 1258 (s), 1232 (s), 1193 (vs), 1095 (vs), 1060 (vs), 1017 (vs), 958 (w), 942 (w), 909 (w), 860 (w), 816 (w), 793 (w), 775 (s), 701 (w), 632 (m), 589 (m), 561 (m), 500 (w), 479 (w), 418 (m) cm^{-1}

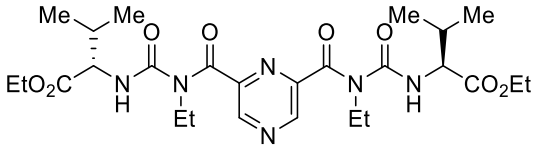


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl)) bis(azanediyl))(2*S*,2'*S*)-bis(3-methylbutanoate) ((*S*)-1k)



(*S*)-**1k**

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (168 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea (*S*)-**S1k** (540 mg, 2.50 mmol, 2.50 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1), afforded the title compound (*S*)-**1k** (360 mg, 638 µmol, 64% yield) as a yellow resin.

$\mathbf{R}_f$ = 0.26 (*n*-pentane:ethyl acetate = 2:1, Detection)

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{24}}$ = -20.6 (c = 0.5 g/100 ml, DCM)

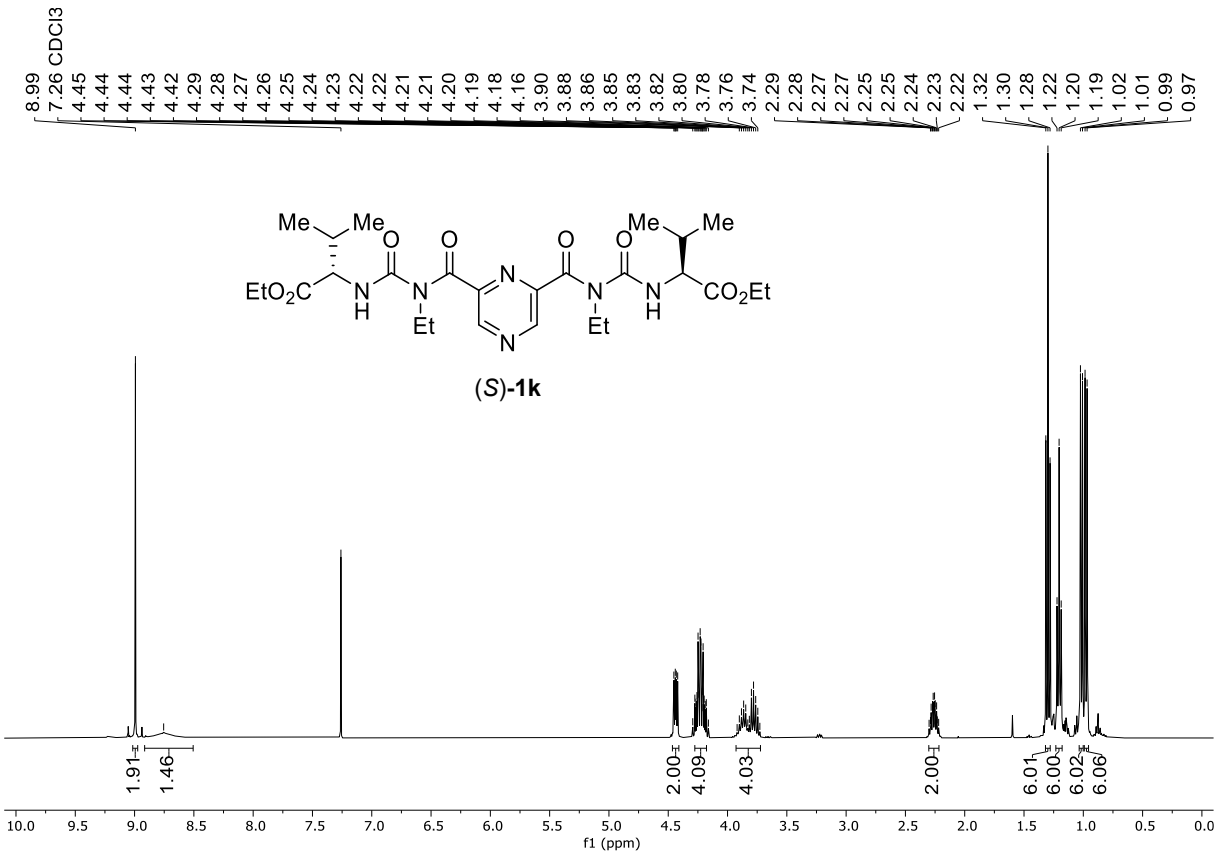
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.99 (s, 2H), 8.75 (s, 2H), 4.44 (dd, *J* = 8.1, 4.7 Hz, 2H), 4.30 – 4.11 (m, 4H), 3.93 – 3.71 (m, 4H), 2.26 (qqd, *J* = 6.9, 6.9, 4.7 Hz, 2H), 1.30 (t, *J* = 7.1 Hz, 6H), 1.20 (t, *J* = 7.0 Hz, 6H), 1.02 (d, *J* = 6.9 Hz, 7H), 0.98 (d, *J* = 6.9 Hz, 7H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 171.8, 168.8, 154.4, 147.1, 145.2, 61.5, 59.1, 41.8, 31.2, 19.3, 17.9, 14.9, 14.4.

MS (EI): *m/z* (%) = 587 (65, $[M+Na]^+$), 420 (100), 394 (10), 310 (8), 302 (8), 249 (4), 223 (4).

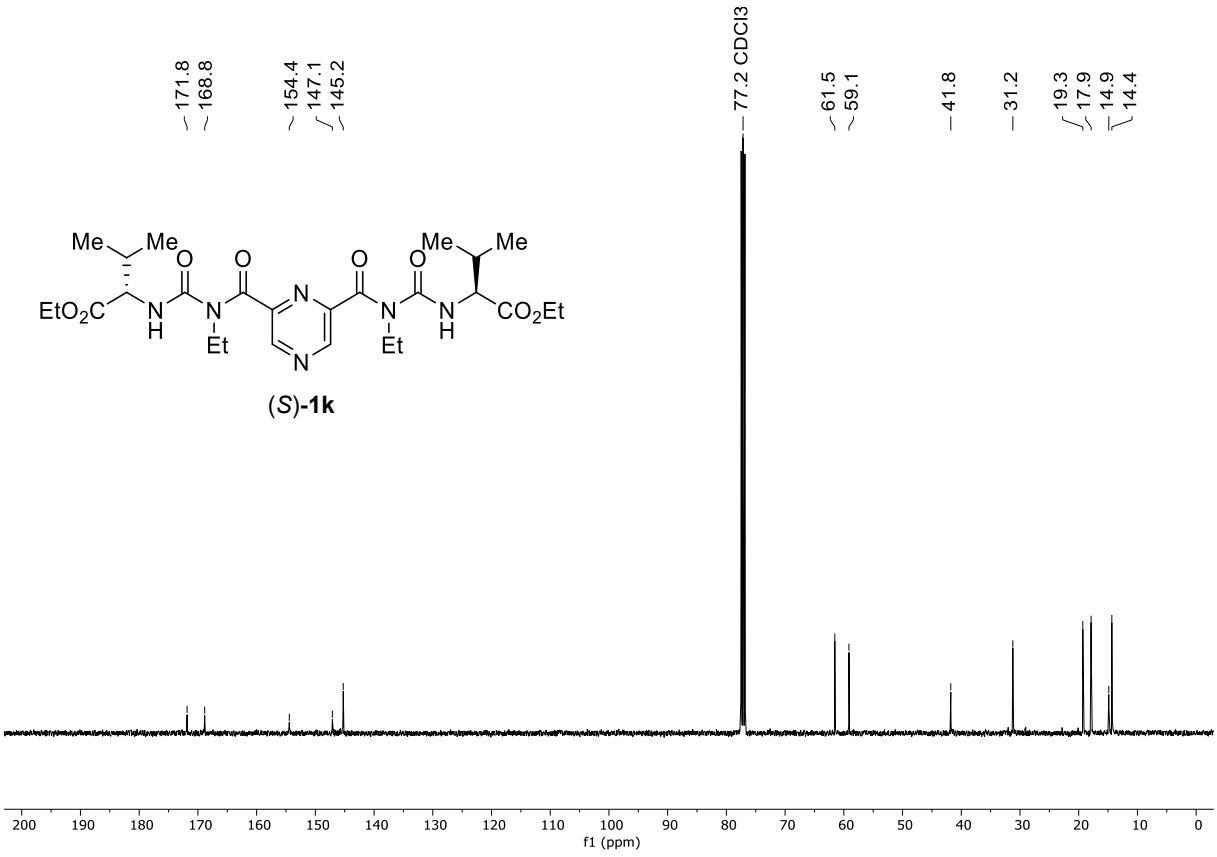
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{26}H_{40}N_6O_8Na]^+$: 587.2800; found: 587.2814 (Error PPM: 2.4).

IR (ATR, neat, $\tilde{\nu}$) = 3305 (w), 2967 (w), 2936 (w), 2877 (vw), 1735 (s), 1705 (vs), 1656 (vs), 1517 (vs), 1464 (m), 1448 (m), 1431 (m), 1393 (s), 1372 (vs), 1356 (s), 1309 (s), 1289 (m), 1248 (s), 1195 (vs), 1154 (vs), 1095 (vs), 1083 (vs), 1038 (s), 1017 (vs), 960 (w), 932 (w), 911 (w), 863 (w), 793 (m), 775 (s), 701 (w), 667 (w), 634 (m), 602 (m), 526 (w), 506 (w), 416 (s) cm^{-1}.



(*S*)-**1k**

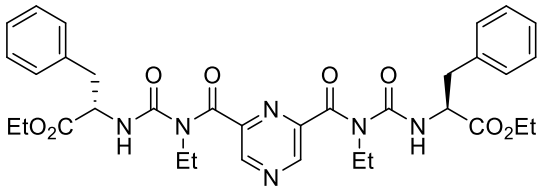
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



(*S*)-**1k**

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis-(azanediyl))(2*S*,2'*S*)-bis(3-phenylpropanoate) ((*S*)-1n)



(*S*)-**1n**

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (168 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea (*S*)-**S1n** (661 mg, 2.50 mmol, 2.50 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1), afforded the title compound (*S*)-**1n** (443 mg, 670 µmol, 67% yield) as a yellow resin.

$\mathbf{R}_f$ = 0.21 (*n*-pentane:ethyl acetate = 2:1, UV)

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23}}$ = +9.4 (c = 0.5 g/100 ml, DCM)

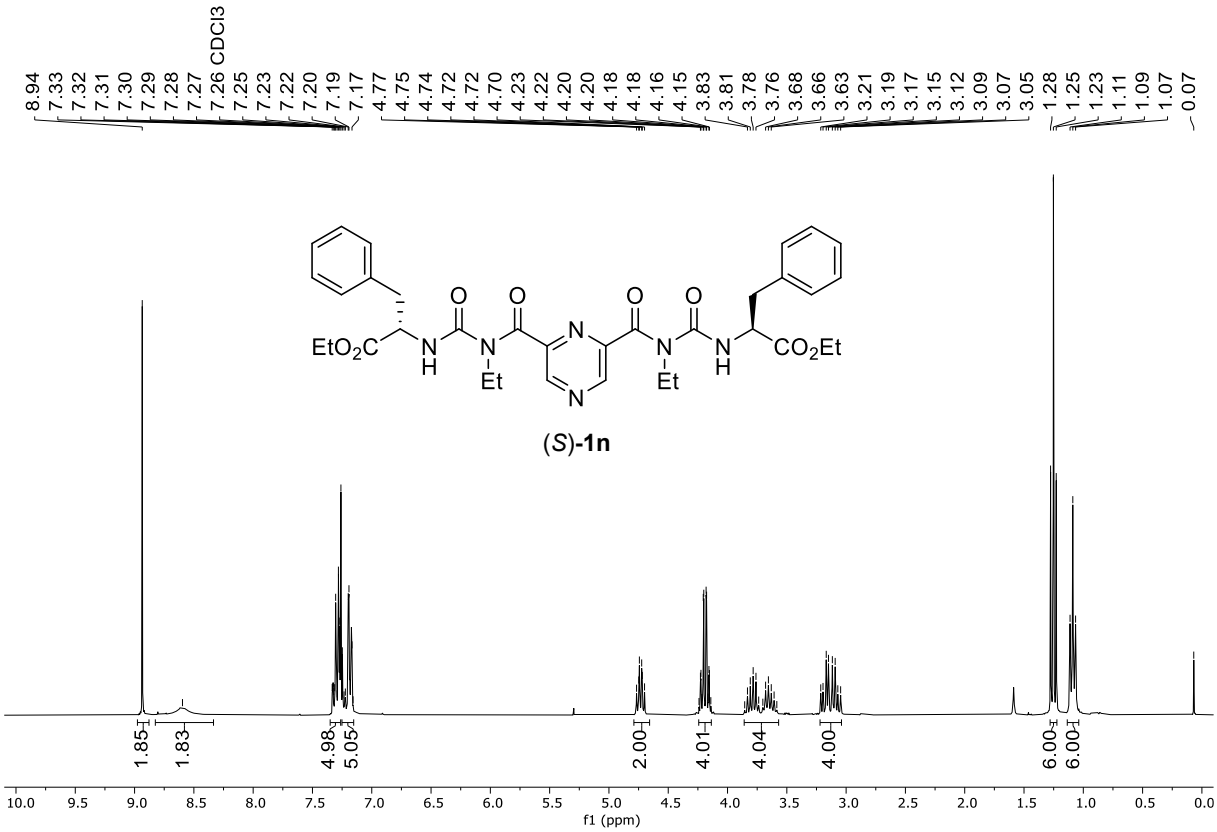
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.94 (s, 2H), 8.60 (s, 1H), 7.35 – 7.26 (m, 5H), 7.25 – 7.15 (m, 5H), 4.73 (ddd, *J* = 7.3, 7.2, 5.7 Hz, 2H), 4.29 – 4.13 (m, 4H), 3.87 – 3.72 (m, 2H), 3.71 – 3.57 (m, 2H), 3.29 – 3.02 (m, 4H), 1.25 (t, *J* = 7.1 Hz, 6H), 1.09 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 171.6, 168.6, 154.0, 147.0, 145.2, 136.0, 129.4, 128.8, 127.4, 61.8, 55.2, 41.7, 38.3, 14.7, 14.2.

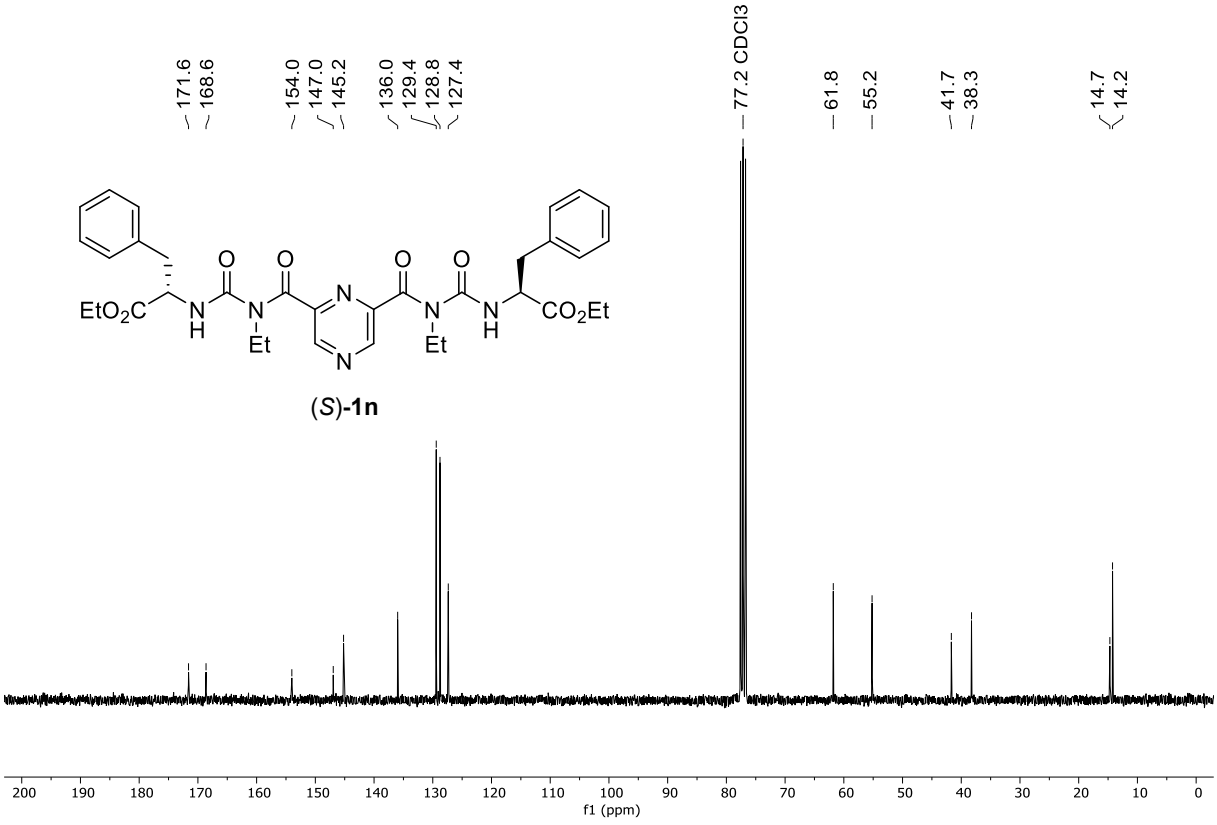
MS (ESI): *m/z* (%) = 683 (24, $[M+Na]^+$), 468 (100), 350 (18), 223 (10).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{34}H_{40}N_6O_8Na]^+$: 683.2800; found: 683.2811 (Error PPM: 1.6).

IR (ATR, neat, $\tilde{\nu}$) = 3295 (w), 2981 (w), 2938 (w), 1737 (s), 1701 (vs), 1654 (vs), 1509 (vs), 1456 (m), 1446 (m), 1395 (s), 1372 (vs), 1352 (s), 1319 (w), 1283 (m), 1254 (s), 1185 (vs), 1095 (s), 1079 (vs), 1062 (vs), 1017 (vs), 964 (w), 913 (w), 860 (w), 818 (w), 791 (w), 775 (m), 759 (m), 744 (s), 699 (vs), 634 (m), 593 (m), 563 (m), 544 (m), 493 (s), 459 (w), 418 (m) cm^{-1}.

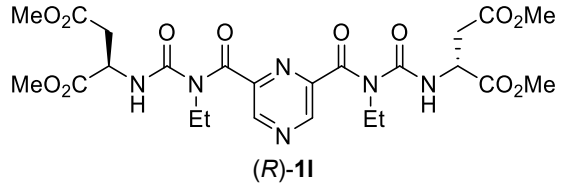


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of tetramethyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis-(azanediyl))(2*R*,2'*R*)-disuccinate ((*R*)-1l)



(*R*)-**1l**

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (168 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea (*R*)-**S1l** (581 mg, 2.50 mmol, 2.50 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 3:5), afforded the title compound (*R*)-**1l** (286 mg, 479 µmol, 48% yield) as a yellow resin.

$\mathbf{R}_f$ = 0.22 (*n*-pentane:ethyl acetate = 3:5, Detection)

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23}}$ = -16.5 (c = 0.5 g/100 ml, DCM)

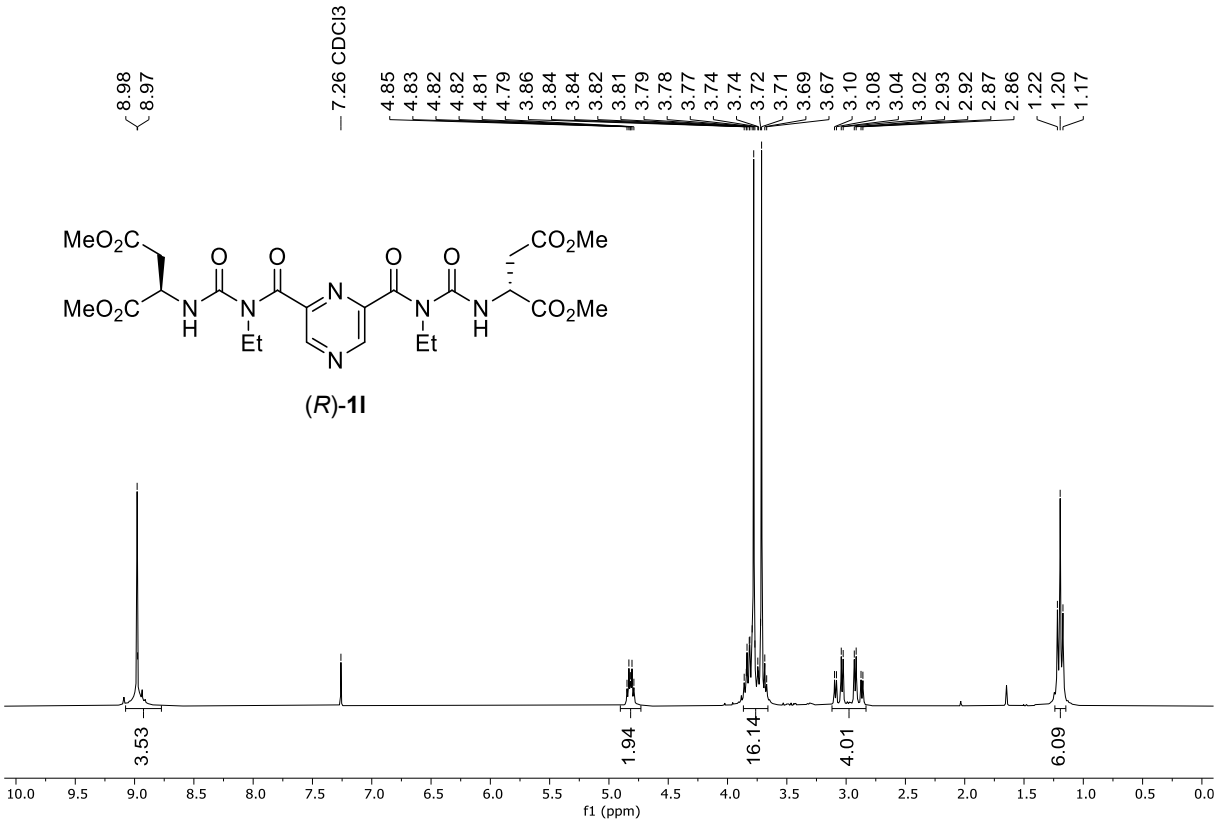
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.08 – 8.77 (m, 4H), 4.82 (dt, *J* = 7.8, 4.8 Hz, 2H), 3.87 – 3.66 (m, 16H), 3.12 – 2.83 (m, 4H), 1.20 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 171.2, 170.9, 168.7, 154.1, 147.0, 145.2, 53.1, 52.3, 50.2, 41.6, 36.2, 14.8.

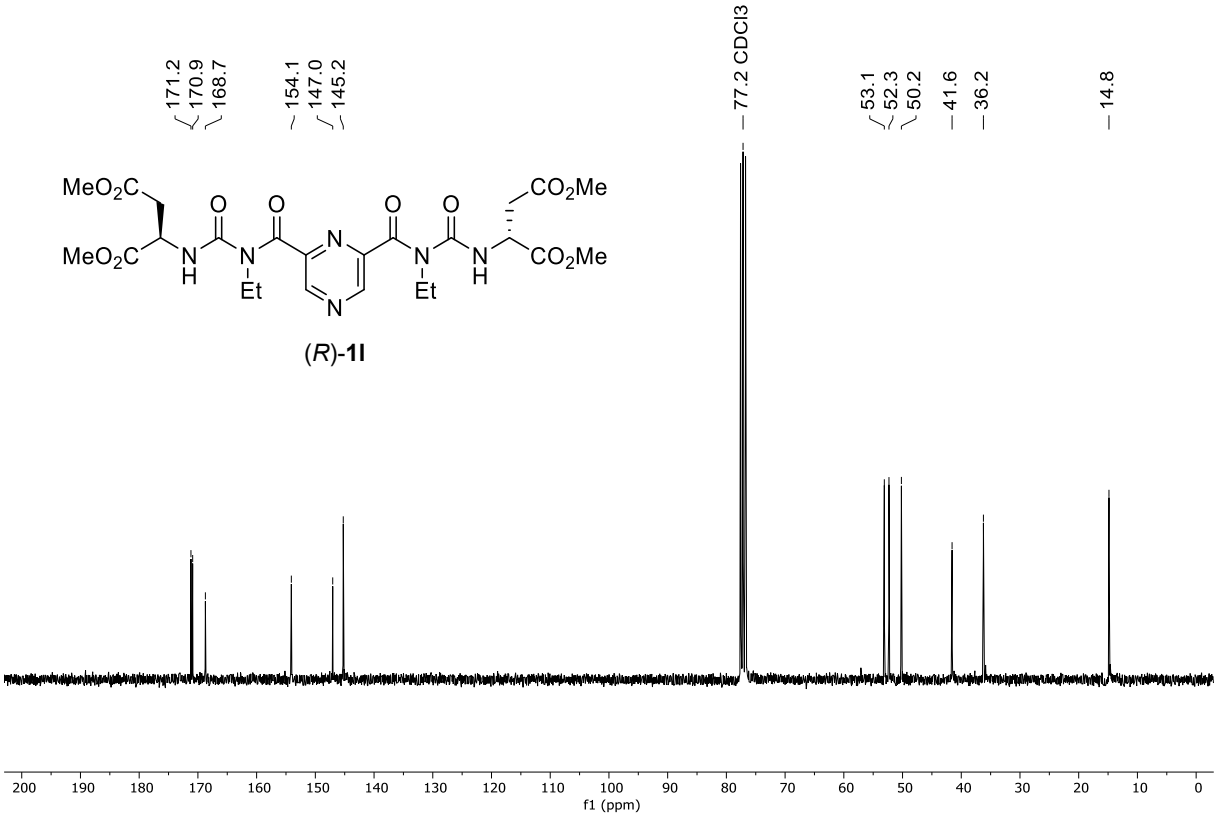
MS (ESI): *m/z* (%) = 619 (43, $[M+Na]^+$), 436 (100), 410 (20), 326 (8), 318 (7), 223 (6).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{24}H_{32}N_6O_{12}Na]^+$: 619.1970; found: 619.1979 (Error PPM: 1.5).

IR (ATR, neat, $\tilde{\nu}$) = 3303 (w), 2957 (w), 1735 (vs), 1701 (vs), 1656 (vs), 1588 (vw), 1509 (vs), 1435 (vs), 1395 (s), 1368 (vs), 1287 (vs), 1197 (vs), 1170 (vs), 1097 (vs), 1068 (vs), 1048 (vs), 1015 (vs), 995 (s), 909 (w), 871 (w), 858 (w), 820 (w), 793 (m), 775 (s), 701 (w), 632 (m), 542 (m), 416 (m) cm^{-1}.

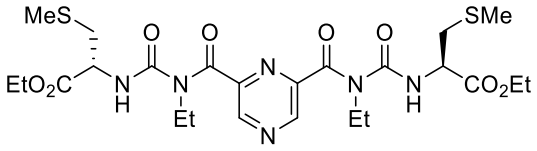


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis-(azanediyl))(2*S*,2'*S*)-bis(4-(methylthio)butanoate) ((*S*)-1o)



(*S*)-**1o**

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (168 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea (*S*)-**S1o** (621 mg, 2.50 mmol, 2.50 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 5:3), afforded the title compound (*S*)-**1o** (422 mg, 670 µmol, 67% yield) as a yellow resin.

$\mathbf{R}_f$ = 0.23 (*n*-pentane:ethyl acetate = 5:3, UV)

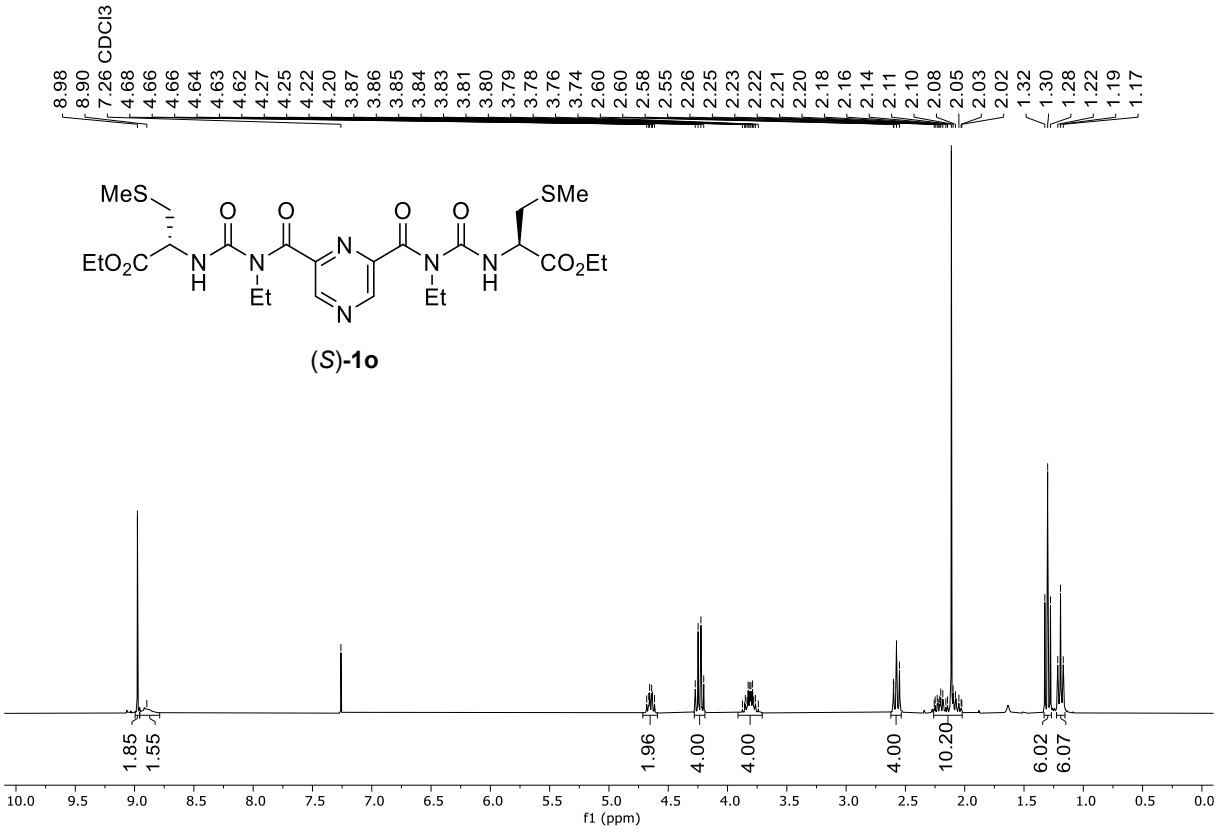
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.98 (s, 2H), 8.90 (bs, 2H), 4.65 (dtd, J = 7.3, 7.3, 5.1 Hz, 2H), 4.24 (q, J = 7.1 Hz, 4H), 3.90 – 3.71 (m, 4H), 2.63 – 2.52 (m, 4H), 2.26 – 2.01 (m, 10H), 1.30 (t, J = 7.1 Hz, 6H), 1.19 (t, J = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 171.7, 168.9, 153.9, 147.2, 145.1, 61.9, 53.3, 41.6, 31.7, 30.2, 15.6, 15.0, 14.3.

MS (ESI): *m/z* (%) = 651 (51, $[M+Na]^+$), 452 (100), 426 (14), 223 (11).

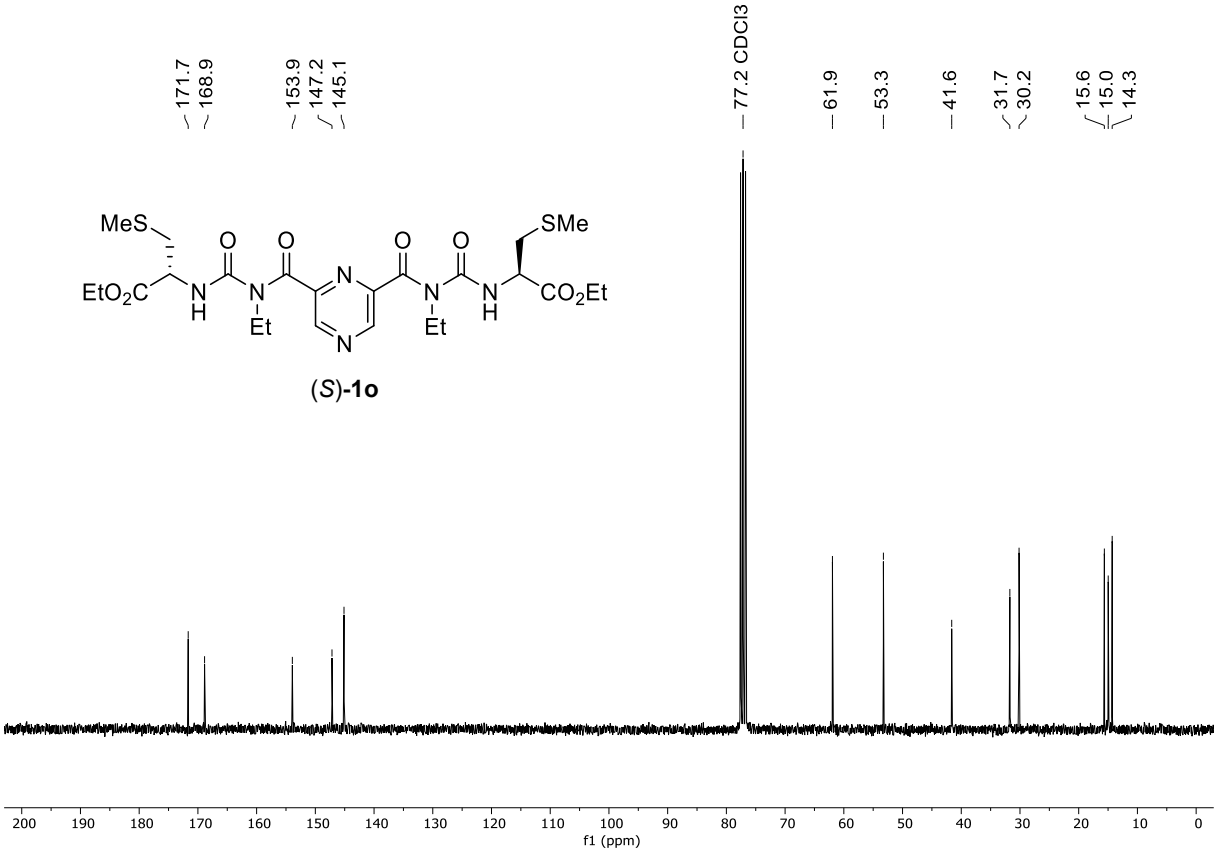
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{26}H_{40}N_6O_8S_2Na]^+$: 651.2241; found: 651.2253 (Error PPM: 1.8).

IR (ATR, neat, $\tilde{\nu}$) = 3303 (w), 2979 (w), 2934 (w), 2918 (w), 1737 (s), 1701 (vs), 1656 (vs), 1511 (vs), 1462 (m), 1444 (s), 1433 (s), 1372 (vs), 1354 (s), 1278 (s), 1254 (s), 1181 (vs), 1095 (vs), 1064 (vs), 1015 (vs), 960 (m), 909 (w), 860 (w), 793 (m), 775 (s), 701 (w), 632 (m), 589 (m), 559 (m), 502 (m), 414 (s) cm^{-1}.



(*S*)-**1o**

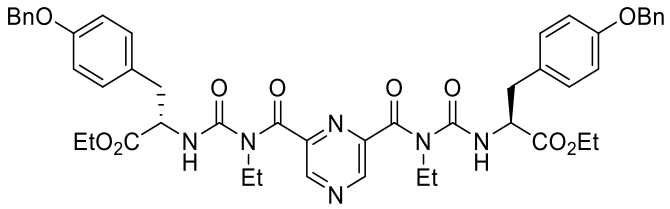
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



(*S*)-**1o**

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-((((pyrazine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis-(azanediyl))(2*S*,2'*S*)-bis(3-(4-(benzyloxy)phenyl)propanoate) ((*S*)-S1m)



(*S*)-**1m**

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (336 mg, 2.00 mmol, 1.00 equiv.) and the corresponding urea (*S*)-**S1m** (499 mg, 2.46 mmol, 2.46 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 3:2), afforded the title compound (*S*)-**S1m** (657 mg, 753 µmol, 38% yield) as a yellow solid.

$\mathbf{R}_f$ = 0.26 (*n*-pentane:ethyl acetate = 3:2, UV)

m.p. = 70-74°C

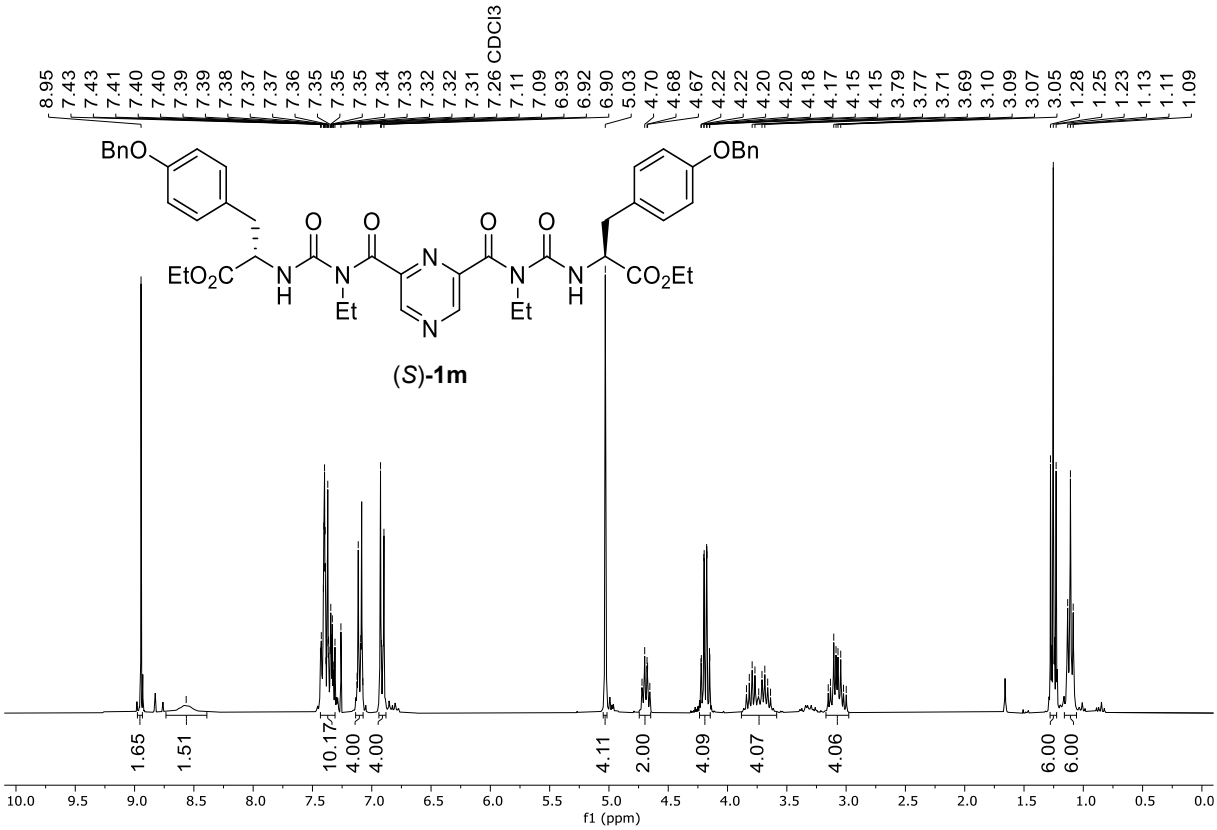
$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{24}}$ = +24,4 (c = 0.5 g/100 ml, DCM)

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.95 (s, 2H), 8.56 (s, 2H), 7.43 – 7.31 (m, 10H), 7.14 – 7.07 (m, 4H), 6.95 – 6.88 (m, 4H), 5.03 (s, 4H), 4.69 (td, *J* = 6.9, 5.6 Hz, 2H), 4.24 – 4.15 (m, 4H), 3.88 – 3.59 (m, 4H), 3.08 (m, 4H), 1.25 (t, *J* = 7.2 Hz, 6H), 1.11 (t, *J* = 7.0 Hz, 6H).

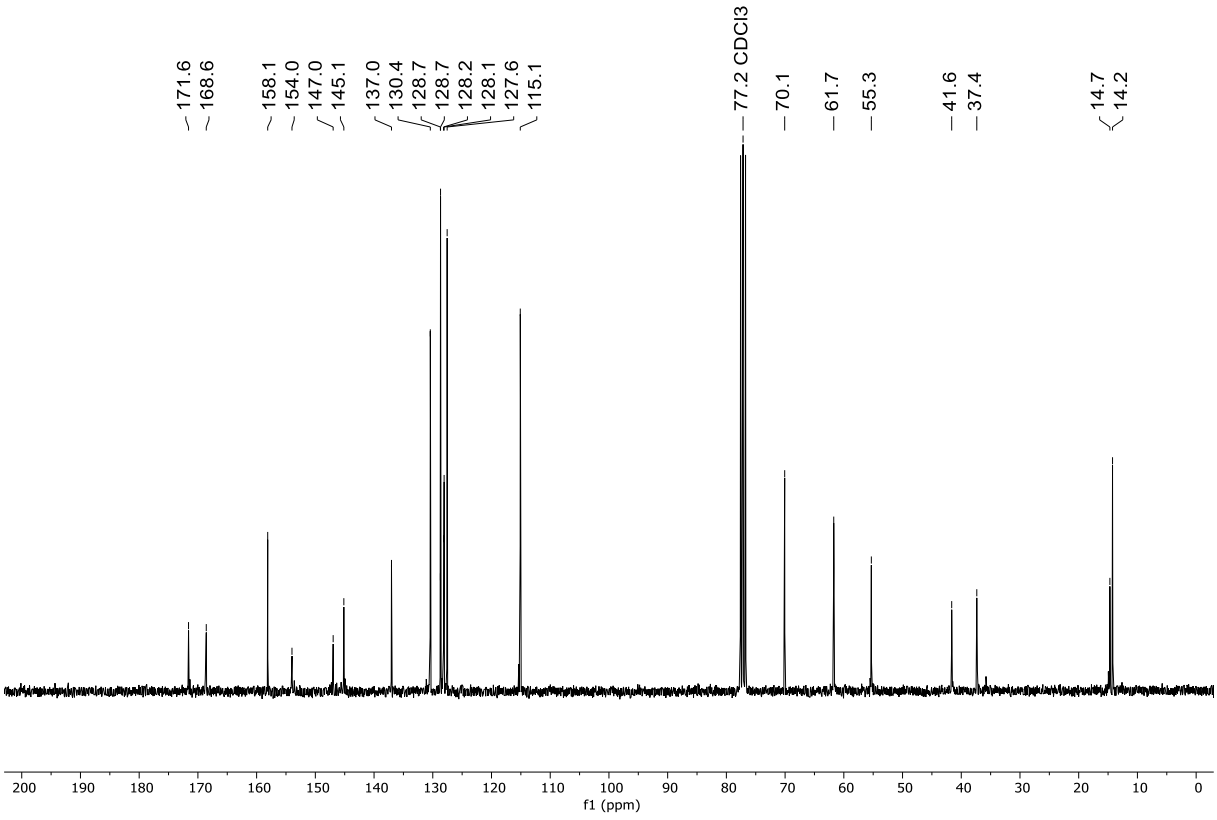
^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 171.6, 168.6, 158.1, 154.0, 147.0, 145.1, 137.0, 130.4, 128.7, 128.7, 128.2, 128.1, 127.6, 115.1, 70.1, 61.7, 55.3, 41.6, 37.4, 14.7, 14.2.

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{48}H_{52}N_6O_{10}Na]^+$: 895.3637; found: 895.3638 (Error PPM: 0.1).

IR (ATR, neat, $\tilde{\nu}$) = 3295 (w), 3032 (vw), 2979 (w), 2934 (w), 2871 (vw), 1735 (m), 1703 (vs), 1656 (s), 1611 (w), 1582 (w), 1509 (vs), 1454 (m), 1372 (vs), 1352 (s), 1297 (m), 1240 (vs), 1219 (vs), 1177 (vs), 1095 (vs), 1064 (vs), 1015 (vs), 909 (w), 860 (m), 844 (m), 818 (m), 793 (m), 773 (s), 734 (vs), 695 (vs), 634 (m), 608 (s), 571 (s), 538 (s), 510 (s), 455 (m), 412 (s) cm^{-1}.

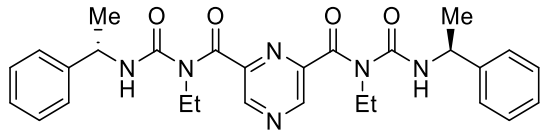


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of N^2,N^6-diethyl-N^2,N^6-bis(((*S*)-1-phenylethyl)carbamoyl)pyrazine-2,6-dicarboxamide ((*S*)-1p) TT1102



(*S*)-**1p**

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (336 mg, 2.00 mmol, 1.00 equiv.) and the corresponding urea (*S*)-**S1p** (961 mg, 5 mmol, 2.49 equiuv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 3:2), afforded the title compound (*S*)-**1p** (711 mg, 1.38 mmol, 69% yield) as a pale yellow solid.

$\mathbf{R}_f$ = 0.27 (*n*-pentane:ethyl acetate = 3:2, UV)

m.p. = 45 – 80 °C (broad)

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{22}}$ = +7,0 (c = 1.0 g/100 ml, DCM)

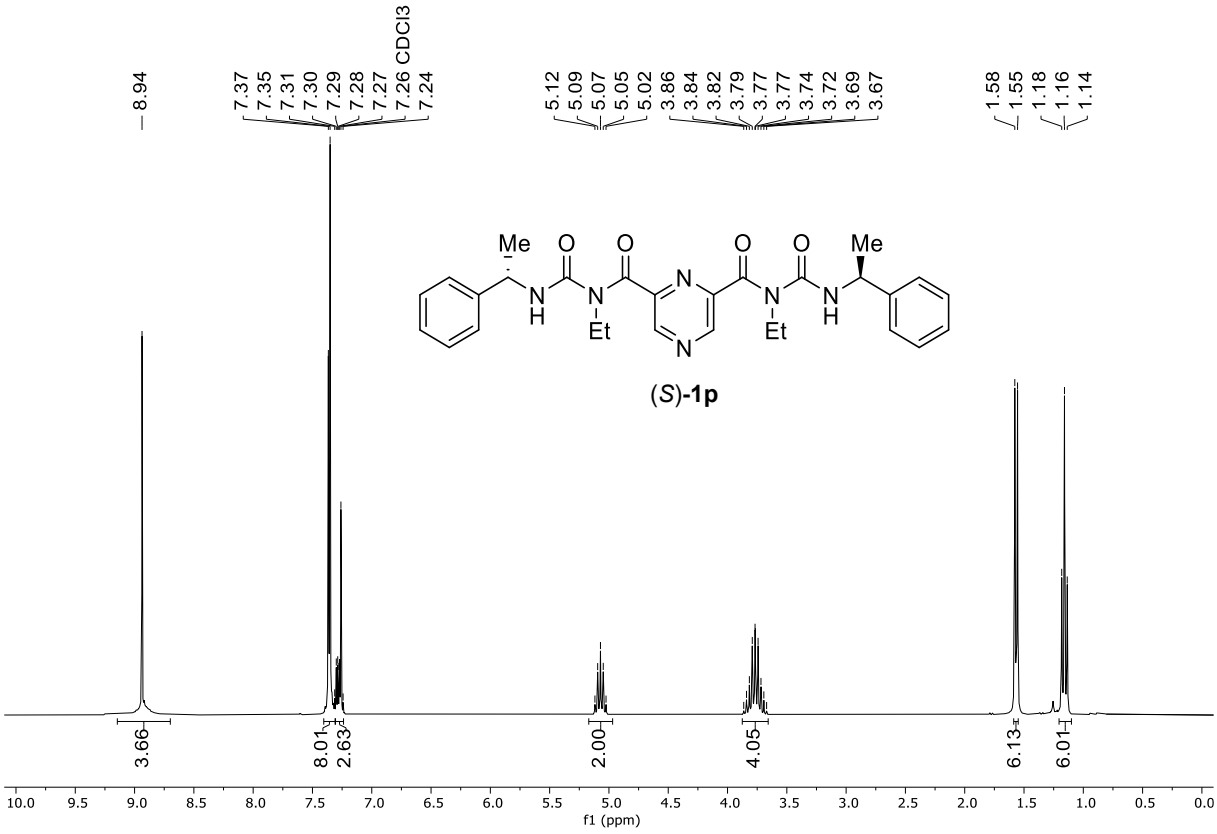
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.15 – 8.70 (m, 4H), 7.36 (m, 8H), 7.33 – 7.21 (m, 2H), 5.07 (dq, *J* = 7.0, 7.0 Hz, 2H), 3.88 – 3.66 (m, 4H), 1.57 (d, *J* = 7.0 Hz, 6H), 1.16 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 168.9, 152.9, 147.5, 144.6, 143.1, 128.8, 127.5, 126.0, 50.8, 41.4, 22.6, 15.1.

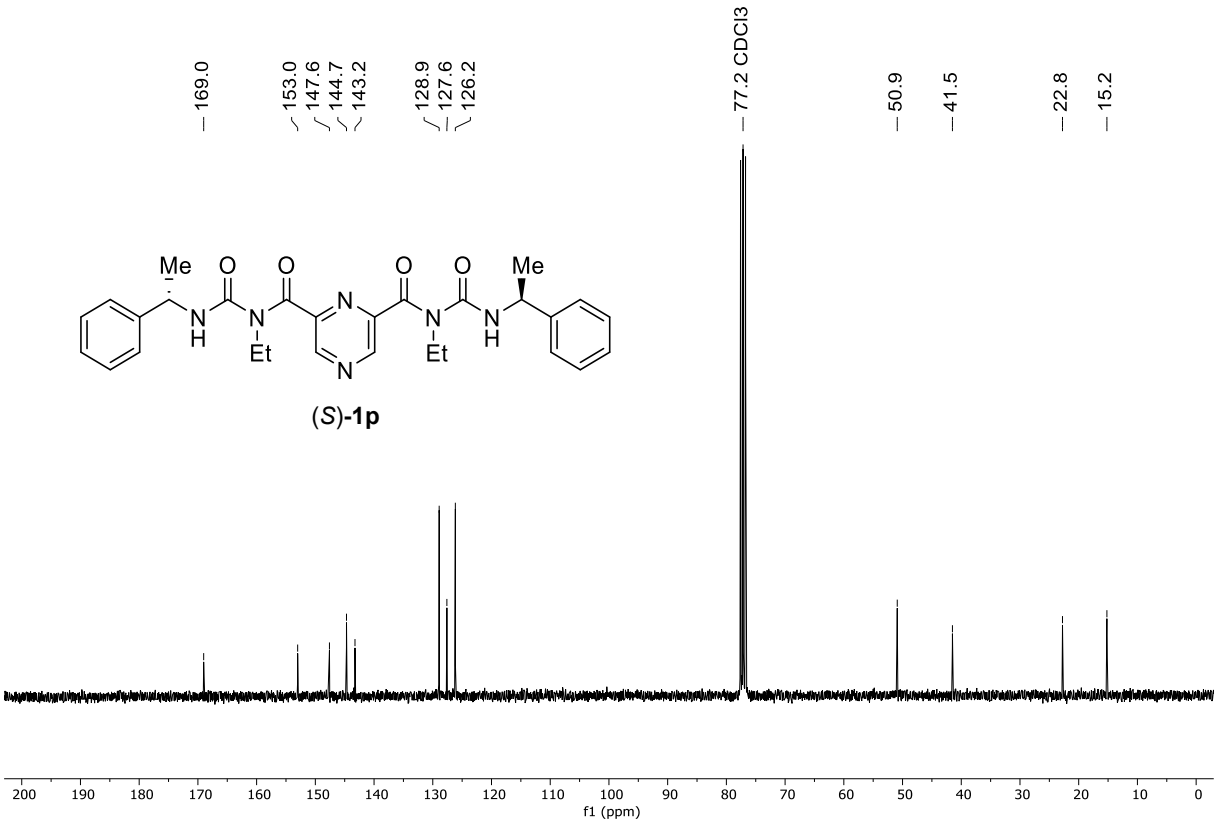
MS (ESI): *m/z* (%) = 539 (56, $[M+Na]^+$), 396 (100), 249 (10), 223 (12).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{28}H_{33}N_6O_4]^+$: 517.2558; found: 517.2567 (Error PPM: 1.7).

IR (ATR, neat, ṽ) = 3293 (w), 2975 (w), 2932 (w), 1686 (vs), 1650 (vs), 1511 (vs), 1495 (vs), 1448 (s), 1374 (vs), 1354 (s), 1283 (m), 1246 (s), 1195 (vs), 1181 (s), 1093 (s), 1070 (vs), 1060 (vs), 1015 (vs), 966 (m), 907 (m), 856 (w), 822 (w), 793 (w), 761 (vs), 697 (vs), 606 (s), 565 (s), 546 (vs), 406 (s) cm^{-1}.

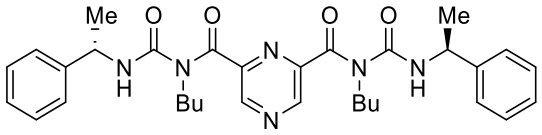


^{1}H NMR (300 MHz, Chloroform-d [77.16 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of N^2,N^6-dibutyl-N^2,N^6-bis(((*S*)-1-phenylethyl)carbamoyl)pyrazine-2,6-dicarboxamide ((*S*)-1q) JM11



(*S*)-**1q**

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (252 mg, 1.50 mmol, 1.00 equiv.) and the corresponding urea (*S*)-**S1q** (832 mg, 3.78 mmol, 2.52 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1), afforded the title compound (*S*)-**1q** (550 mg, 960 µmol, 64% yield) as a as a yellow resin.

$\mathbf{R}_f$ = 0.42 (*n*-pentane:ethyl acetate = 2:1, UV)

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{22}}$ = -12,8 (c = 0.5 g/100 ml, DCM)

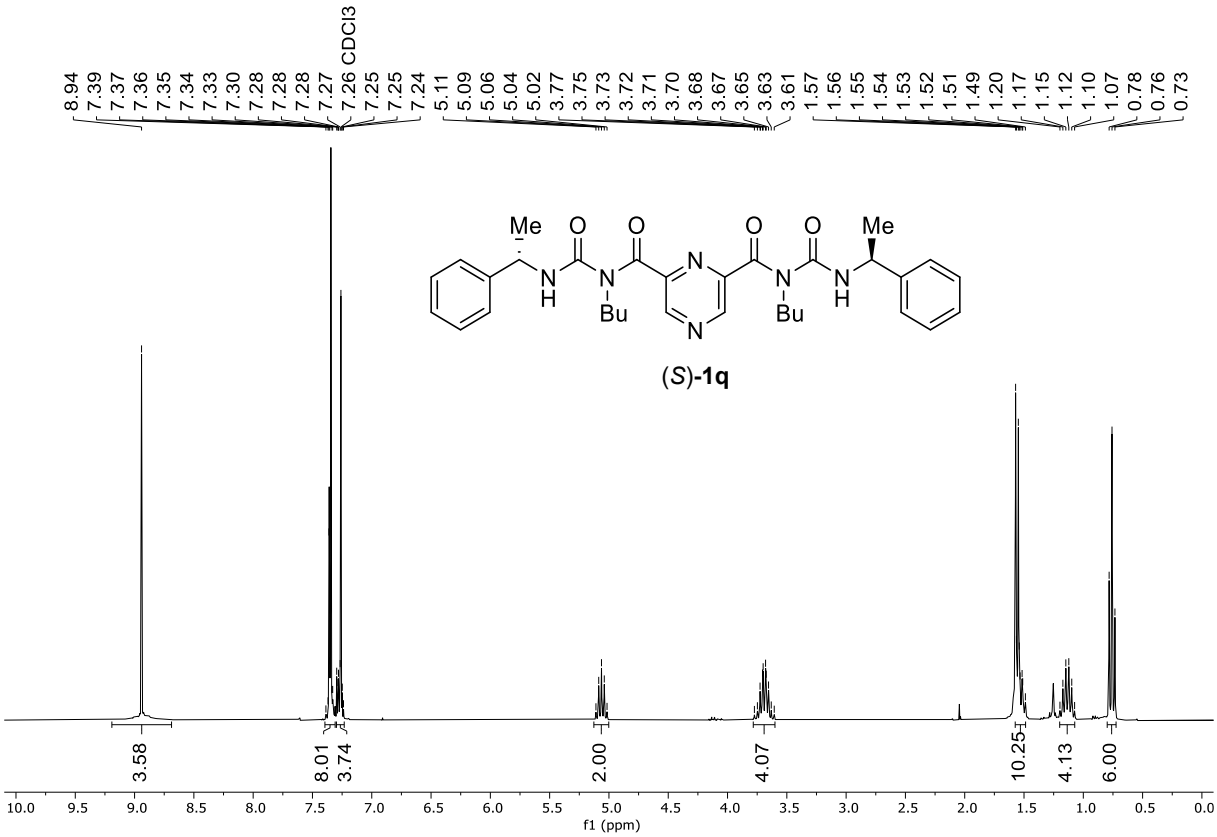
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.94 (s, 4H), 7.35 (m, 8H), 7.31 – 7.21 (m, 2H), 5.06 (qd, *J* = 7.0, 7.0 Hz, 2H), 3.80 – 3.58 (m, 4H), 1.60 – 1.52 (m, 10H), 1.21 – 1.07 (m, 4H), 0.76 (t, *J* = 7.3 Hz, 6H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 169.0, 153.2, 147.5, 144.8, 143.2, 128.9, 127.6, 126.1, 50.9, 46.1, 32.0, 22.7, 19.9, 13.7.

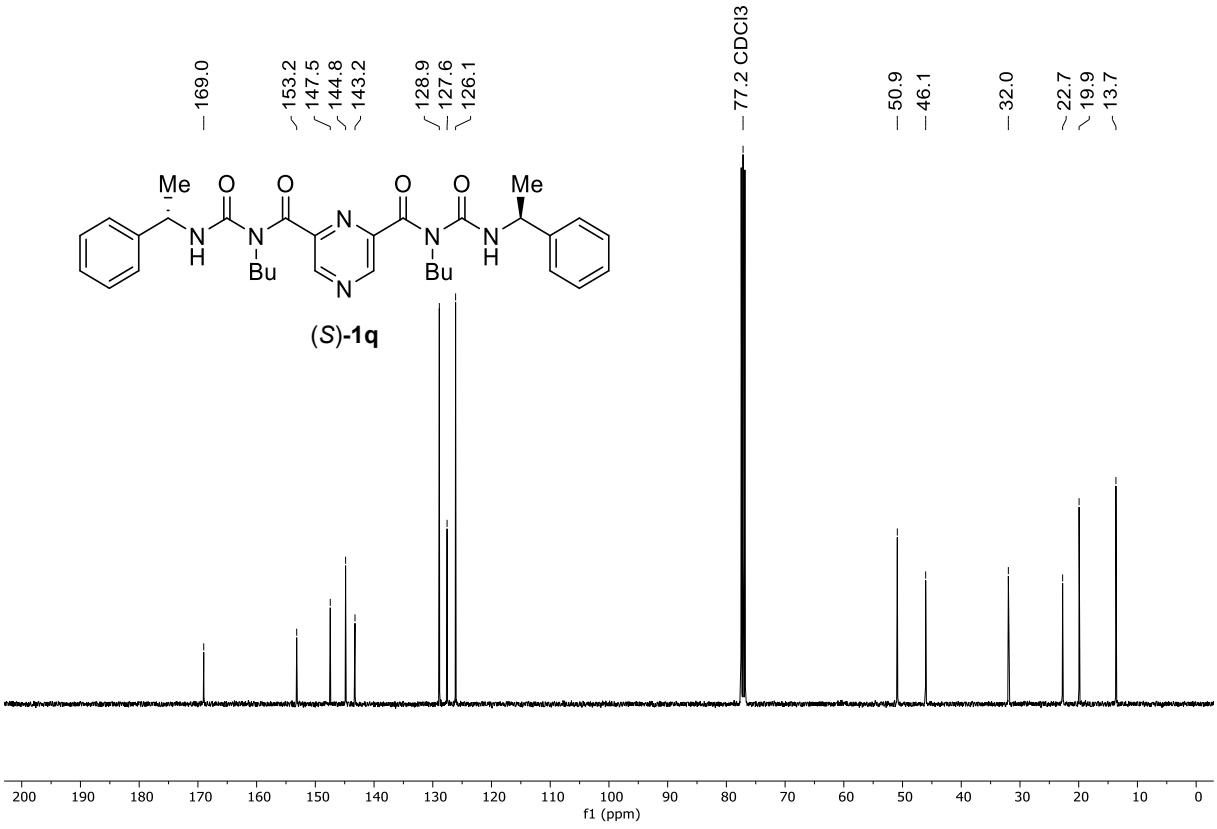
MS (ESI): *m*/*z* (%) =

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{32}H_{40}N_6O_4Na]^+$: 595.3003; found: 595.3018 (Error PPM: 2.5).

IR (ATR, neat, ṽ) = 3297 (w), 2961 (w), 2932 (w), 2873 (w), 1690 (vs), 1652 (vs), 1586 (w), 1513 (vs), 1495 (vs), 1448 (s), 1397 (s), 1368 (vs), 1297 (m), 1230 (s), 1177 (s), 1103 (s), 1079 (vs), 1015 (s), 948 (w), 928 (m), 909 (m), 761 (vs), 697 (vs), 604 (s), 548 (vs), 406 (s) cm^{-1}.

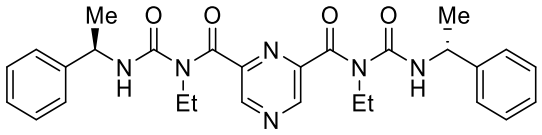


^{1}H NMR (300 MHz, Chloroform-d [77.16 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of N^2,N^6-diethyl- N^2,N^6-bis(((*R*)-1-phenylethyl)carbamoyl)pyrazine-2,6-dicarboxamide ((*R*)-1r) TT1108



(*R*)-**1r**

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (337 mg, 2.00 mmol, 1.00 equiv.) and the corresponding urea (*R*)-**S1r** (964 mg, 5.01 mmol, 2.50 equiv.), and purification by column chromatography ((*n*-pentane:ethyl acetate = 3:2), afforded the title compound (*R*)-**1r**(688 mg, 1.33 mmol, 66% yield) as a pale yellow solid.

R_f = 0.27 (*n*-pentane:ethyl acetate = 3:2, UV)

m.p. = broad 45 – 80 °C

$[\alpha]_D^{21}$ = -9.0 (c = 1 g/100 ml, DCM)

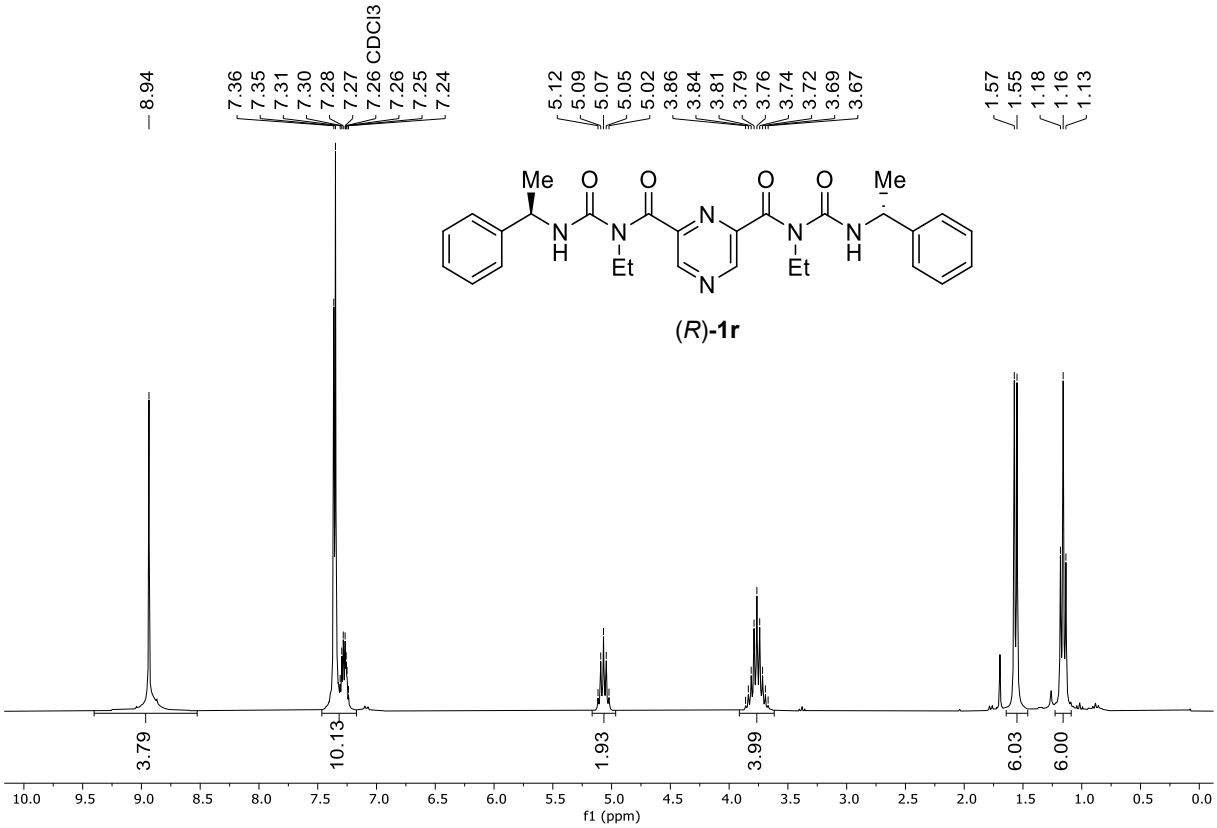
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.11 – 8.81 (m, 4H), 7.40 – 7.29 (m, 8H), 7.31 – 7.23 (m, 2H), 5.07 (dq, *J* = 7.0, 7.0 Hz, 2H), 3.96 – 3.60 (m, 4H), 1.56 (d, *J* = 6.9 Hz, 6H), 1.16 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 169.0, 153.0, 147.5, 144.7, 143.2, 128.9, 127.6, 126.1, 50.8, 41.5, 22.7, 15.1.

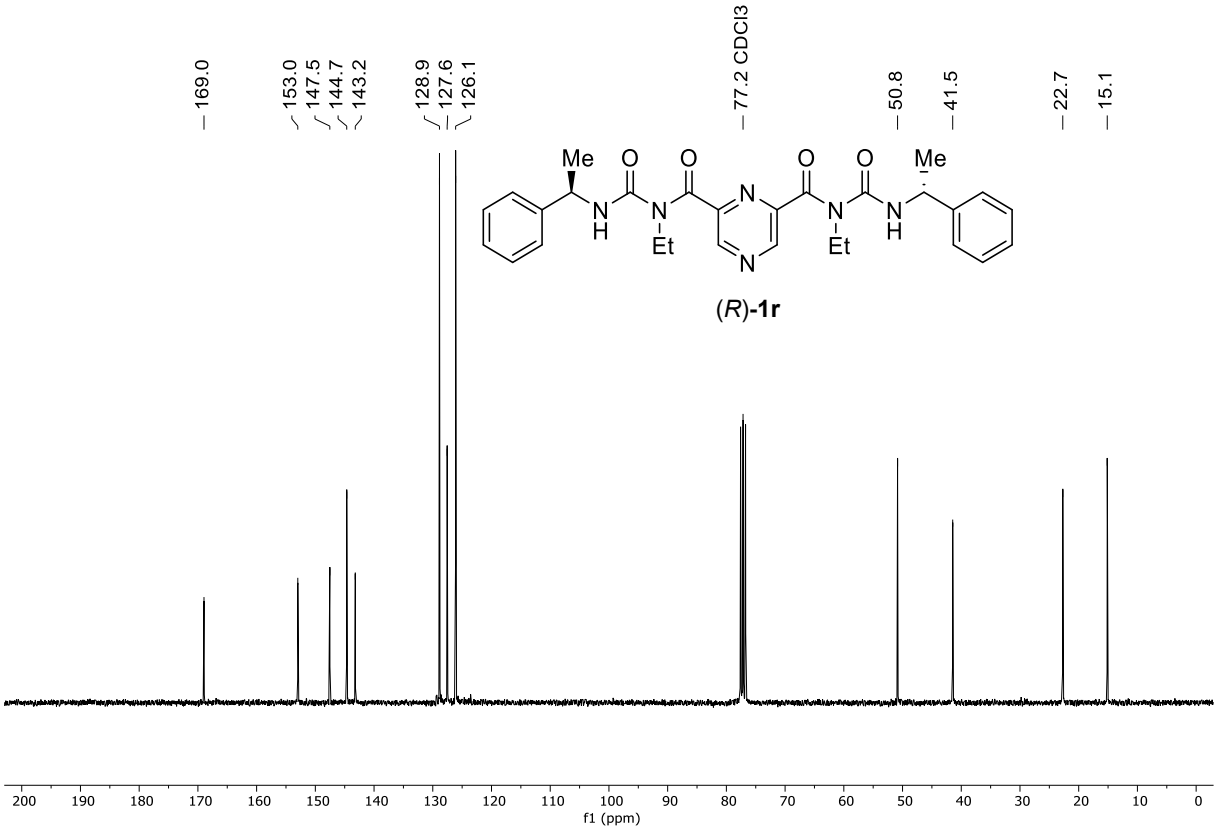
MS (ESI): *m/z* (%) = 539 (67, $[M+Na]^+$), 396 (100), 249 (8), 223 (11).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{28}H_{33}N_6O_4]^+$: 517.2558; found: 517.2568 (Error PPM: 1.9).

IR (ATR, neat, $\tilde{\nu}$) = 3293 (w), 2975 (w), 2932 (w), 1688 (vs), 1650 (vs), 1513 (vs), 1495 (vs), 1448 (s), 1372 (vs), 1354 (s), 1283 (m), 1246 (s), 1195 (vs), 1181 (s), 1093 (s), 1070 (vs), 1060 (vs), 1015 (vs), 966 (w), 907 (w), 856 (w), 822 (w), 793 (w), 761 (vs), 697 (vs), 602 (s), 567 (s), 546 (vs), 402 (s) cm^{-1}.

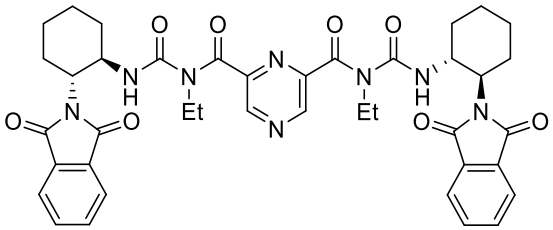

8.94
7.36
7.35
7.31
7.30
7.28
7.27
7.26 CDCl3
7.26
7.25
7.24
5.12
5.09
5.07
5.05
5.02
3.86
3.84
3.81
3.79
3.76
3.74
3.72
3.69
3.67
1.57
1.55
1.18
1.16
1.13
Me
O
O
O
O
Me
N
H
N
Et
N
N
N
Et
N
H
3.79
10.13
1.93
3.99
6.03
6.00
f1 (ppm)

(*R*)-**1r**


169.0
153.0
147.5
144.7
143.2
128.9
127.6
126.1
77.2 CDCl3
50.8
41.5
22.7
15.1
Me
O
O
O
O
Me
N
H
N
Et
N
N
N
Et
N
H
f1 (ppm)

(*R*)-**1r**

Synthesis of N^2,N^6-bis(((1*R*,2*R*)-2-(1,3-dioxoisoindolin-2-yl)cyclohexyl)carbamoyl)-N^2,N^6-diethylpyrazine-2,6-dicarboxamide ((1*R*,2*R*)-1s)



(1*R*,2*R*)-**1s**

Following the general procedure GP2 adding the corresponding urea (1*R*,2*R*)-**S1s** (1.18 g, 3.75 mmol, 2.50 equiv.) as solid due to poor solubilty in CH_2Cl_2, using 2,6-pyrazinedicarboxylic acid (252 mg, 1.50 mmol, 1.00 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 1:1), afforded the title compound (1*R*,2*R*)-**1s** (585 mg, 767 µmol, 51% yield) as a pale yellow crystalline solid.

R_f = 0.31 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = 130-134 °C

$[\alpha]_D^{26}$ = -108.2 (c = 0.5 g/100 ml, DCM)

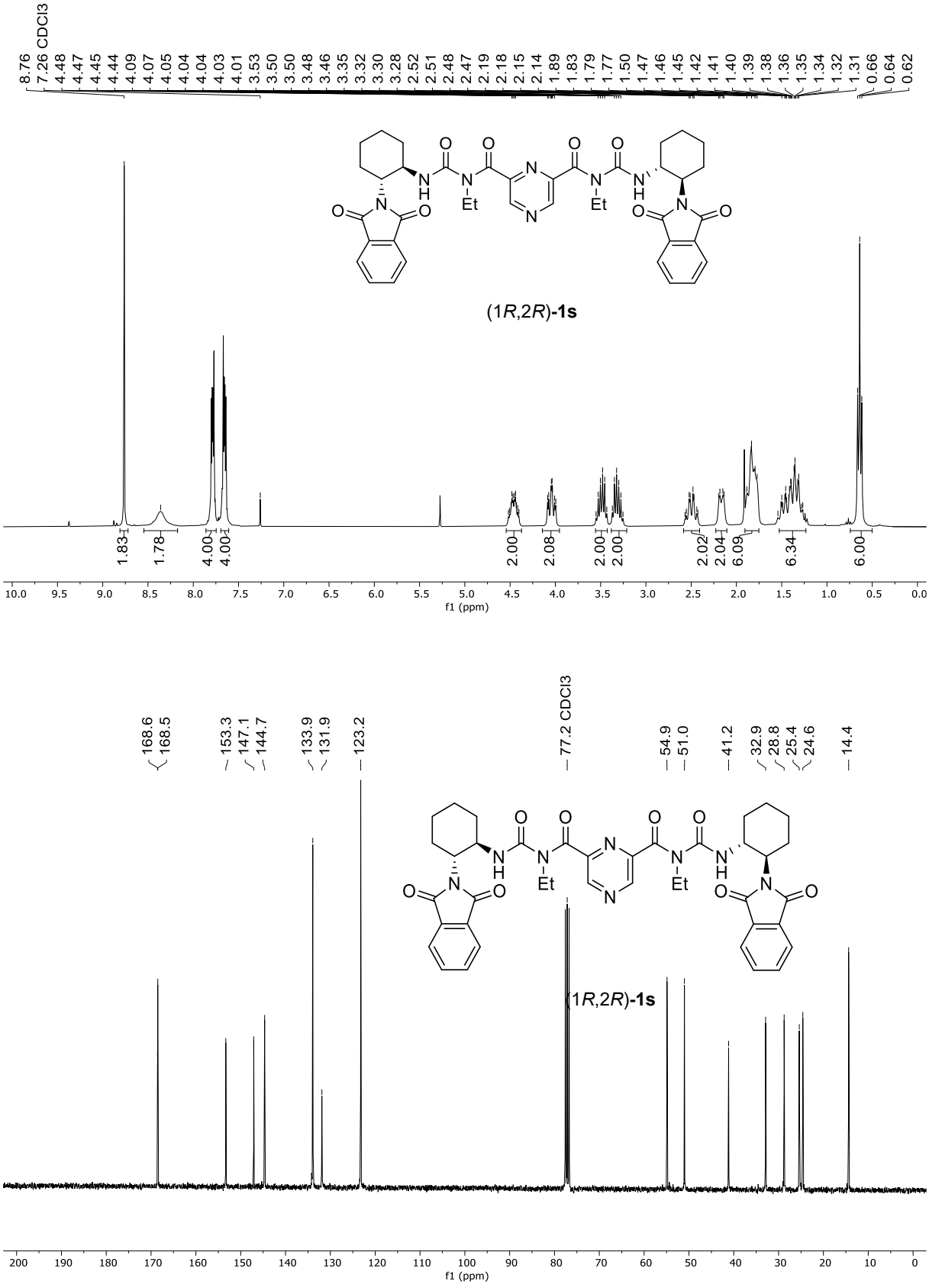
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.76 (s, 2H), 8.36 (bs, 2H), 7.86 – 7.75 (m, 4H), 7.70 – 7.61 (m, 4H), 4.46 (ddd, *J* = 11.1, 8.4, 4.1 Hz, 2H), 4.04 (ddd, *J* = 12.2, 10.9, 3.9 Hz, 2H), 3.56 – 3.43 (m, 2H), 3.38 – 3.21 (m, 2H), 2.50 (ddt, *J* = 13.9, 12.3, 4.0 Hz, 2H), 2.23 – 2.11 (m, 2H), 1.91 – 1.75 (m, 6H), 1.59 – 1.17 (m, 6H), 0.64 (t, *J* = 6.9 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 168.6, 168.5, 153.3, 147.1, 144.7, 133.9, 131.9, 123.2, 54.9, 51.0, 41.2, 32.9, 28.8, 25.4, 24.6, 14.4.

MS (EI): *m/z* (%) = 758 (9, $[M]^+$), 611 (6), 532 (100), 295 (30), 226 (33), 148 (42), 118 (24).

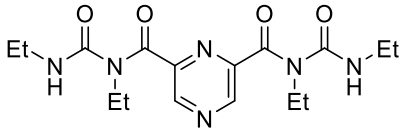
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for : $[C_{40}H_{42}N_8O_8Na]^+$: 785.3018; found: 785.3037. (Error PPM: 2.4)

IR (ATR, neat, $\tilde{\nu}$) = 3293 (vw), 2936 (w), 2863 (w), 1701 (vs), 1650 (s), 1521 (s), 1466 (w), 1431 (w), 1374 (vs), 1358 (vs), 1329 (s), 1289 (m), 1252 (m), 1189 (s), 1156 (s), 1077 (vs), 1048 (s), 1015 (s), 1003 (w), 956 (w), 907 (w), 871 (m), 791 (m), 775 (m), 718 (vs), 697 (s), 638 (s), 565 (m), 530 (vs), 481 (m), 461 (m) cm^{-1}.



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of N^2,N^6-dipropyl-N^2,N^6-bis(propylcarbamoyl)pyrazine-2,6-dicarboxamide (1u) RT36



1u

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (336 mg, 2.00 mmol, 1.00 equiv.) and 1,3-diethylurea (336 mg, 2.00 mmol, 1.00 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 1:1), afforded the title compound **1u** (555 mg, 1.52 mmol, 76% yield) as a yellow solid.

R_f = 0.18 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = 126-128°C

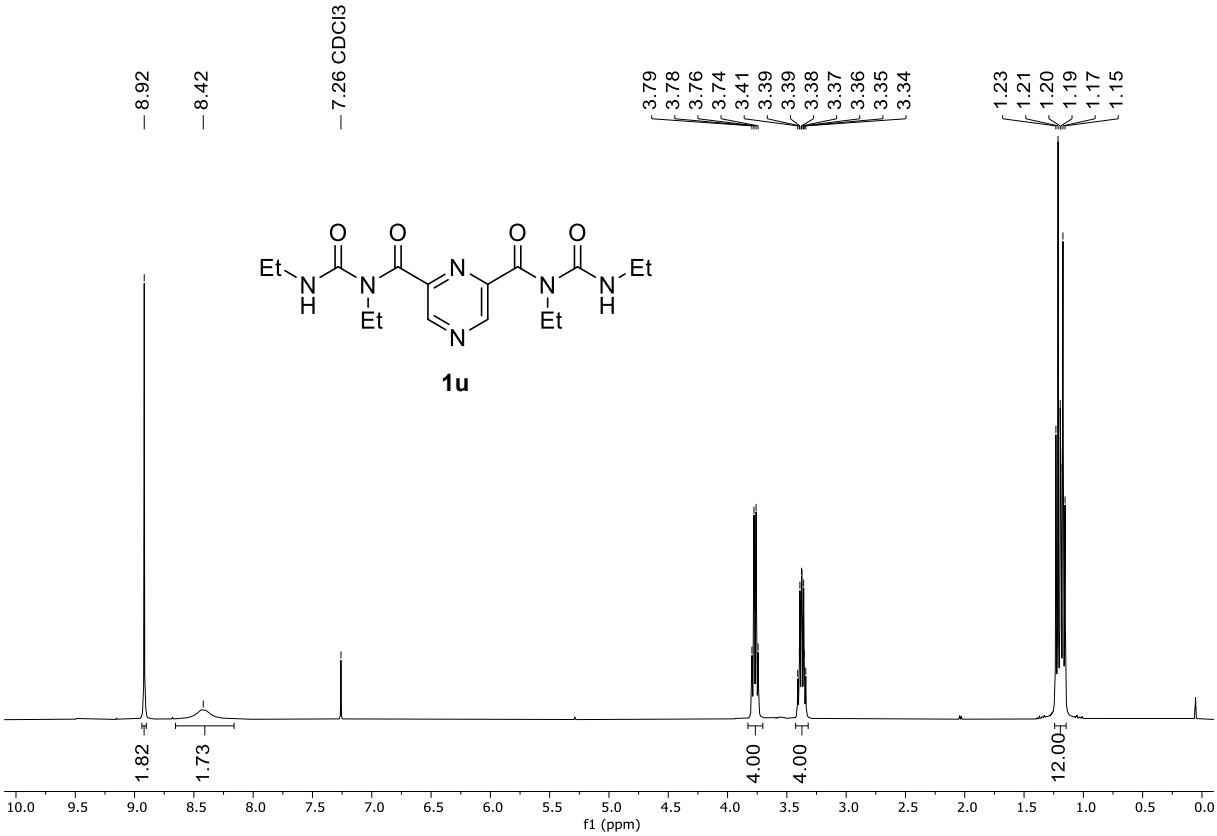
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.92 (s, 2H), 8.42 (bs, 2H), 3.77 (q, *J* = 7.0 Hz, 4H), 3.37 (qd, *J* = 7.3, 5.4 Hz, 4H), 1.21 (t, *J* = 7.3 Hz, 6H), 1.17 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 168.8, 153.8, 147.6, 144.6, 41.4, 35.8, 15.1, 14.8.

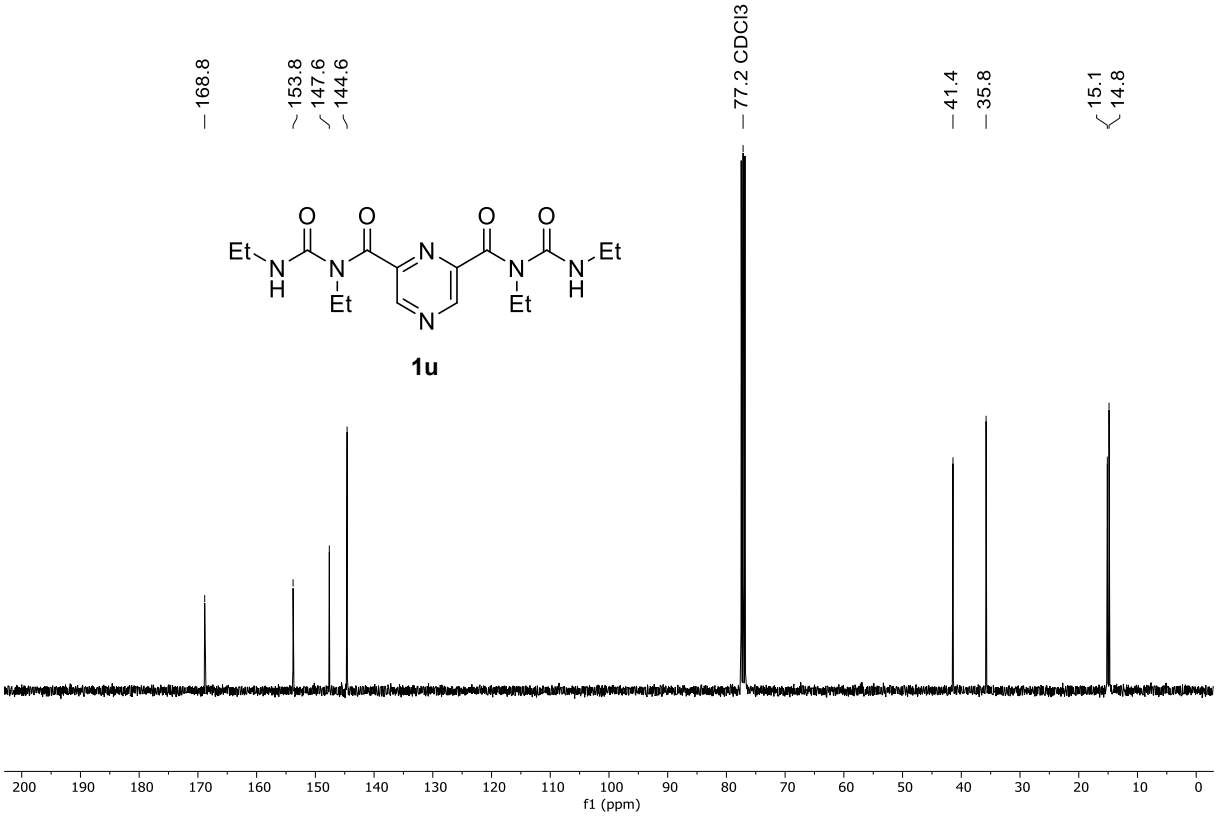
MS (ESI): *m/z* (%) = 387 (37, $[M+Na]^+$), 320 (100), 294 (26), 223 (29).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{16}H_{25}N_6O_4]^+$: 365.1932; found 365.1933 (Error PPM: 0.3).

IR (ATR, neat, $\tilde{\nu}$) = 3326 (w), 2977 (w), 2936 (w), 2875 (vw), 1709 (vs), 1672 (vs), 1637 (vs), 1531 (vs), 1462 (m), 1433 (s), 1403 (vs), 1366 (vs), 1354 (s), 1317 (m), 1297 (s), 1268 (vs), 1230 (m), 1197 (s), 1183 (vs), 1148 (s), 1103 (s), 1087 (s), 1073 (s), 1042 (s), 1017 (s), 966 (w), 942 (w), 918 (w), 891 (w), 856 (w), 822 (m), 799 (m), 775 (s), 740 (w), 652 (m), 636 (s), 624 (s), 583 (s), 553 (m), 512 (m), 473 (w), 410 (s) cm^{-1}.

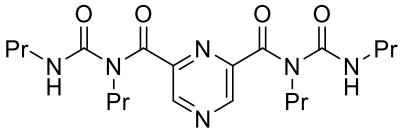


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of N^2,N^6-dipropyl-N^2,N^6-bis(propylcarbamoyl)pyrazine-2,6-dicarboxamide (1v)



1v

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (336 mg, 2.00 mmol, 1.00 equiv.) and the corresponding urea **S1v** (722 mg, 5.01 mmol, 2.50 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1), afforded the title compound **1v** (589 mg, 1.40 mmol, 70% yield) as a yellow solid.

R_f = 0.27 (*n*-pentane:ethyl acetate = 2:1, UV)

m.p. = 84-85 °C

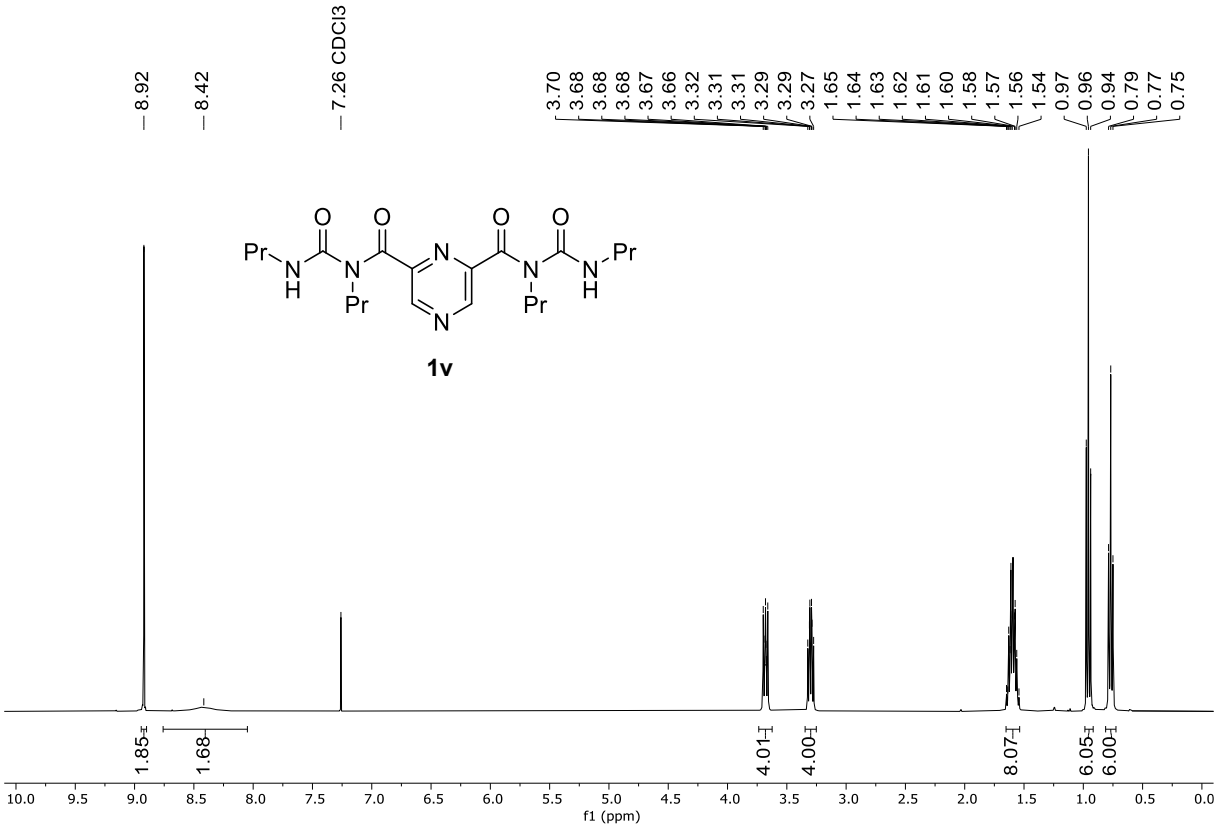
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.92 (s, 2H), 8.42 (bs, 2H), 3.75–3.61 (m, 4H), 3.30 (m, *J* = 7.1, 5.6 Hz, 4H), 1.67–1.52 (m, 8H), 0.96 (t, *J* = 7.4 Hz, 6H), 0.77 (t, *J* = 7.4 Hz, 6H).

^{13}C NMR(101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 168.9, 154.2, 147.6, 144.8, 47.7, 42.6, 23.2, 22.8, 11.5, 11.1.

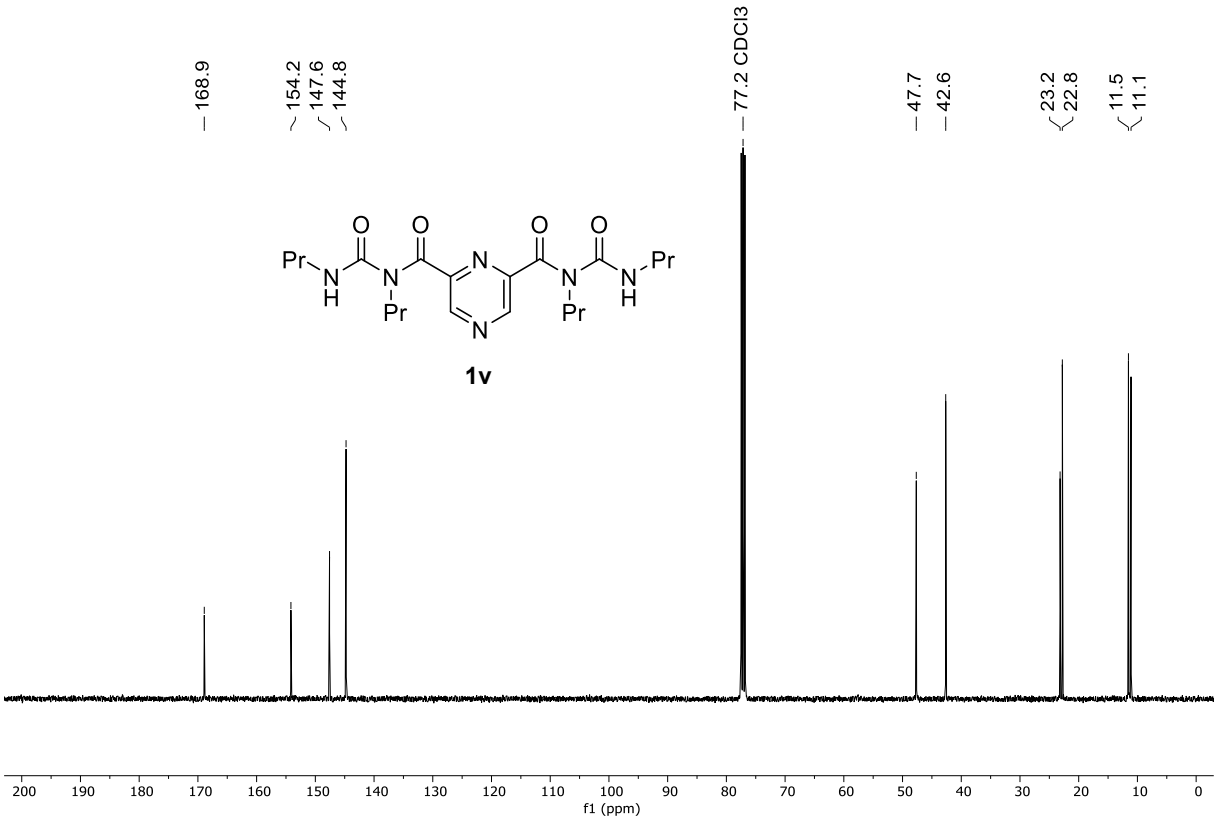
MS (ESI): *m/z* (%) 443 (74, [M+Na^+]), 362 (100), 336 (21), 251 (38).

HRMS (ESI-TOF): *m/z* [M+Na]$^+$ calcd for [$C_{20}H_{32}N_6O_4Na$]$^+$: 443.2377; found: 443.2385 (Error PPM: 1.8).

IR (ATR, neat, cm^{-1}): 3285(w), 2963(w), 2935(w), 2874(w), 1707(w), 1686(s), 1636(s), 1530(s), 1457(w), 1436(m), 1401(m), 1377(m), 1362(m), 1324(w), 1301(w), 1283(m), 1244(s), 1188(m), 1097(s), 1019(m), 974(w), 915(w), 889(w), 778(m), 768(w), 714(m), 655(m), 637(m), 598(w), 557(w), 507(w), 471(w), 425(m).

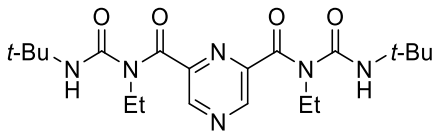


^{1}H NMR (400 MHz, Chloroform-d [77.16 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of N^2,N^6-bis(tert-butylcarbamoyl)-N^2,N^6-diethylpyrazine-2,6-dicarboxamide (1x)



1

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (84.2 mg, 501 µmol, 1.00 equiv.) and the corresponding urea **S1x** (180 mg, 1.25 mmol, 2.50 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1), afforded the title compound **1x** (176 mg, 417 µmol, 83% yield) as a colorless solid.

R_f = 0.28 (*n*-pentane:ethyl acetate = 3:1, UV)

m.p. = 110-112°C

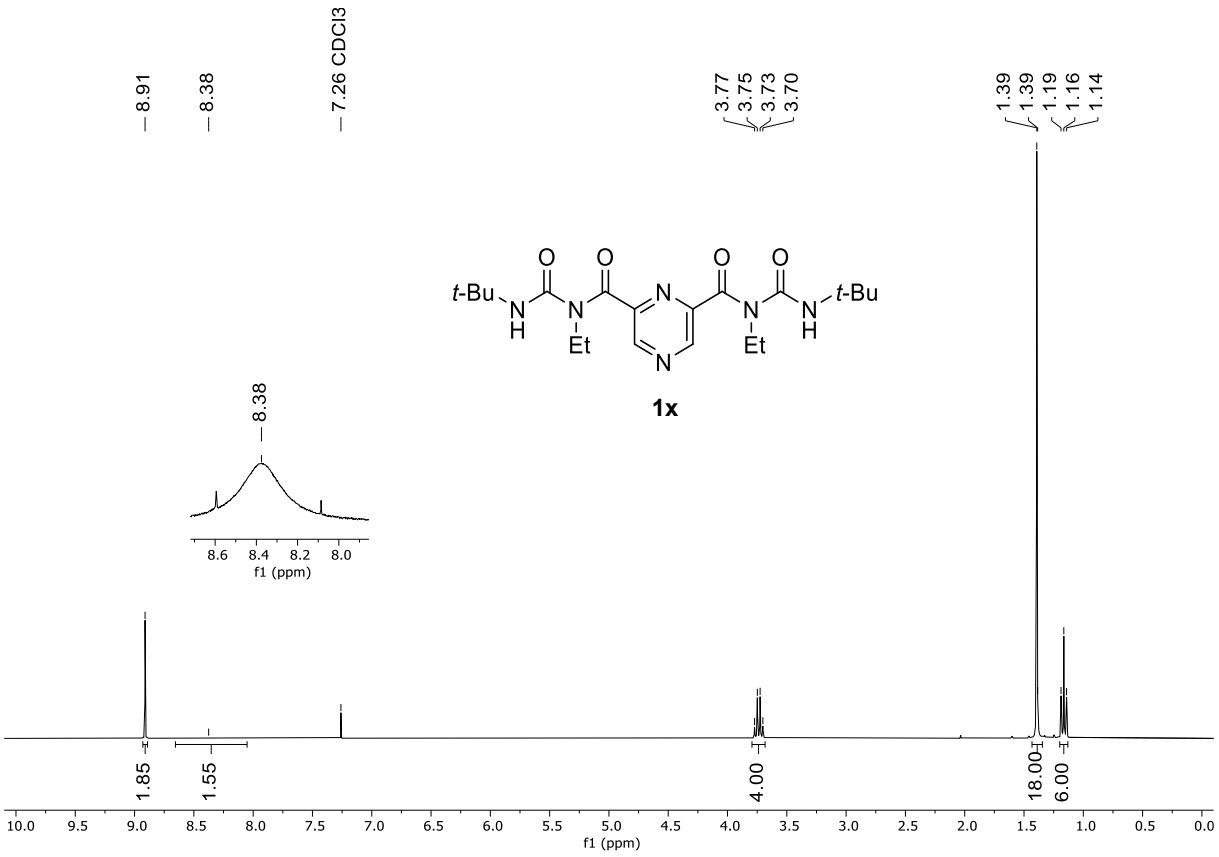
H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.91 (s, 2H), 8.38 (bs, 2H), 3.74 (q, *J* = 7.0 Hz, 4H), 1.39 (s, 18H), 1.16 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 168.8, 152.1, 147.8, 144.4, 51.7, 41.2, 28.8, 15.1.

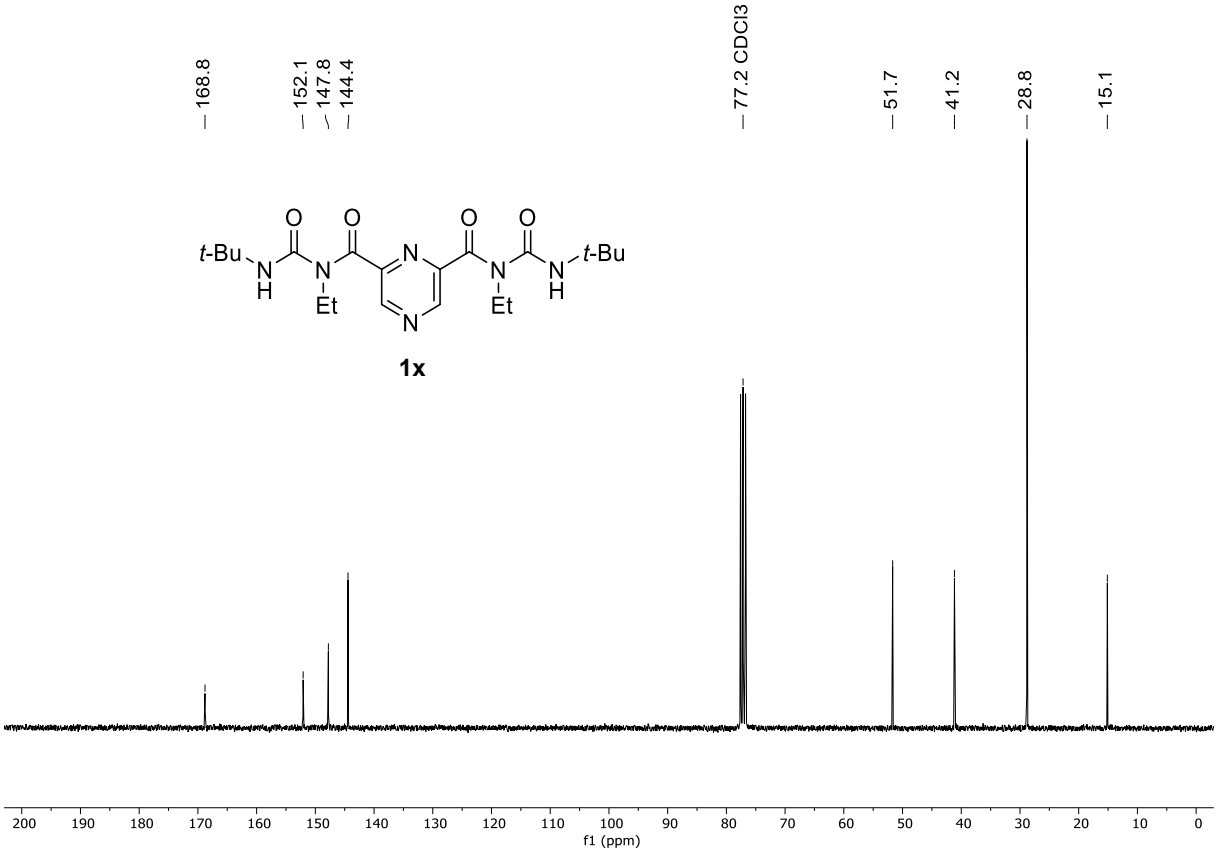
MS (ESI): *m/z* (%) = 443 (100, [M+Na^+]), 348 (11), 223 (26).

HRMS (ESI-TOF): *m/z* [M+Na]$^+$ calcd. for [$C_{20}H_{32}N_6O_4Na$]$^+$: 443.2377; found: 443.2371 (Error PPM: -1.4).

IR (ATR, $\tilde{\nu}$) = 3271 (w), 2975 (w), 2938 (w), 1709 (vs), 1678 (w), 1643 (s), 1523 (vs), 1482 (w), 1460 (s), 1446 (m), 1427 (s), 1401 (s), 1376 (vs), 1364 (vs), 1354 (vs), 1301 (w), 1254 (s), 1225 (m), 1181 (vs), 1105 (vs), 1075 (vs), 1011 (s), 973 (m), 950 (m), 916 (m), 899 (w), 848 (w), 803 (w), 791 (s), 773 (vs), 748 (w), 728 (w), 703 (w), 665 (w), 636 (vs), 626 (s), 614 (vs), 565 (w), 532 (w), 516 (w), 489 (w), 473 (w), 442 (m), 406 (s) cm^{-1}.

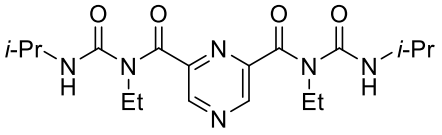


^{1}H NMR (300 MHz, Chloroform-d [77.16 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of N^2,N^6-dipropyl-N^2,N^6-bis(isopropylcarbamoyl)pyrazine-2,6-dicarboxamide (1w)



1w

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (168 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea **S1w** (325 mg, 2.50 mmol, 2.50 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 3:1), afforded the title compound **1w** (226 mg, 576 µmol, 58% yield) as a yellow resin.

R_f = 0.15 (*n*-pentane:ethyl acetate = 3:1, UV)

m.p. = N/A

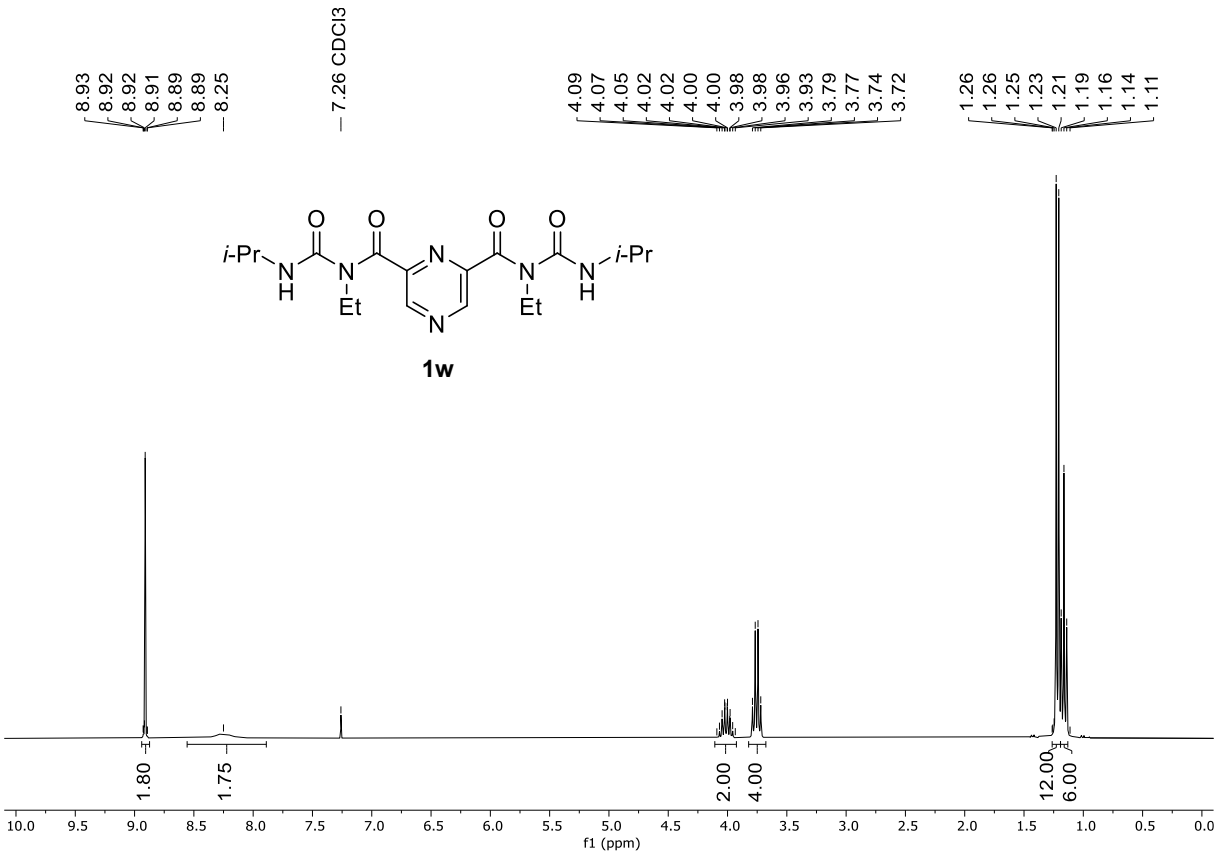
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.91 (s, 2H), 8.25 (bs, 2H), 4.01 (dhept, *J* = 6.9, 6.6 Hz, 2H), 3.75 (q, *J* = 7.0 Hz, 4H), 1.22 (d, *J* = 6.6 Hz, 12H), 1.16 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 168.8, 153.0, 147.6, 144.5, 43.1, 41.3, 22.7, 15.1.

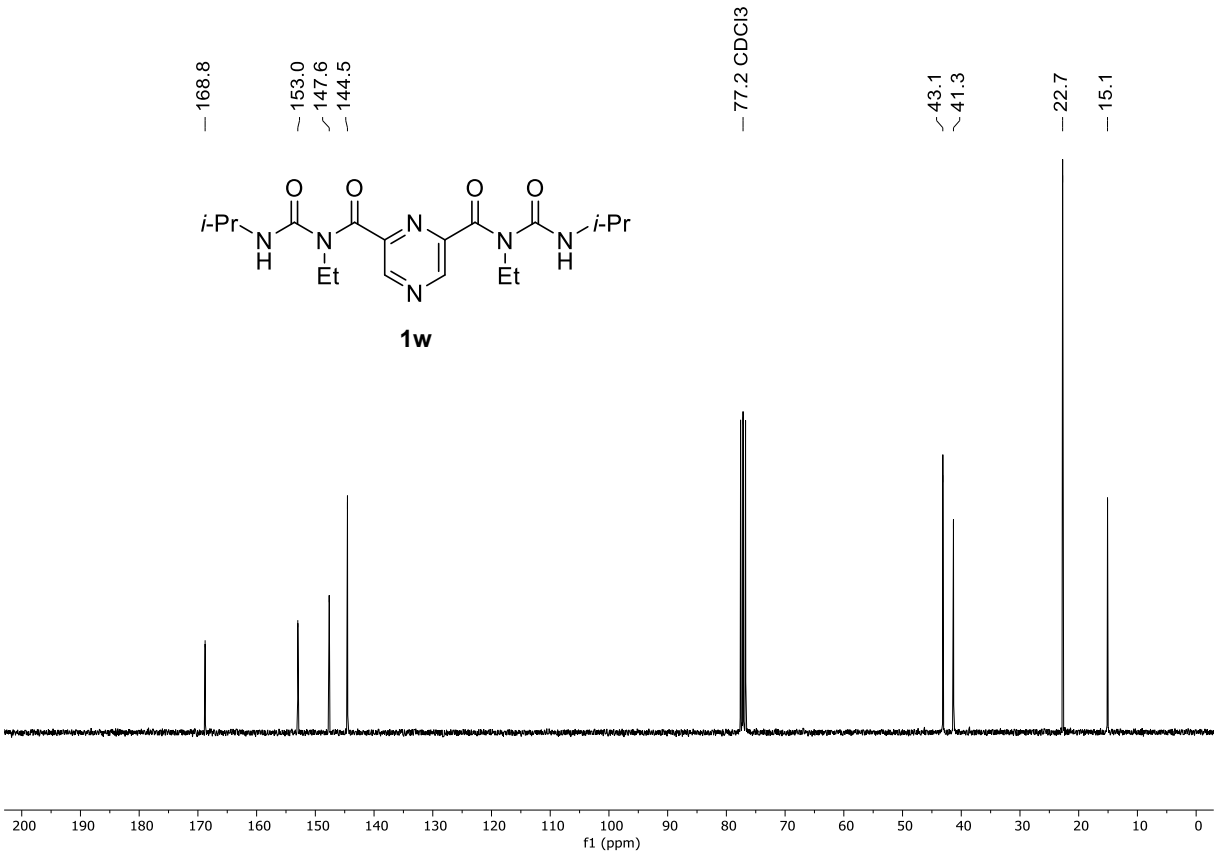
MS (ESI): *m/z* (%) = 415 (100, $[M+Na]^+$), 393 (25, $[M+H]^+$), 334 (32), 223 (19).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{18}H_{28}N_6O_4Na]^+$: 415.2064; found: 415.2061 (Error PPM: -0.7).

IR (ATR, $\tilde{\nu}$) = 3305 (w), 2973 (w), 2936 (w), 2875 (w), 1686 (vs), 1648 (vs), 1519 (vs), 1460 (s), 1429 (s), 1366 (vs), 1321 (s), 1293 (m), 1250 (vs), 1199 (vs), 1183 (vs), 1166 (vs), 1152 (vs), 1130 (s), 1095 (vs), 1066 (vs), 1017 (vs), 987 (w), 952 (w), 930 (w), 909 (w), 873 (w), 809 (m), 795 (m), 769 (s), 701 (m), 628 (s), 585 (s), 520 (m), 495 (m), 418 (vs) cm^{-1}.

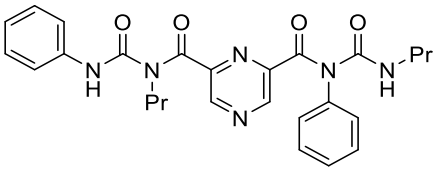


^{1}H NMR (300 MHz, Chloroform-d [77.16 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of N^2-phenyl-N^6-(phenylcarbamoyl)-N^6-propyl-N^2-(propylcarbamoyl)pyrazine-2,6-dicarboxamide (1t) TT1109



1t

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (337 mg, 2.00 mmol, 1.00 equiv.) and the corresponding urea **S1t** (892 mg, 5.00 mmol, 2.50 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1, UV), afforded the title compound **1t** (370 mg, 757 µmol, 38% yield as a pale yellow solid.

R_f = 0.21 (*n*-pentane:ethyl acetate = 2:1, UV)

m.p. = 122-125 °C

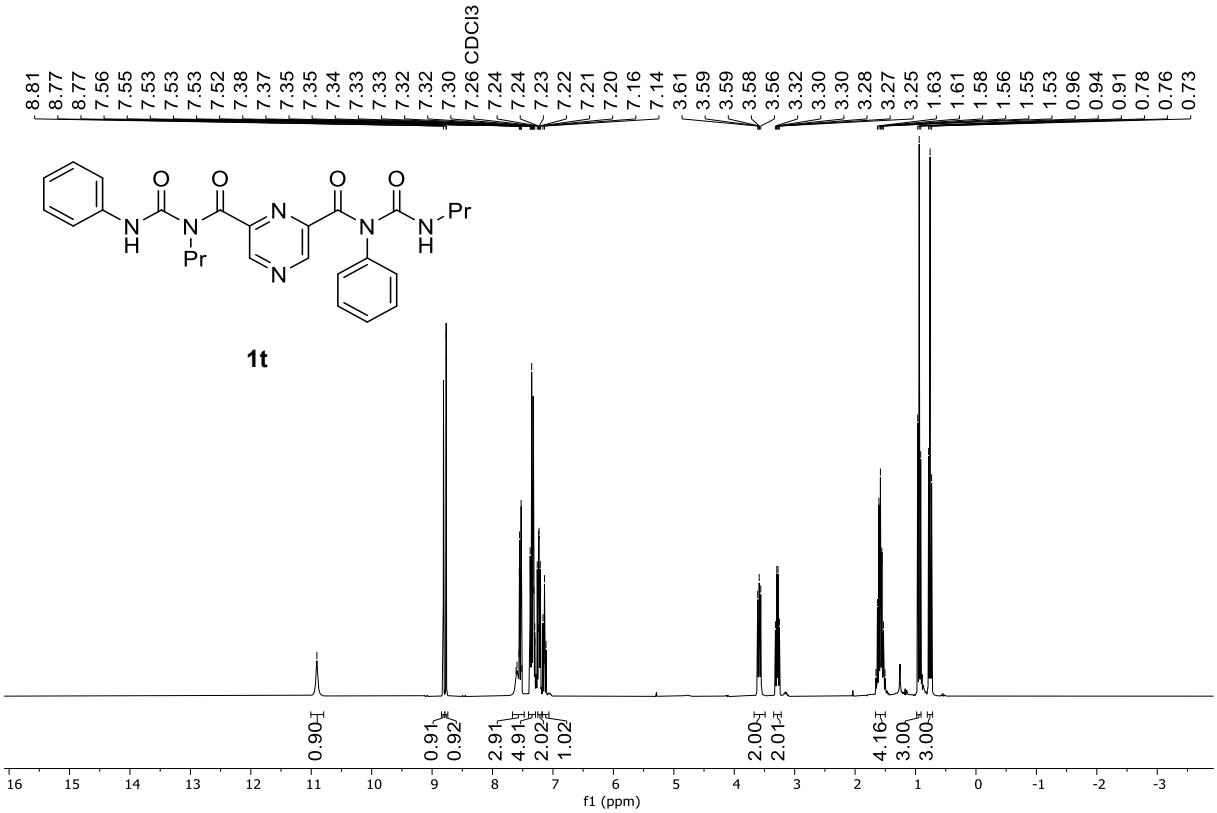
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 10.91 (s, 1H), 8.81 (d, *J* = 0.5 Hz, 1H), 8.77 (d, *J* = 0.5 Hz, 1H), 7.63 – 7.50 (m, 3H), 7.41 – 7.29 (m, 5H), 7.25 – 7.19 (m, 2H), 7.18 – 7.10 (m, 1H), 3.63 – 3.53 (m, 2H), 3.29 (td, *J* = 7.1, 5.7 Hz, 2H), 1.93 – 1.37 (m, 4H), 0.94 (t, *J* = 7.4 Hz, 3H), 0.76 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 169.5, 168.1, 153.9, 151.4, 147.7, 147.2, 144.7, 144.1, 137.4, 137.3, 129.6, 129.6, 129.3, 129.2, 124.7, 120.7, 47.5, 42.7, 23.1, 22.7, 11.4, 11.1.

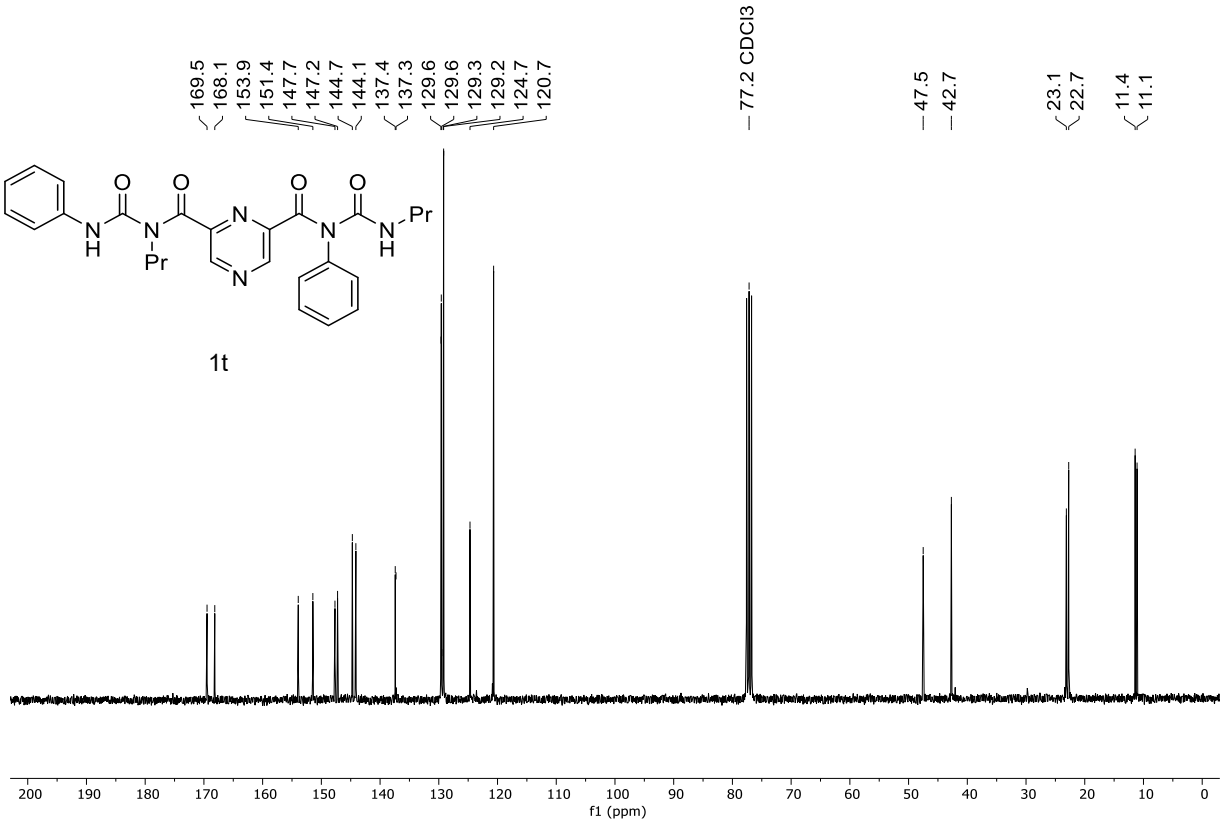
MS (ESI): *m/z* (%) = 511 (34, $[M+Na]^+$), 396 (100), 311 (17).

HRMS (EI): *m/z* $[M]^+$ calcd. for $[C_{26}H_{28}O_4N_6]^+$: 488.21666; found: 488.21723 (Error PPM: 1.2).

IR (ATR, neat, ṽ) = 3279 (w), 3216 (w), 3136 (w), 3061 (w), 2963 (w), 2936 (w), 2873 (w), 1717 (vs), 1703 (vs), 1688 (vs), 1635 (m), 1595 (vs), 1554 (vs), 1515 (vs), 1486 (s), 1460 (m), 1448 (s), 1440 (s), 1433 (s), 1393 (vs), 1370 (s), 1334 (vs), 1301 (s), 1285 (s), 1258 (vs), 1246 (vs), 1221 (s), 1185 (vs), 1177 (vs), 1154 (m), 1136 (s), 1107 (vs), 1091 (vs), 1081 (vs), 1017 (s), 979 (w), 950 (w), 909 (w), 899 (m), 881 (m), 846 (w), 814 (w), 791 (w), 767 (vs), 752 (vs), 740 (vs), 714 (vs), 691 (vs), 673 (s), 650 (s), 628 (s), 616 (m), 593 (s), 567 (m), 526 (w), 510 (s), 453 (m), 426 (vs) cm^{-1}.

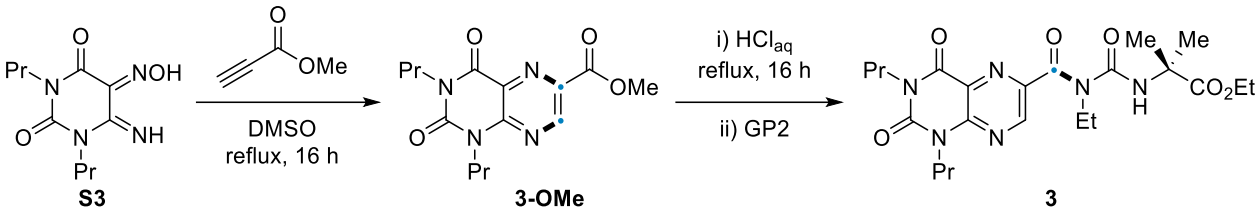


^{1}H NMR (300 MHz, Chloroform-d [77.16 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

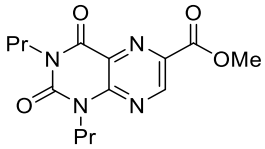
Synthesis of ethyl 2-(3-(2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropteridine-6-carbonyl)-3-ethylureido)-2-methylpropanoate (3)



S3 3-OMe 3

The title compound **3** was synthesized in 3 steps from 6-amino-5-nitroso-1,3-dipropylpyrimidine-2,4-dione (**S3**), synthesized in a previous work of this group.[8]

Step 1: Synthesis of methyl 2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropteridine-6-carboxylate (3-OMe)



3-OMe

The title compound was synthesized according to an modified version of an literature known procedure.[9] 6-amino-5-nitroso-1,3-dipropylpyrimidine-2,4-dione (**S3)** (1.01 g, 4.22 mmol, 1.0 equiv) was dissolved in DMSO (8.5 ml, 0.5 M) and methyl prop-2-ynoate (879 mg, 10.5 mmol, 2.5 equiv) was added. The reaction mixture was stirred under reflux for 16 h. The DMSO was removed *in vacuo* and the crude residue was prufied by column chromatography (*n*-pentane:ethyl acetate = 2:1) yielding the title compound **3-OMe** (393 mg, 1.28 mmol, 30 % yield) as a yellow cristalline solid.

R_f = 0.23 (*n*-pentane:ethyl acetate = 2:1, Detection)

m.p. = 161-164 °C

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.30 (s, 1H), 4.38 – 4.20 (m, 2H), 4.13 – 4.04 (m, 2H), 4.02 (s, 3H), 1.83 – 1.66 (m, 4H), 0.98 (m, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 163.9, 158.8, 150.0, 149.5, 149.3, 138.7, 126.9, 53.2, 44.7, 44.3, 21.1, 21.0, 11.4, 11.3.

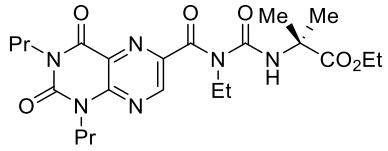
MS (EI): *m/z* (%) = 306 (52, $[M]^+$), 246 (100), 233 (19), 204 (22), 178 (16), 164 (14), 137 (14).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{14}H_{18}N_4O_4Na]^+$: 329.1220; found: 329.1220 (Error PPM: 1.5).

IR (ATR, neat, $\tilde{\nu}$) = 2961 (w), 2930 (w), 2875 (w), 1717 (s), 1670 (vs), 1578 (m), 1541 (s), 1499 (vs), 1448 (s), 1435 (vs), 1391 (s), 1366 (m), 1340 (s), 1291 (vs), 1268 (vs), 1242 (vs), 1234 (vs), 1189 (vs),

1162 (s), 1144 (vs), 1099 (s), 1036 (m), 1003 (s), 964 (m), 946 (m), 932 (m), 889 (w), 875 (m), 816 (s), 785 (m), 763 (m), 750 (vs), 718 (s), 691 (s), 589 (w), 567 (m), 536 (vs), 528 (vs), 451 (vs) cm^{-1}.

Steps 2 and 3: Synthesis of ethyl 2-(3-(2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropteridine-6-carbonyl)-3-ethylureido)-2-methylpropanoate (3)



3

Step 2: Following and adapted version of a literature known procedure ethyl 2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropteridine-6-carboxylate (**3-OMe**) (550 mg, 1.80 mmol, 1.0 equiv.) was suspended in 8 ml of concentrated aqueous HCl under ambient conditions and was refluxed for 16 h.[9] The reaction mixture was thoroughly dried *in vacuo* yielding the crude 2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropteridine-6-carboxylic acid (**3-OH**) in quantitative yield. **Step 3:** Following general procedure GP2', using the crude 2,4-dioxo-1,3-dipropyl-1,2,3,4-tetrahydropteridine-6-carboxylic acid (**3-OH**) (168 mg, 1.00 mmol, 1.0 equiv.) and the corresponding urea **S1a** (271 mg, 1.34 mmol, 1.25 equiv.), column chromatography (*n*-pentane:ethyl acetate = 3:1 to 2:1) afforded the title compound **3** (224 mg, 471 µmol, 67% yield) as a yellow resin.

$\mathbf{R}_f$ = 0.38 (*n*-pentane:ethyl acetate = 2:1, UV)

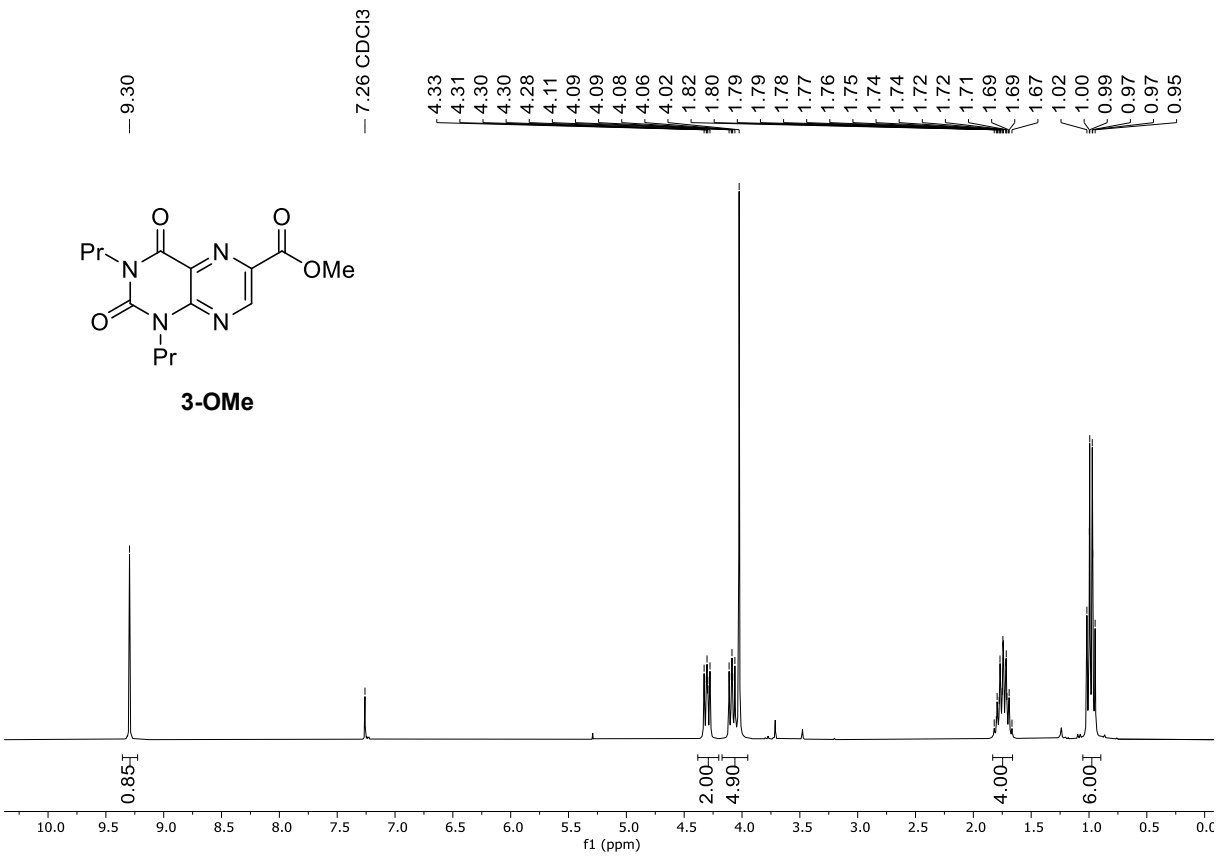
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.95 (s, 1H), 8.87 (s, 1H), 4.36 – 4.25 (m, 2H), 4.20 (q, *J* = 7.1 Hz, 2H), 4.12 – 4.00 (m, 2H), 3.88 (q, *J* = 6.9 Hz, 2H), 1.81 – 1.67 (m, 4H), 1.58 (s, 6H), 1.30 (t, *J* = 7.0 Hz, 3H), 1.26 (t, *J* = 7.1 Hz, 3H), 0.98 (q, *J* = 7.7 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.2, 168.8, 159.0, 153.0, 150.0, 148.4, 148.2, 144.2, 125.0, 61.6, 57.0, 44.6, 44.3, 41.9, 25.0, 21.2, 21.0, 15.0, 14.3, 11.4, 11.3.

MS (ESI): *m/z* (%) = 499 (100, $[M+Na]^+$), 420 (13), 320 (83), 266 (17).

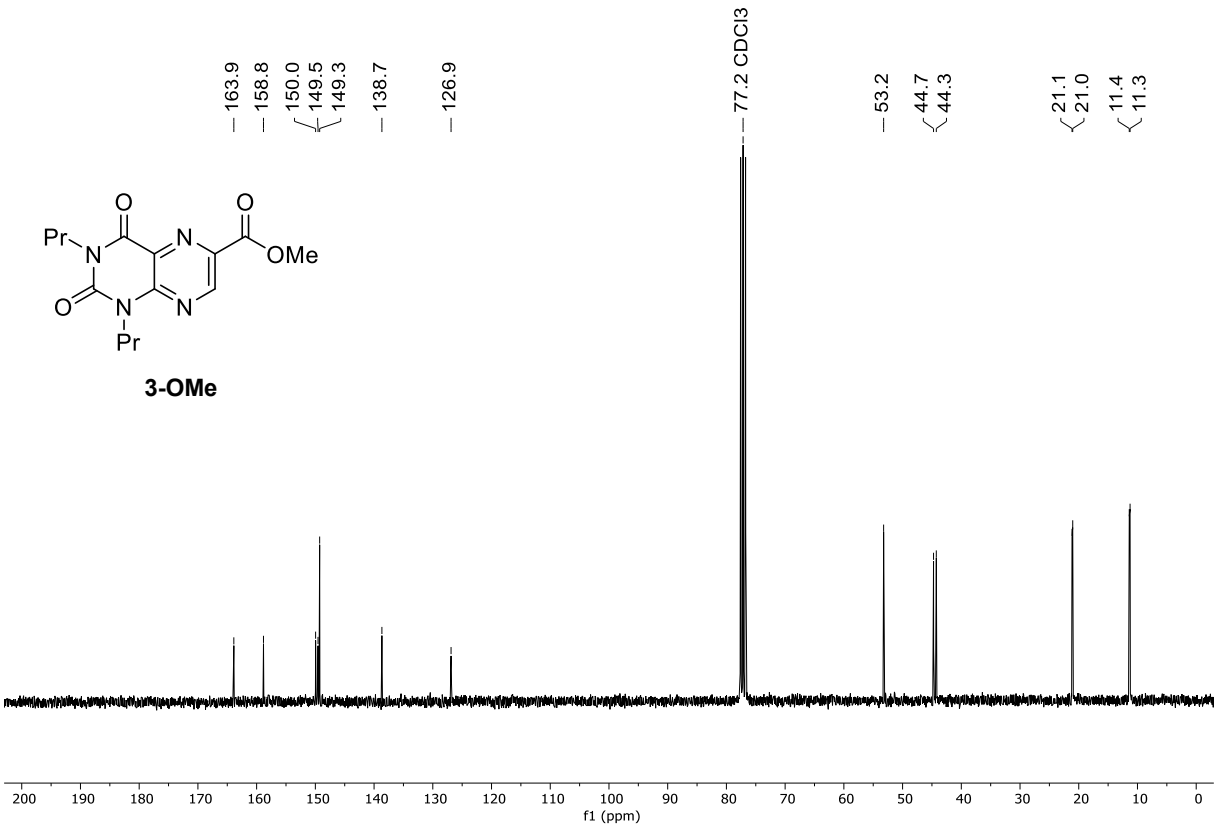
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{22}H_{32}N_6O_6Na]^+$: 499.2275; found: 499.2281 (Error PPM: 1.2).

IR (ATR, neat, $\tilde{\nu}$) = 3305 (vw), 2965 (w), 2936 (w), 2877 (w), 1723 (s), 1707 (s), 1672 (vs), 1578 (m), 1544 (s), 1497 (vs), 1446 (vs), 1403 (vs), 1380 (vs), 1321 (s), 1283 (vs), 1242 (vs), 1172 (vs), 1148 (vs), 1105 (s), 1093 (s), 1077 (vs), 1028 (m), 1005 (m), 938 (w), 893 (w), 863 (w), 814 (s), 777 (m), 754 (s), 712 (w), 667 (w), 622 (m), 585 (w), 532 (m), 516 (m), 445 (s) cm^{-1}.



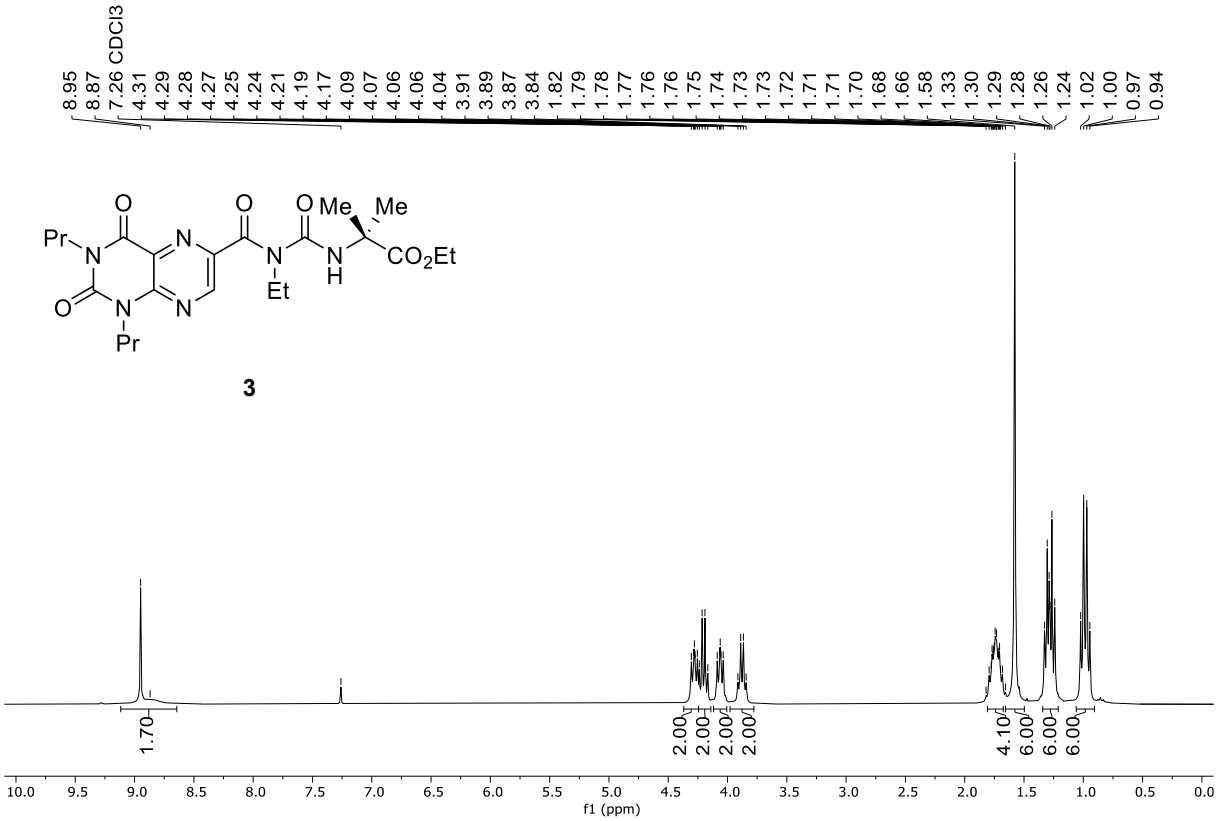
3-OMe

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)

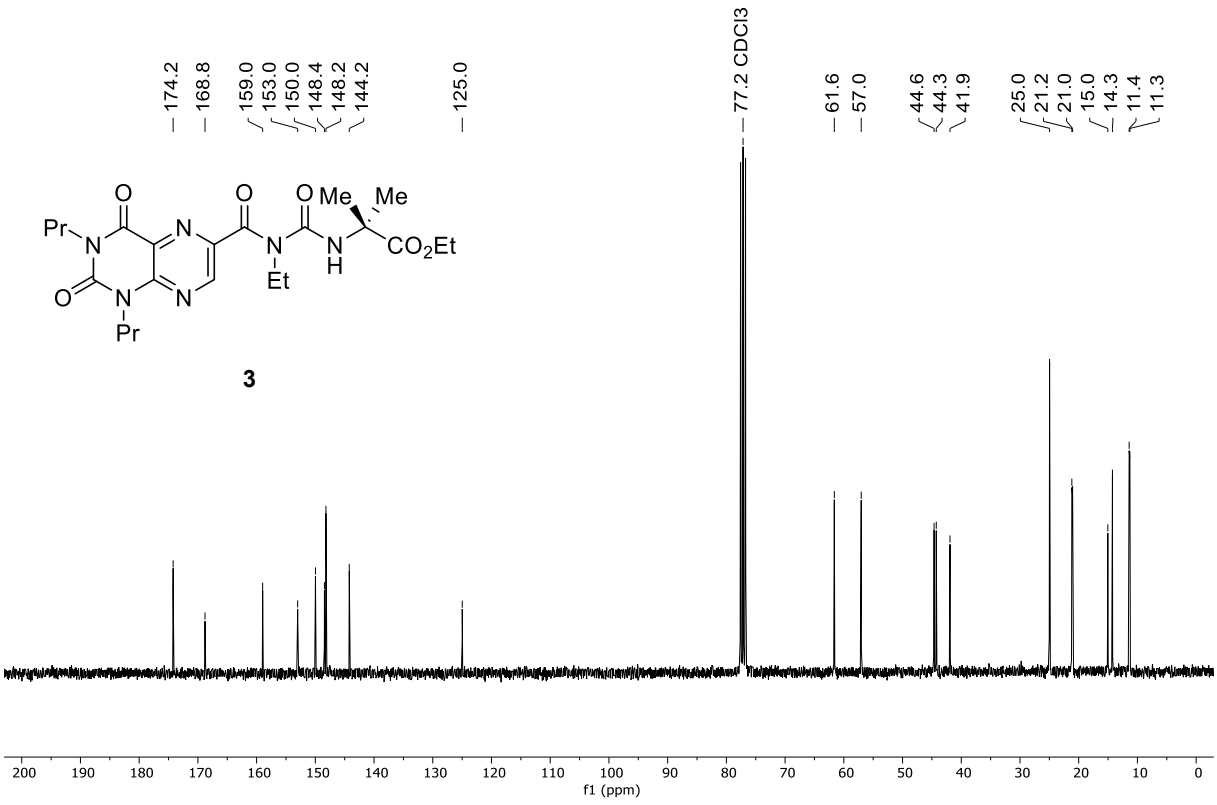


3-OMe

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)



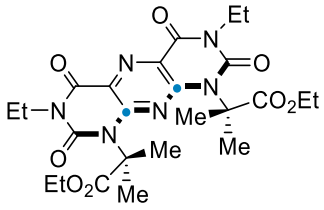
[1]H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



[13]C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

4.3 Synthesis of pyrimidopteridines according to GP3

Synthesis of diethyl 2,2'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(2-methylpropanoate) (2a)



2a

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **1a** (107 mg, 200 µmol, 1.00 equiv.) purification by column chromatography (n-pentane:ethyl acetate = 1:1), afforded the title compound **2a** (66.3 mg, 124 µmol, 62% yield) a pale orange solid.

R_f = 0.25 (*n*-pentane/EtOAc = 1:1)

m.p. = 163-165 °C

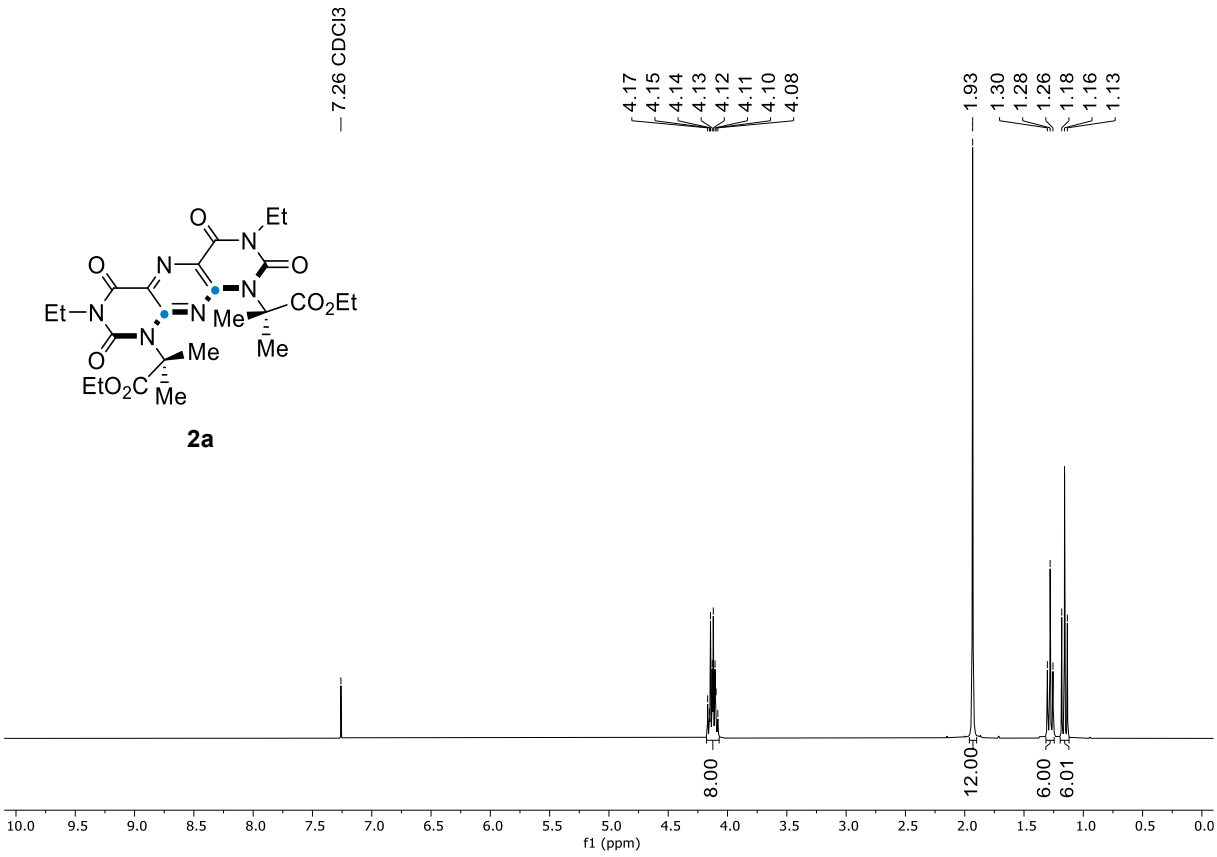
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.18 – 4.07 (m, 4H), 1.93 (s, 6H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.16 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, Chloroform-d [77.2 ppm], ppm) δ = 172.3, 157.5, 150.4, 148.3, 124.9, 66.0, 61.9, 38.1, 24.8, 14.1, 13.0.

MS (GC-MS): *m/z* (%) = 532 (10, $[M]^+$) 413 (100), 388 (17), 345 (33), 314 (15), 304 (18), 274 (62), 175 (15).

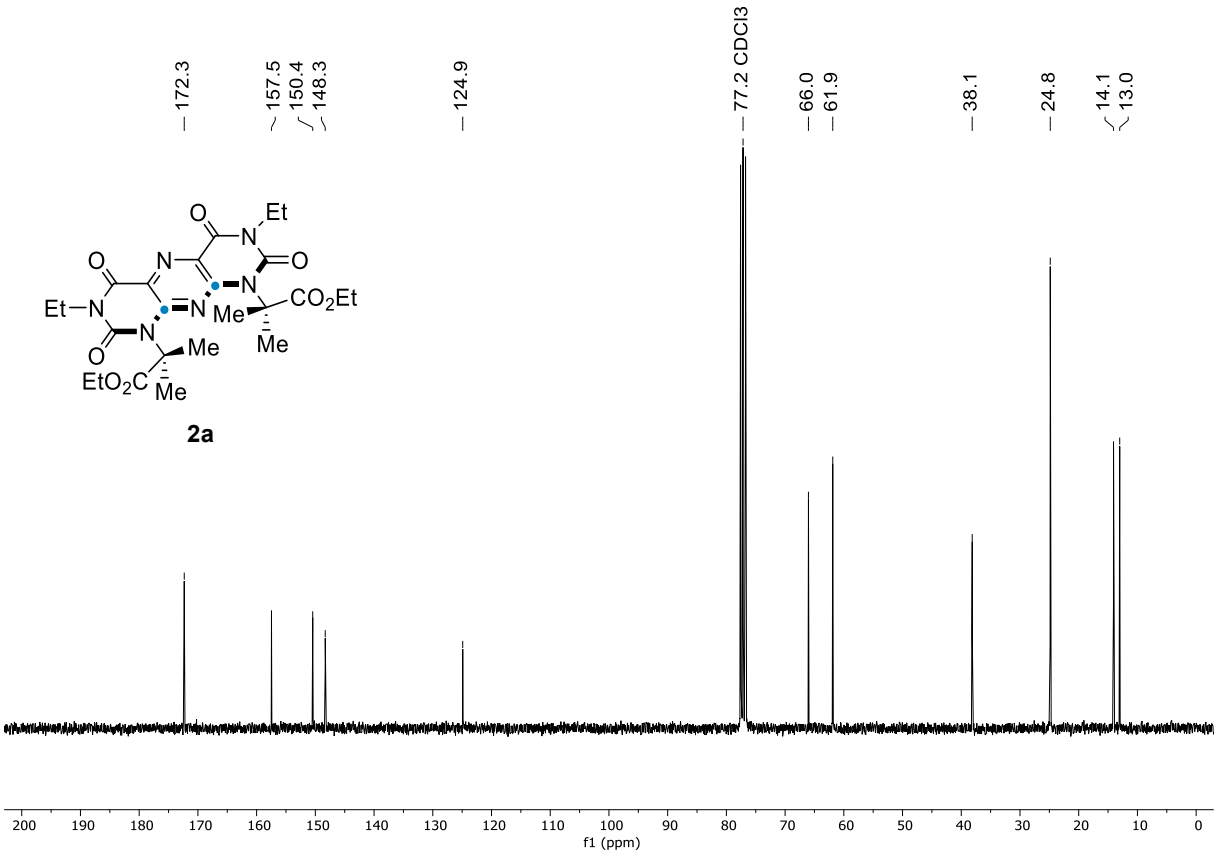
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{24}H_{32}N_6O_8Na]^+$: 555.2173; found: 555.2178 (Error PPM: 0.9).

IR (ATR, neat, $\tilde{\nu}$) = 2985 (w), 2942 (w), 1725 (s), 1713 (s), 1676 (vs), 1552 (vs), 1511 (w), 1466 (w), 1444 (m), 1425 (w), 1391 (vs), 1370 (vs), 1329 (vs), 1281 (vs), 1262 (vs), 1232 (s), 1221 (vs), 1207 (s), 1172 (vs), 1144 (vs), 1081 (vs), 1073 (s), 1030 (s), 969 (m), 932 (m), 899 (w), 858 (m), 814 (s), 803 (m), 767 (m), 754 (vs), 716 (m), 689 (m), 585 (w), 551 (w), 491 (vs), 475 (s), 457 (m), 445 (m), 430 (m), 408 (vs) cm^{-1}.



2a

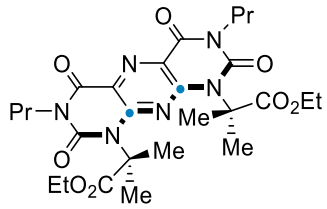
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



2a

^{13}C NMR (100 MHz, Chloroform-d [77.2 ppm], ppm)

Synthesis of diethyl 2,2'-(2,4,6,8-tetraoxo-3,7-dipropyl-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(2-methylpropanoate) (2b)



2b

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **1b** (113 mg, 200 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 2:1), afforded the title compound **2b** (68.5 mg, 122 µmol, 61% yield) as an off-white solid.

R_f = 0.22 (*n*-pentane:ethyl acetate = 2:1, UV)

m.p. = 95-98 °C

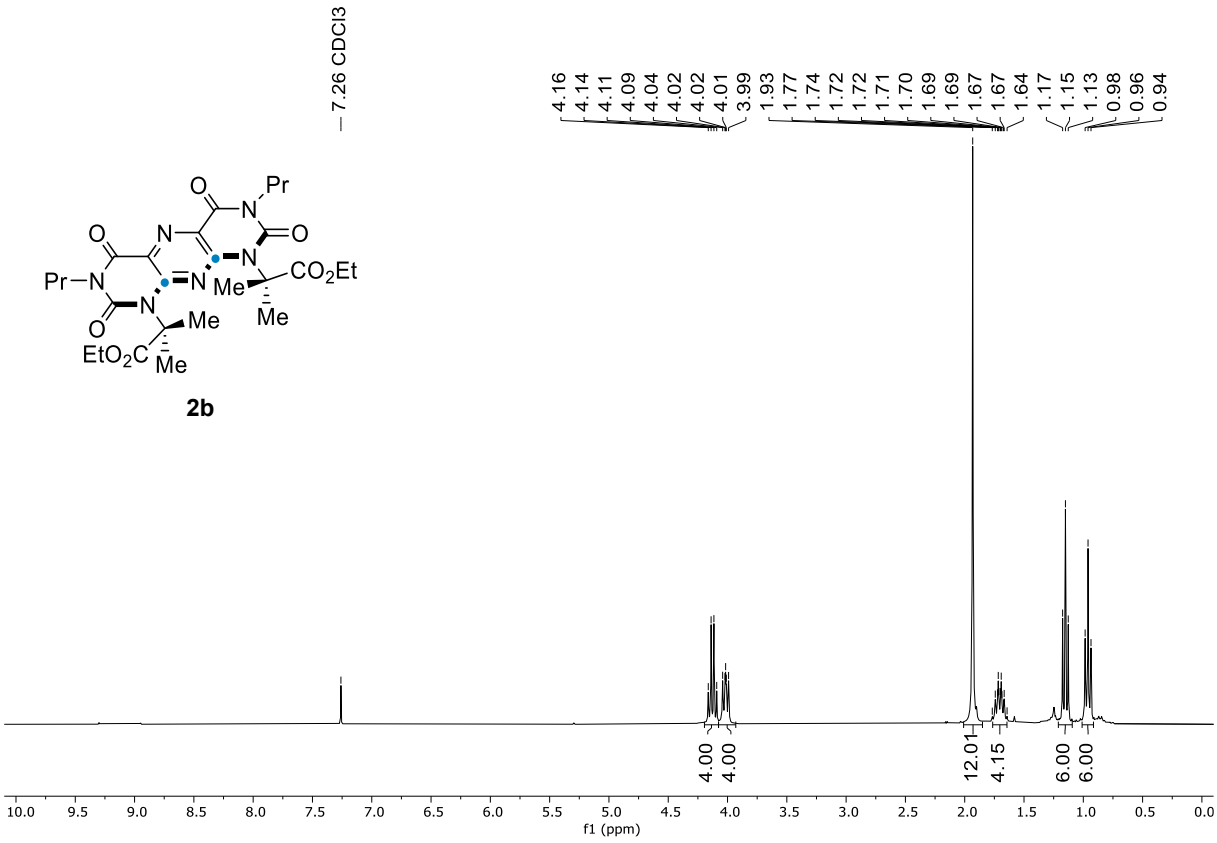
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.13 (q, *J* = 7.1 Hz, 4H), 4.08 – 3.93 (m, 4H), 1.93 (s, 12H), 1.76 – 1.64 (m, 4H), 1.15 (t, *J* = 7.1 Hz, 6H), 0.96 (t, *J* = 7.4 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 172.3, 157.7, 150.6, 148.3, 124.9, 66.0, 61.9, 44.3, 24.8, 21.0, 14.1, 11.3.

MS (EI): *m/z* (%) = 560 (78, $[M]^+$), 446 (42), 441 (100), 402 (55), 373 (38), 331 (61), 288 (94), 175 (22).

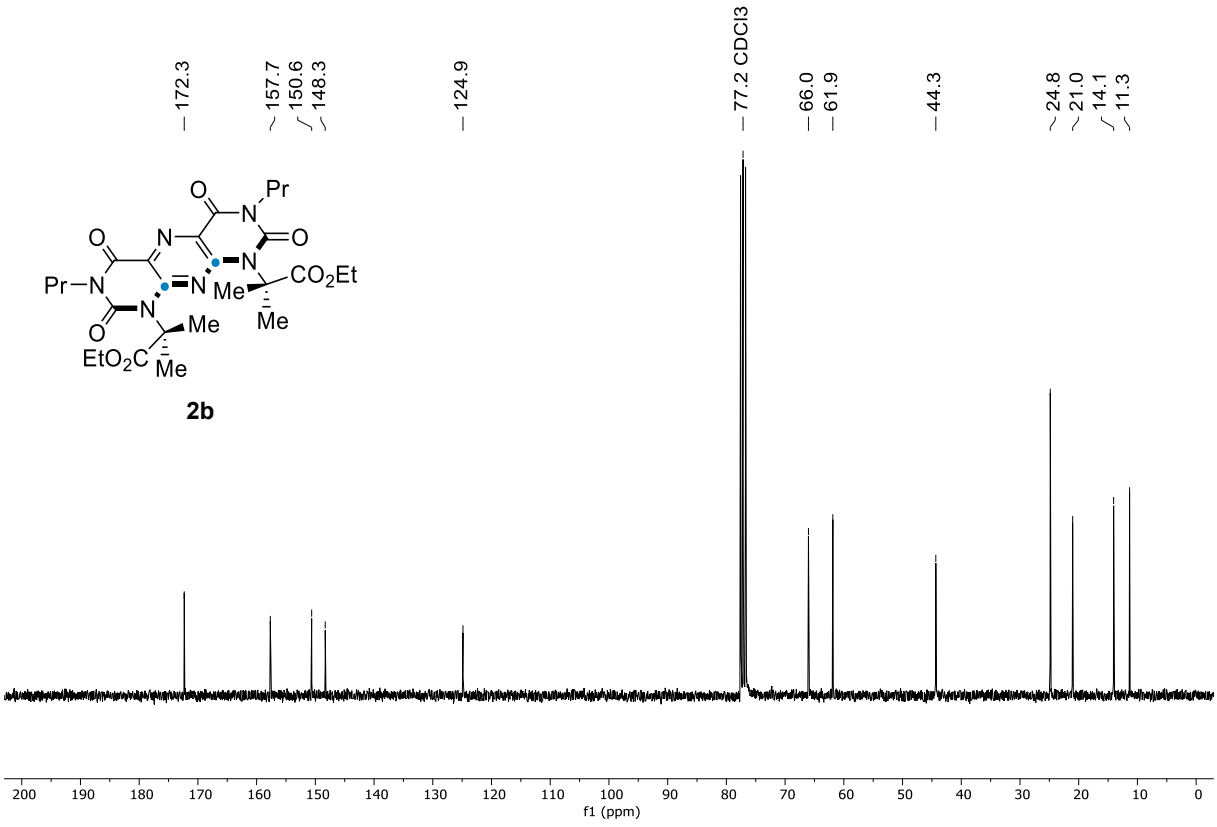
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{26}H_{36}N_6O_8Na]^+$: 583.2487; found: 583.2477 (Error PPM: 0.3).

IR (ATR, neat, $\tilde{\nu}$) = 2965 (w), 2938 (w), 1725 (s), 1674 (vs), 1550 (vs), 1517 (w), 1458 (w), 1427 (m), 1393 (vs), 1366 (vs), 1338 (s), 1276 (vs), 1258 (vs), 1227 (vs), 1174 (s), 1138 (vs), 1091 (s), 1022 (s), 954 (w), 907 (w), 891 (w), 860 (w), 812 (m), 754 (s), 716 (w), 687 (w), 667 (w), 597 (w), 544 (w), 506 (s), 491 (m), 479 (m) cm^{-1}.



2b

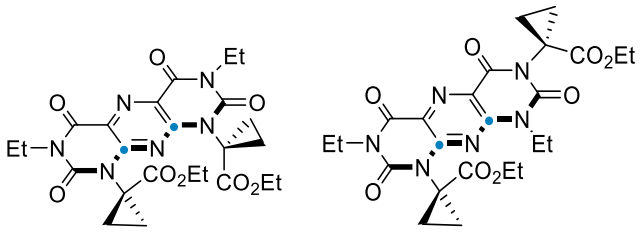
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



2b

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydro- pyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(cyclopropane-1-carboxylate) (2c) and diethyl 1,1'-(3,9-diethyl-2,4,6,8-tetraoxo-3,4,8,9-tetrahydropyrimido[5,4-*g*]pteridine-1,7(2*H*,6*H*)-diyl)bis(cyclopropane-1-carboxylate) (2c‘)



1c **1c‘**

Following the general procedure GP3, using the corresponding regioisomer mixture of pyrazine-2,6-dicarboxamides **1c** and **1c'** (106 mg, 200 µmol, 1.00 equiv, **1c:1c**' = 2:1) purification by column chromatography (*n*-pentane:ethyl acetate = 1:2) afforded the title compounds as an inseparable mixture (78.0 mg, 148 µmol, 74% yield) as an off white solid.

R_f = 0.32 (*n*-pentane:ethyl acetate = 1:2, UV)

m.p. = 204-206°C

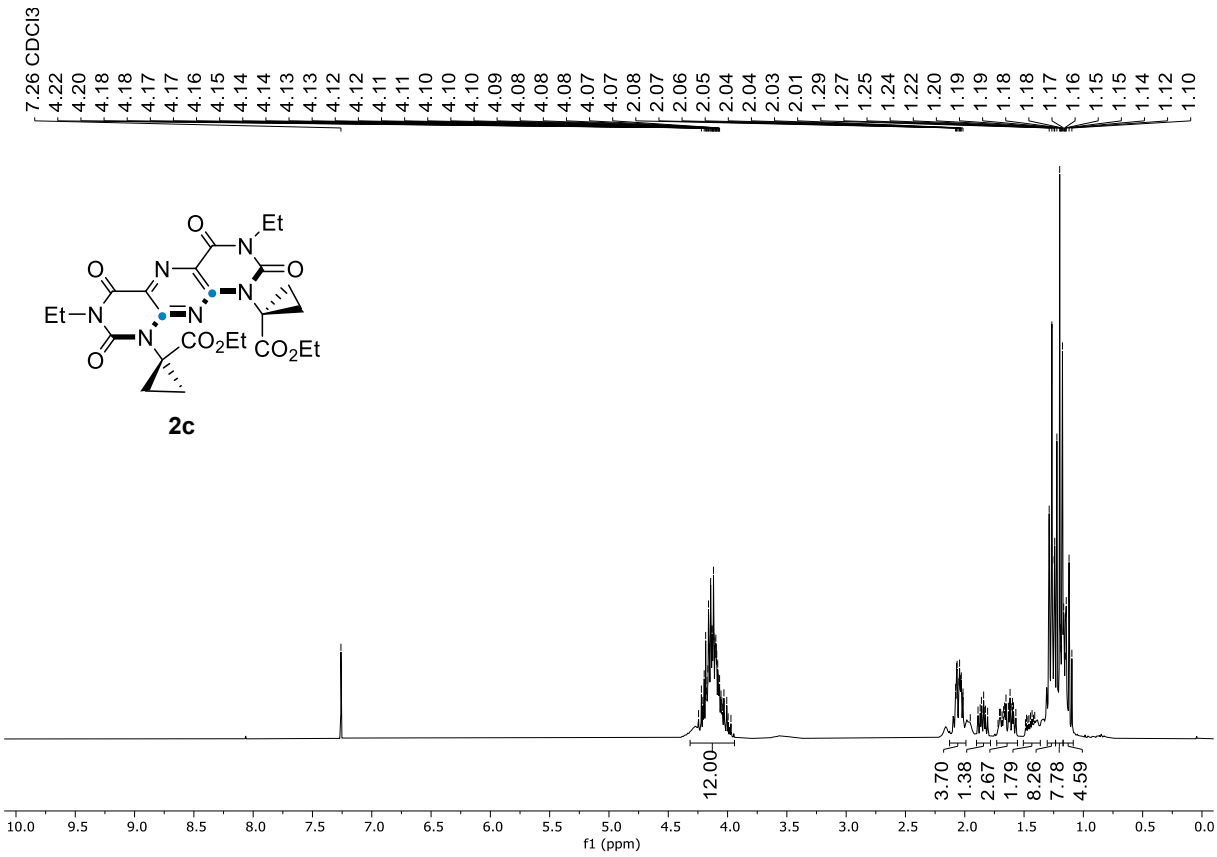
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.32 – 3.94 (m, 12H), 2.13 – 1.99 (m, 4H), 1.90 – 1.78 (m, 1H), 1.73 – 1.56 (m, 3H), 1.51 – 1.36 (m, 2H), 1.30 – 1.23 (m, 8H), 1.20 (t, *J* = 7.1 Hz, 8H), 1.17 – 1.09 (m, 4H). Collection of peaks of both isomers.

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 170.3, 170.3, 170.1, 170.0, 158.1, 157.9, 157.7, 150.2, 150.0, 150.0, 149.8, 149.8, 149.8, 149.7, 149.1, 124.7, 124.6, 124.0, 62.2, 62.0, 61.9, 39.0, 38.0, 38.0, 37.4, 37.3, 36.7, 20.4, 20.0, 19.8, 19.3, 14.3, 14.3, 14.2, 14.1, 13.0, 12.8. Collection of peaks of both isomers.

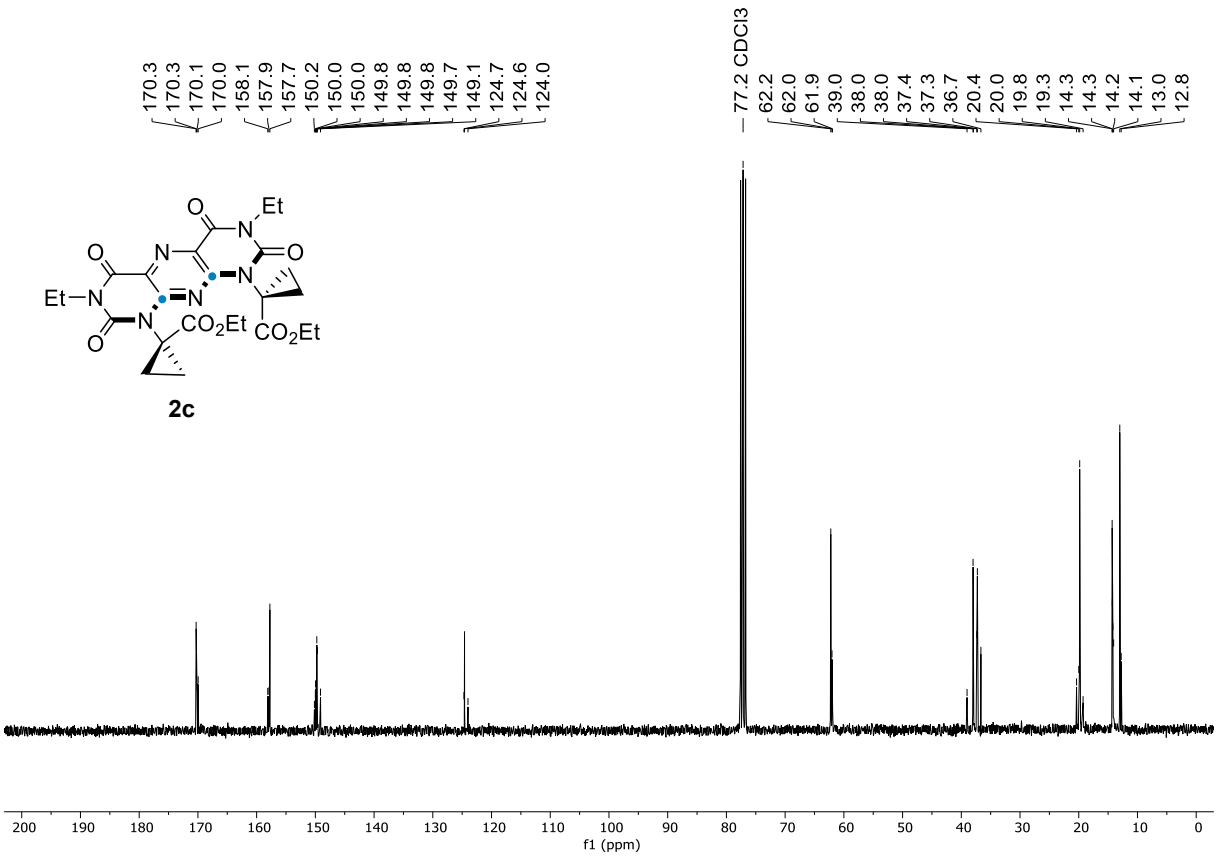
MS (EI): *m/z* (%) = 528 (100 ,$[M]^+$), 482 (28), 455 (92), 384 (14), 255 (11), 189 (12).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{24}H_{29}N_6O_8]^+$: 529.2042; found: 529.2051 (Error PPM: 1.7).

IR (ATR, neat, $\tilde{\nu}$) = 2983 (w), 1731 (vs), 1694 (vs), 1678 (vs), 1556 (vs), 1523 (w), 1438 (s), 1419 (s), 1393 (vs), 1372 (vs), 1340 (vs), 1323 (vs), 1293 (m), 1276 (vs), 1232 (s), 1187 (vs), 1172 (s), 1154 (vs), 1117 (w), 1093 (m), 1075 (m), 1058 (m), 1024 (s), 983 (w), 946 (m), 918 (w), 869 (w), 858 (w), 814 (m), 756 (s), 750 (s), 718 (w), 705 (w), 687 (w), 663 (w), 579 (w), 557 (w), 522 (m), 500 (vs), 479 (m), 465 (w), 445 (w), 408 (w) cm^{-1}.

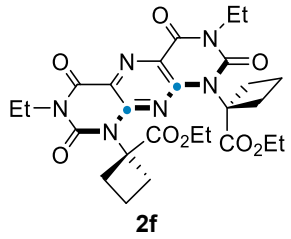


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm).



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm).

Synthesis of diethyl 1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-g]pteridine-1,9(2H,6H)-diyl)bis(cyclobutane-1-carboxylate) (2d)



2f

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **1d** (112 mg, 200 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:1) afforded the title compound **2d** (89.5 0mg, 161 µmol, 80% yield) as an ofwhite solid.

R_f = 0.22 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = 262-263 °C

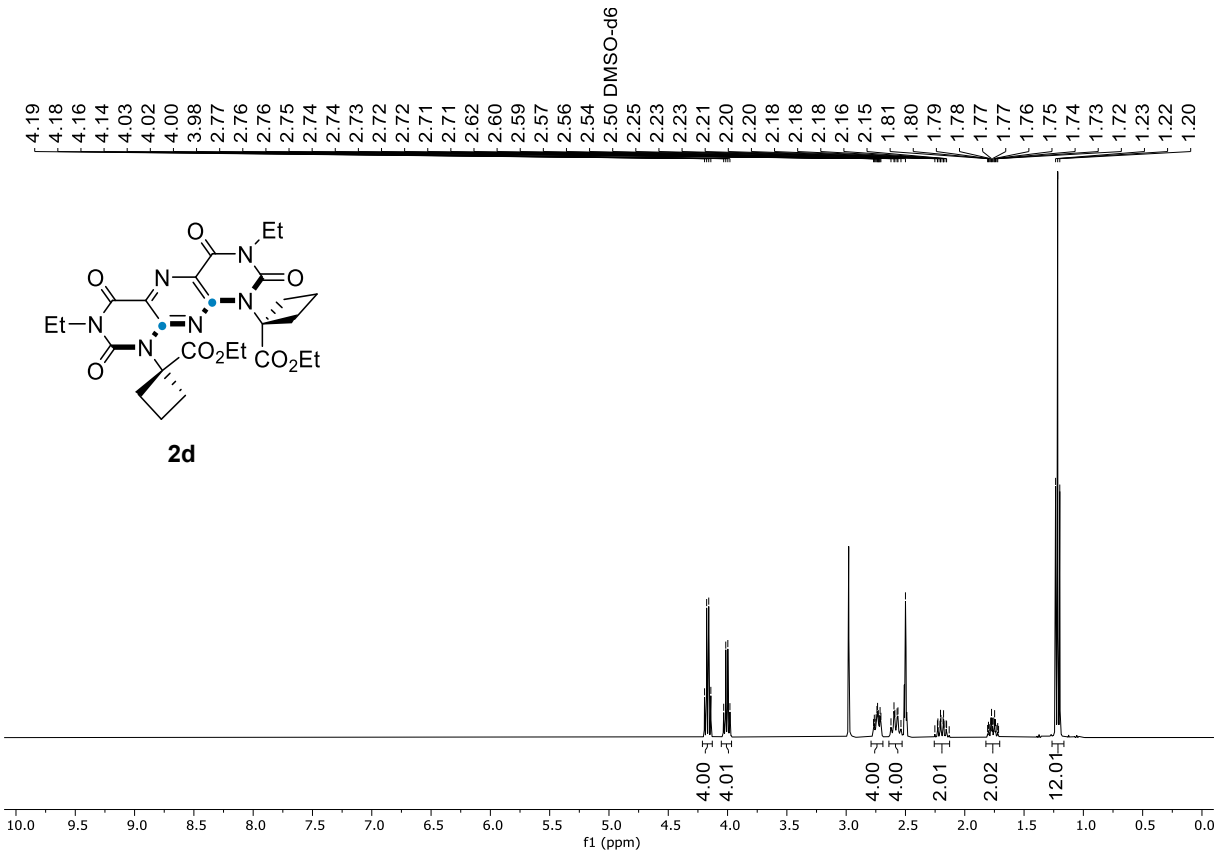
^{1}H NMR (400 MHz, 373 K, DMSO-d_6 [2.50 ppm], ppm) δ = 4.17 (q, *J* = 7.1 Hz, 4H), 4.01 (q, *J* = 7.0 Hz, 4H), 2.79 – 2.69 (m, 4H), 2.66 – 2.53 (m, 4H), 2.26 – 2.12 (m, 2H), 1.95 – 1.66 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 12H).

^{13}C NMR (101 MHz, 373 K, DMSO-d_6 [39.5 ppm], ppm) δ = 170.9, 157.5, 148.0, 147.0, 123.6, 63.7, 60.8, 36.2, 32.5, 15.1, 13.2, 12.1.

MS (EI): *m/z* (%) = hab ich find ich relative complex Molpeak ist aber zu sehen

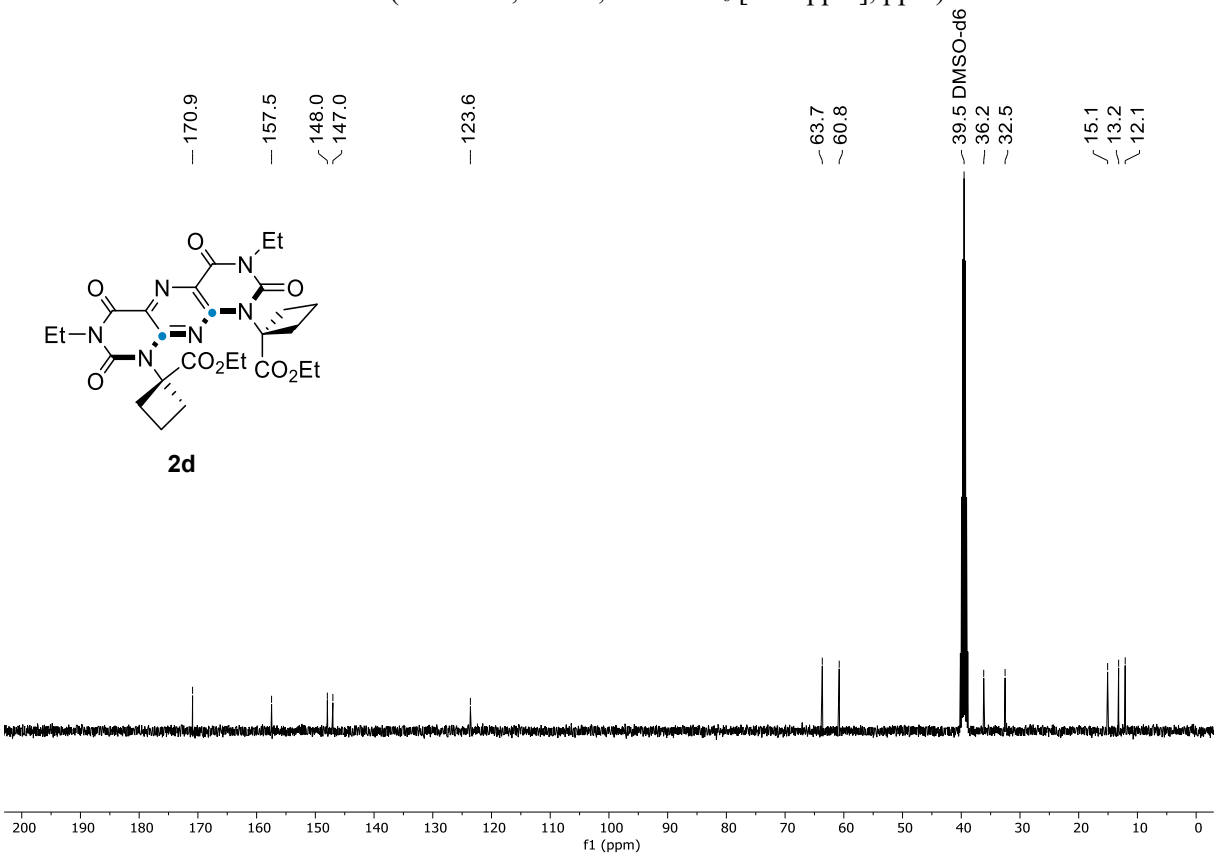
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{26}H_{32}N_6O_8Na]^+$: 579.2173; found: 579.2184 (Error PPM: 0.9).

IR (ATR, neat, $\tilde{\nu}$) = 3012 (vw), 2983 (w), 2965 (w), 2942 (vw), 1725 (vs), 1678 (vs), 1550 (vs), 1433 (s), 1413 (s), 1391 (vs), 1376 (vs), 1336 (vs), 1305 (s), 1270 (vs), 1248 (s), 1234 (s), 1205 (vs), 1150 (m), 1136 (m), 1115 (s), 1093 (s), 1070 (s), 1052 (m), 1013 (s), 975 (m), 942 (m), 901 (w), 871 (w), 854 (w), 814 (m), 779 (w), 759 (s), 748 (m), 726 (m), 716 (s), 708 (m), 683 (w), 638 (w), 579 (w), 557 (w), 500 (s), 483 (m), 463 (m), 451 (w), 408 (m) cm^{-1}.



2d

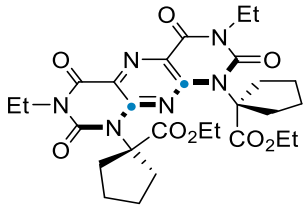
^{1}H NMR (400 MHz, 373 K, DMSO-d_6 [2.50 ppm], ppm)



2d

^{13}C NMR (101 MHz, 373 K DMSO-d_6 [39.5 ppm], ppm)

Synthesis of dimethyl 1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(cyclopentane-1-carboxylate) (2e)



2e

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **1e** (112 mg, 200 µmol, 1.00 equiv.) afforded the title compound **2e** (72.7 mg, 131 µmol, 65% yield) as a light orange solid after purification by column chromatography (*n*-pentane:ethyl acetate = 1:1).

R_f = 0.24 (*n*-pentane:ethyl acetate = 1:1, UV)

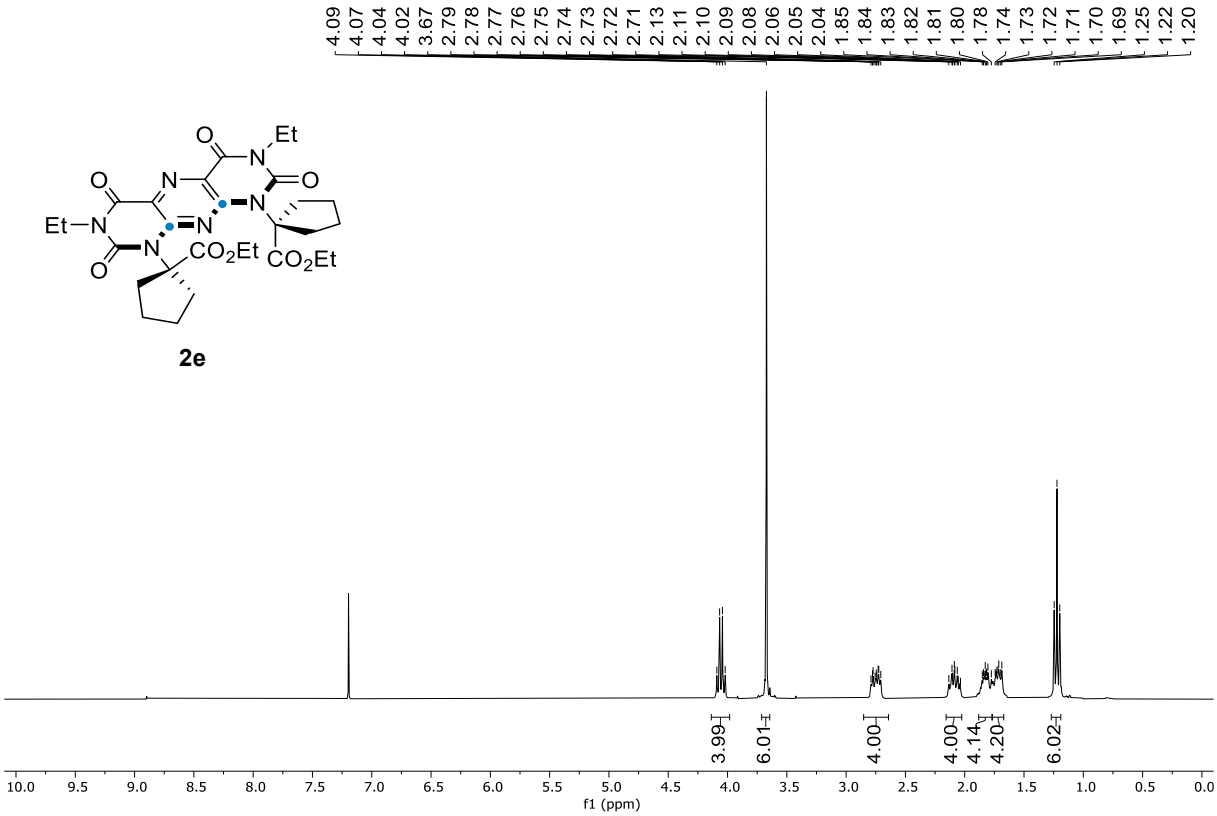
m.p. = degradation starting at 255 °C

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.05 (q, *J* = 7.0 Hz, 4H), 3.67 (s, 6H), 2.85 – 2.64 (m, 4H), 2.16 – 2.03 (m, 4H), 1.88 – 1.77 (m, 4H), 1.76 – 1.67 (m, 4H), 1.22 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 173.0, 157.7, 150.5, 148.8, 125.1, 74.1, 53.1, 39.0, 38.2, 24.8, 13.2.

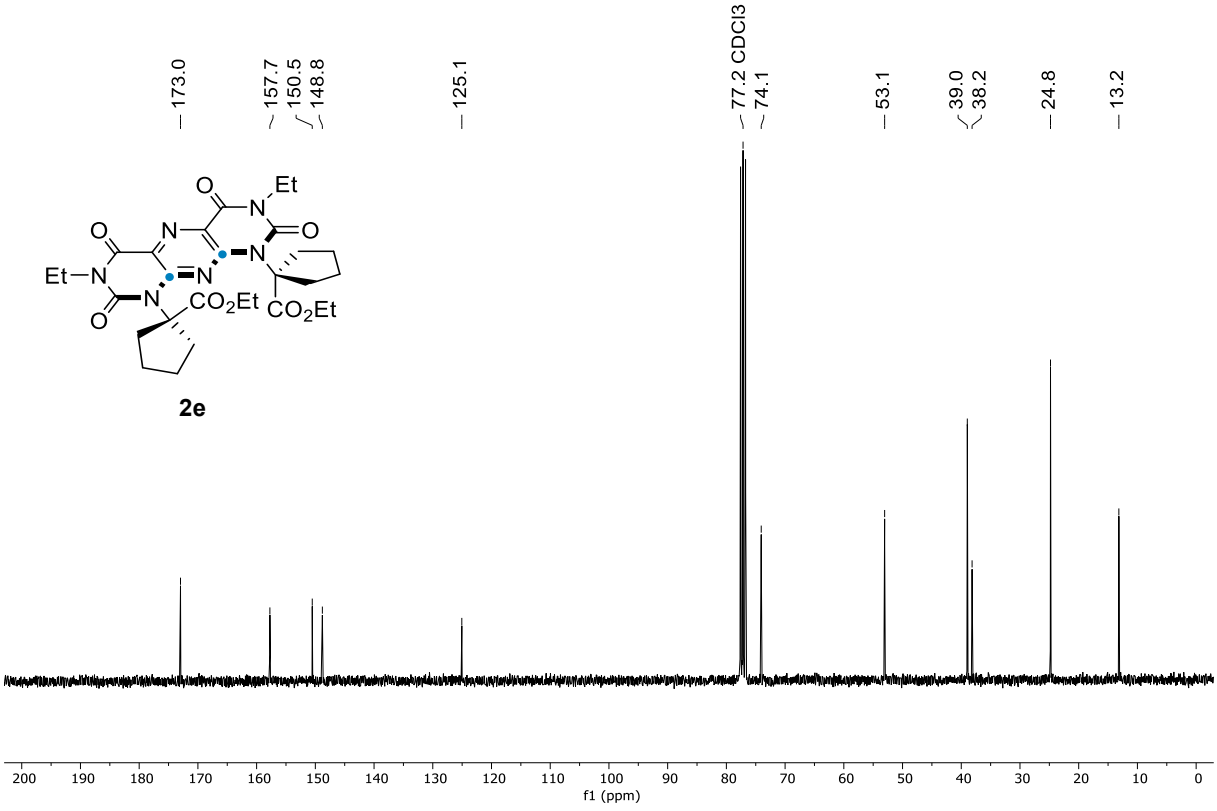
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{26}H_{32}N_6O_8Na]^+$: 579.2173; found: 579.2178 (Error PPM: 0.9).

IR (ATR, neat, $\tilde{\nu}$) = 2940 (w), 2875 (w), 1727 (vs), 1680 (vs), 1552 (vs), 1429 (s), 1389 (vs), 1327 (vs), 1264 (vs), 1225 (vs), 1193 (vs), 1170 (vs), 1089 (s), 1013 (m), 950 (w), 932 (m), 911 (m), 867 (w), 809 (m), 752 (s), 720 (m), 693 (m), 659 (w), 581 (w), 504 (m), 493 (m), 473 (s), 432 (m), 414 (w) cm^{-1}.



2e

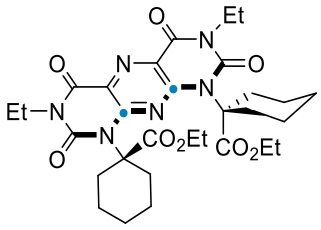
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



2e

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dimethyl 1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(cyclohexane-1-carboxylate) (2f)



2f

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **1f** (118 mg, 200 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:1).afforded the title compound **2f** (31.1 mg, 51.1 µmol, 27% yield) as a light orange solid.

R_f = 0.29 (*n*-pentane:ethyl acetate = 1:1, UV)

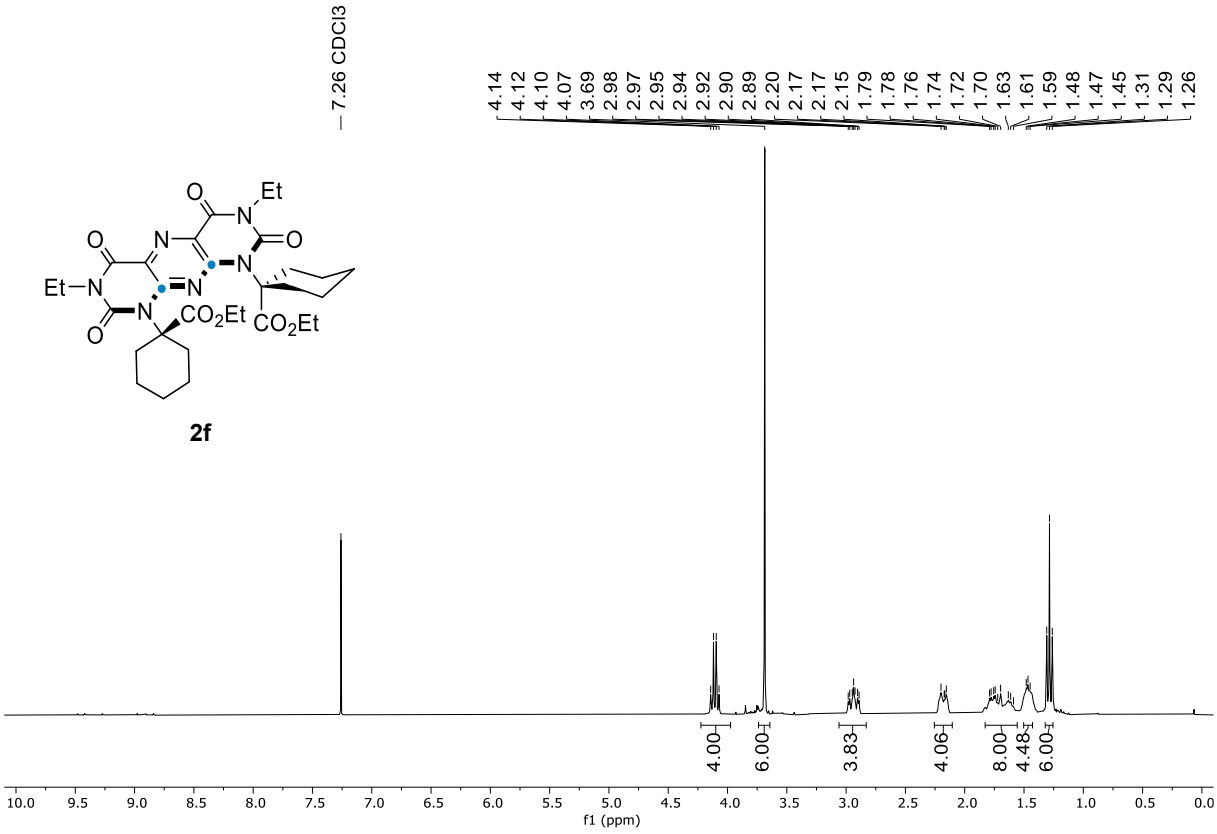
m.p. = broad starting at 110 °C

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.11 (q, *J* = 7.1 Hz, 4H), 3.69 (s, 6H), 2.94 (ddd, *J* = 13.8, 10.2, 3.8 Hz, 4H), 2.26 – 2.10 (m, 4H), 1.83 – 1.56 (m, 8H), 1.50 – 1.43 (m, 4H), 1.29 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 171.6, 157.7, 150.8, 148.2, 125.4, 70.7, 52.6, 38.2, 32.1, 24.5, 22.6, 13.

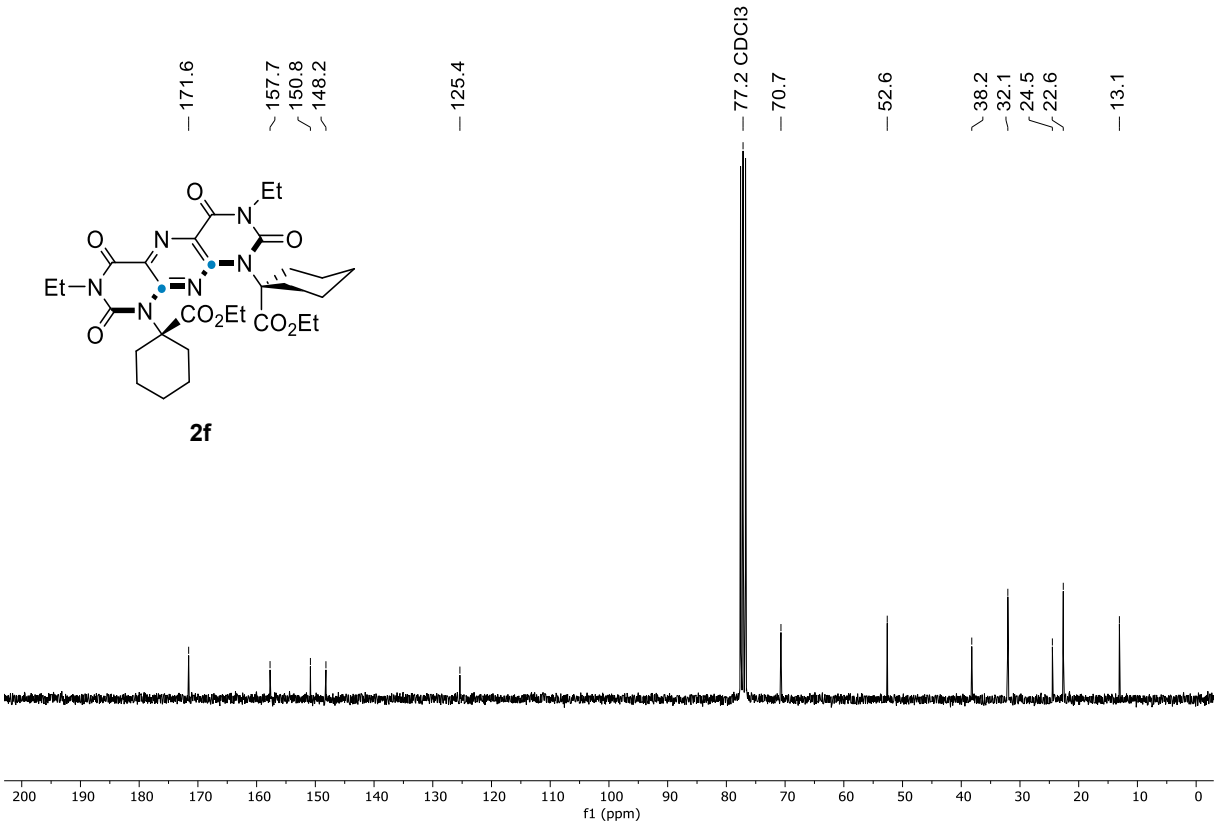
HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{28}H_{37}N_6O_8]^+$: 585.2668; found: 585.2673 (Error PPM: 0.9).

IR (ATR, neat, $\tilde{\nu}$) = 2936 (w), 2863 (w), 1725 (s), 1678 (vs), 1550 (vs), 1446 (m), 1431 (m), 1393 (vs), 1376 (vs), 1360 (s), 1327 (s), 1309 (s), 1256 (vs), 1223 (vs), 1174 (s), 1152 (m), 1134 (s), 1087 (m), 1066 (m), 1044 (m), 989 (w), 966 (w), 924 (w), 903 (w), 869 (w), 852 (w), 830 (w), 801 (w), 752 (m), 718 (m), 677 (w), 657 (w), 640 (w), 620 (w), 599 (w), 577 (w), 559 (w), 540 (w), 518 (w), 463 (m), 445 (w), 424 (w) cm^{-1}.



2f

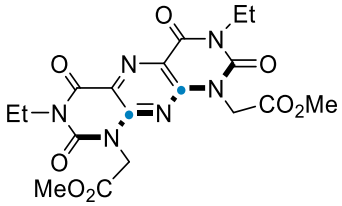
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



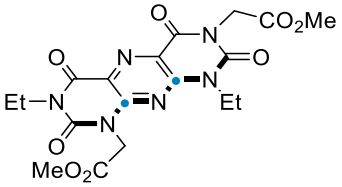
2f

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dimethyl 2,2'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)diacetate (2g) and dimethyl 2,2'-(3,9-diethyl-2,4,6,8-tetraoxo-3,4,8,9-tetrahydropyrimido[5,4-*g*]pteridine-1,7(2*H*,6*H*)-diyl)diacetate (2g')



2g (major isomere)



2g' (minor isomere)

Following the general procedure GP3, using the corresponding regioisomer mixture of pyrazine-2,6-dicarboxamides **1g** and **1g' (1g**:**1g**' = 6.9:1) (90.5 mg, 200 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:2) afforded the title compounds **2g** and **2g'** (40.1 mg, 89.4 µmol, 45% yield, **2g**:**2g**' = 6.6:1) as an pale orange solid.

R$_f$ = 0.22 (*n*-pentane:ethyl acetate = 1:2, UV)

m.p. =°236-239°C

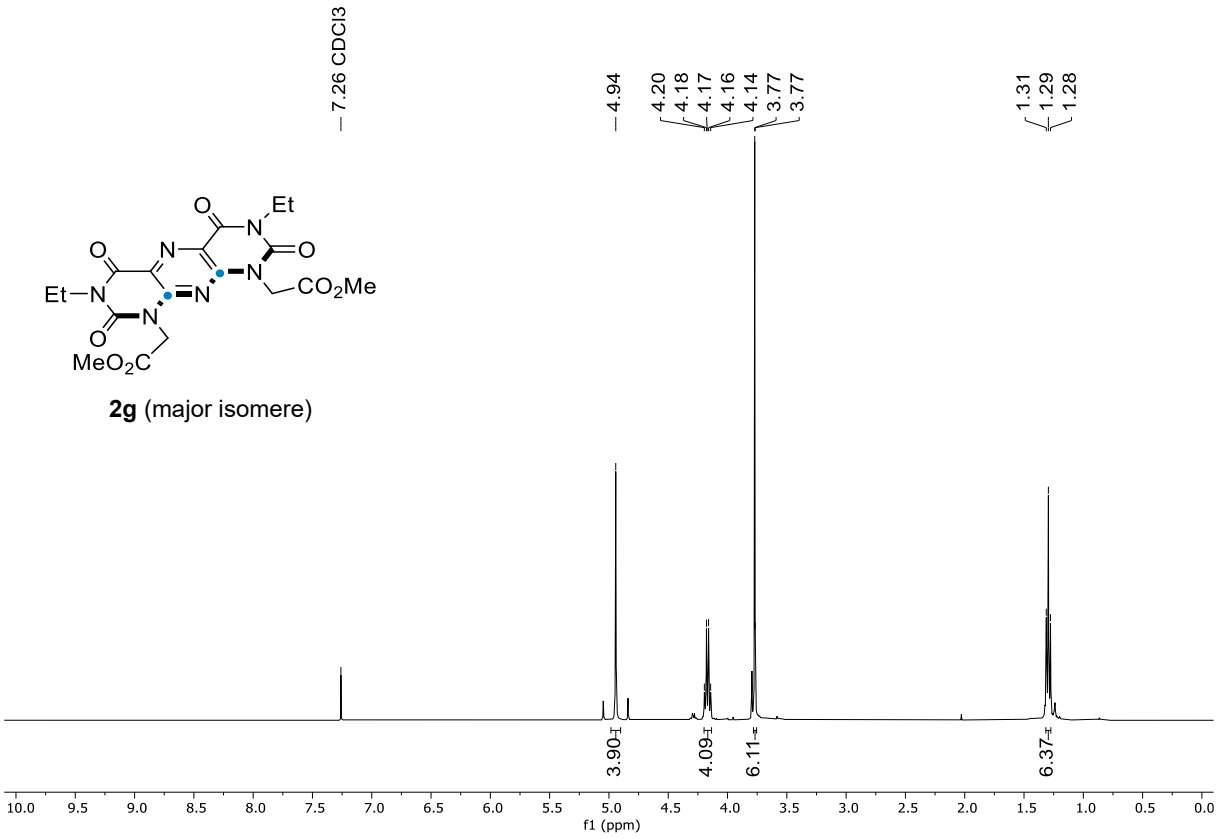
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.94 (s, 4H), 4.17 (q, *J* = 7.1 Hz, 4H), 3.77 (s, 6H), 1.29 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 167.5, 157.3, 149.5, 148.7, 124.4, 53.0, 43.8, 38.3, 13.0.

MS (GC-EI): *m*/*z* (%) = 448 (100, $[M]^+$), 318 (77), 191 (15), 137 (14).

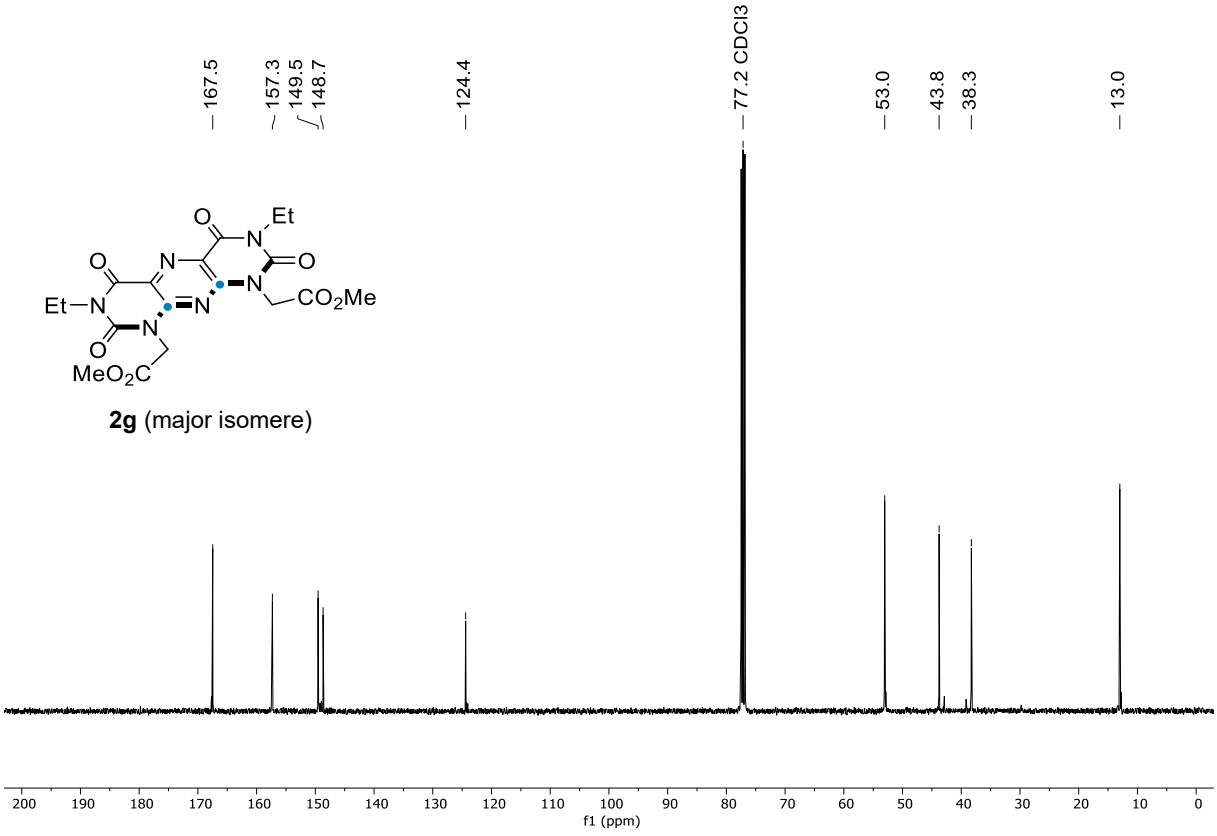
HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for: $[C_{18}H_{21}N_6O_8]^+$: 449.1415; found: 449.1423 (Error PPM: 1.8).

IR (ATR, neat, ṽ) = 3014 (vw), 2981 (w), 2959 (w), 1749 (m), 1719 (s), 1674 (vs), 1556 (vs), 1438 (vs), 1403 (vs), 1378 (vs), 1352 (s), 1301 (s), 1268 (vs), 1213 (vs), 1121 (m), 1089 (s), 1060 (m), 1013 (m), 985 (m), 954 (m), 909 (m), 879 (w), 848 (w), 814 (s), 754 (vs), 728 (s), 703 (s), 648 (m), 546 (m), 489 (vs), 406 (m) cm^{-1}.



2g (major isomere)

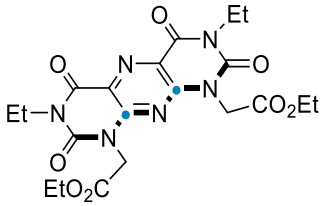
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



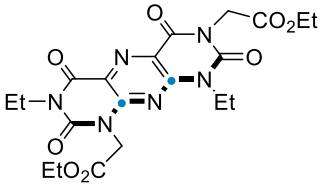
2g (major isomere)

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dimethyl 2,2'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)diacetate (2h) and diethyl 2,2'-(3,9-diethyl-2,4,6,8-tetraoxo-3,4,8,9-tetrahydropyrimido[5,4-*g*]pteridine-1,7(2*H*,6*H*)-diyl)diacetate (2h')



2h (major isomere)



2h' (minor isomere)

Following the general procedure GP3, using the corresponding regioisomer mixture of pyrazine-2,6-dicarboxamides **1h** and **1h' (1h**:**1h**' = 8.7:1) (96.1 mg, 200 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:1) afforded the title compounds **2h** and **2h'** (42.1 mg, 88.4 µmol, 44% yield, **2h**:**2h**' = 8.8:1) as a pale orange solid.

$\mathbf{R}_f$ = 0.19 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = 239-242°C

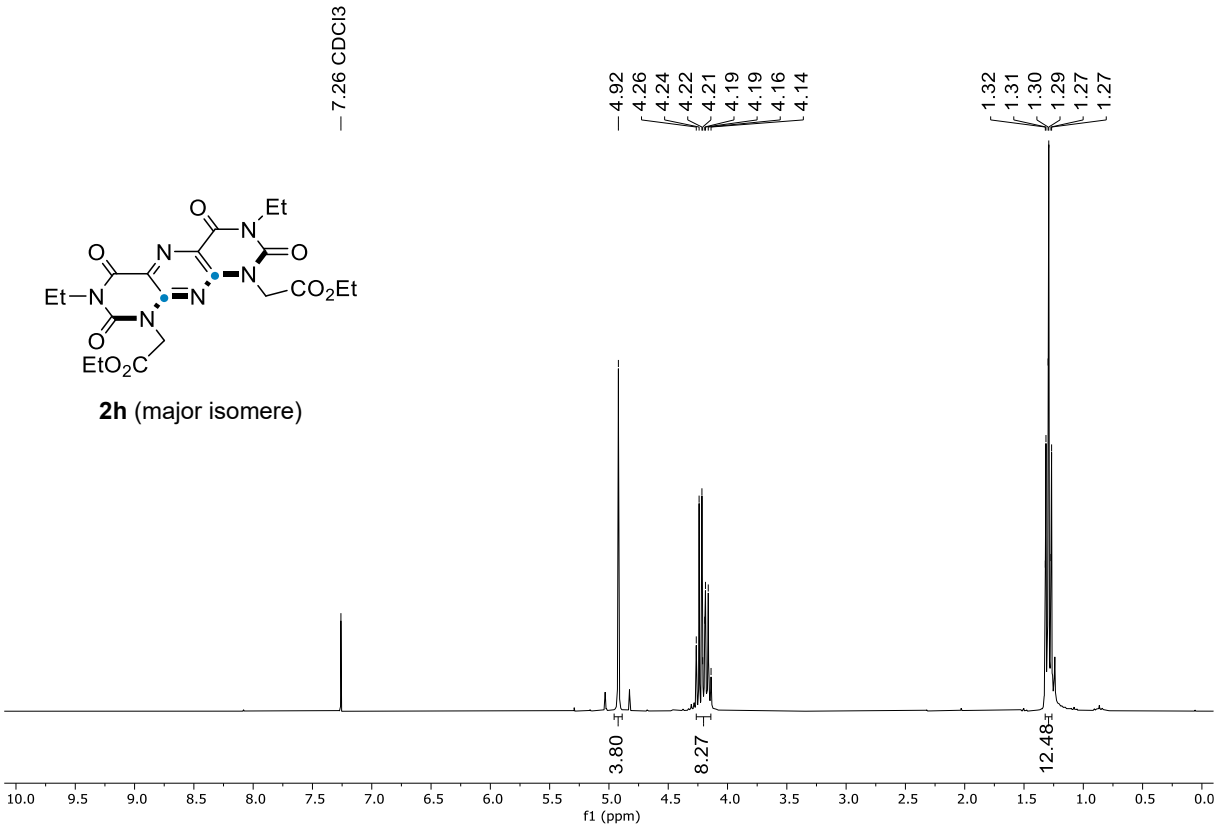
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.92 (s, 4H), 4.23 (q, *J* = 7.1 Hz, 4H), 4.17 (q, *J* = 7.1 Hz, 4H), 1.30 (t, *J* = 7.1 Hz, 6H), 1.29 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 167.0, 157.3, 149.6, 148.7, 124.4, 62.3, 43.9, 38.3, 14.2, 13.0.

MS (GC-EI): *m*/*z* (%) = 477 (24, $[M+H]^+$) 476 (100, $[M]^+$) 333 (14) 332 (79) 330 (19) 205 (10) 177 (10).

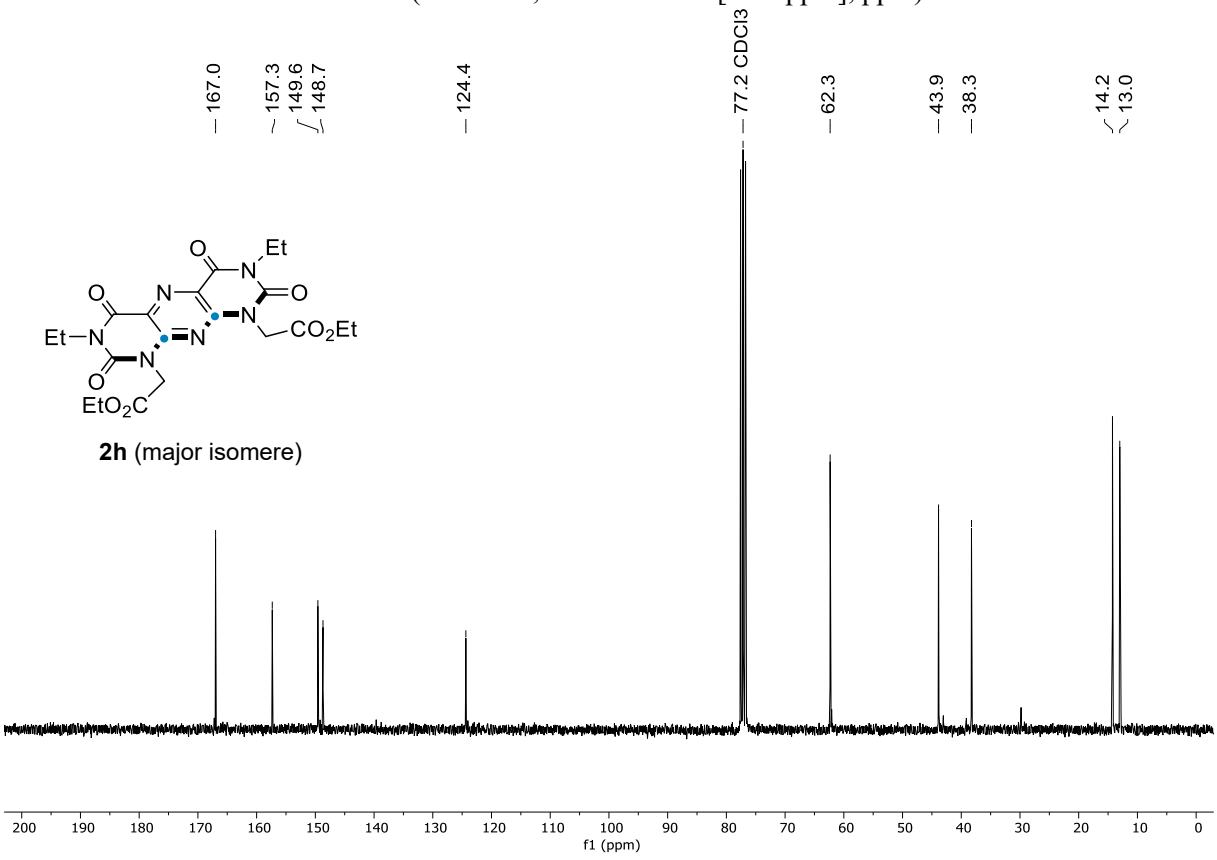
HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{20}H_{25}N_6O_8]^+$: 477.1728; found: 477.1727 (Error PPM: -0.2).

IR (ATR, neat, $\tilde{\nu}$) = 2987 (w), 2942 (vw), 2914 (vw), 1760 (s), 1715 (s), 1672 (vs), 1560 (vs), 1435 (vs), 1411 (vs), 1378 (s), 1340 (m), 1305 (s), 1272 (vs), 1238 (m), 1201 (vs), 1160 (s), 1123 (m), 1089 (s), 1062 (m), 1026 (s), 952 (m), 932 (w), 916 (w), 879 (w), 867 (w), 840 (w), 814 (s), 756 (vs), 708 (m), 648 (m), 551 (w), 502 (s), 489 (s), 469 (m), 459 (m) cm^{-1}.



2h (major isomere)

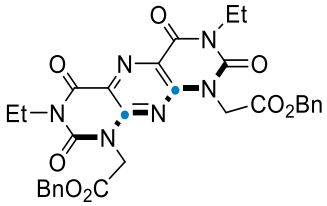
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



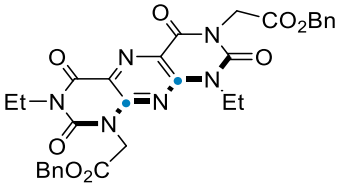
2h (major isomere)

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dibenzyl 2,2'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)diacetate (2i) and dibenzyl 2,2'-(3,9-diethyl-2,4,6,8-tetraoxo-3,4,8,9-tetrahydropyrimido[5,4-*g*]pteridine-1,7(2*H*,6*H*)-diyl)diacetate (2i')



2i (major isomere)



2i' (minor isomere)

Following the general procedure GP3, using the corresponding regioisomer mixture of pyrazine-2,6-dicarboxamides **1i** and **1i' (1i**:**1i'** = 8.1:1) (115 mg, 190 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:1) afforded the title compounds **2i** and **2i'** (54.2 mg, 90.2 µmol, 47% yield, **2i**:**2i**' = 7.8:1) as a pale orange solid.

R_f = 0.19 (methylene chloride:ethyl acetate = 9:1, UV)

m.p. = 176-180 °C

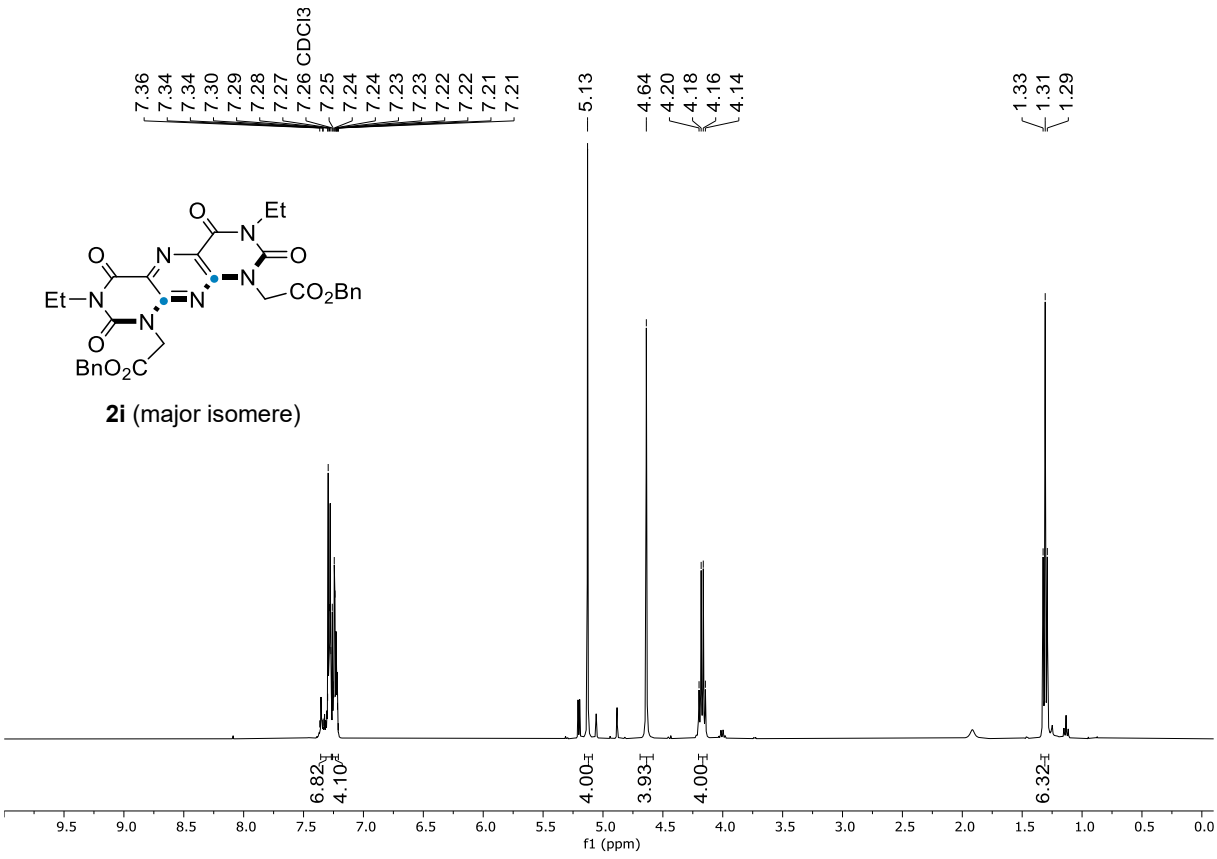
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.36 – 7.24 (m, 6H), 7.27 – 7.18 (m, 4H), 5.13 (s, 4H), 4.64 (s, 4H), 4.17 (q, *J* = 7.1 Hz, 4H), 1.31 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 166.8, 157.3, 149.4, 148.4, 135.0, 128.8, 128.8, 124.2, 67.7, 43.8, 38.3, 13.0.

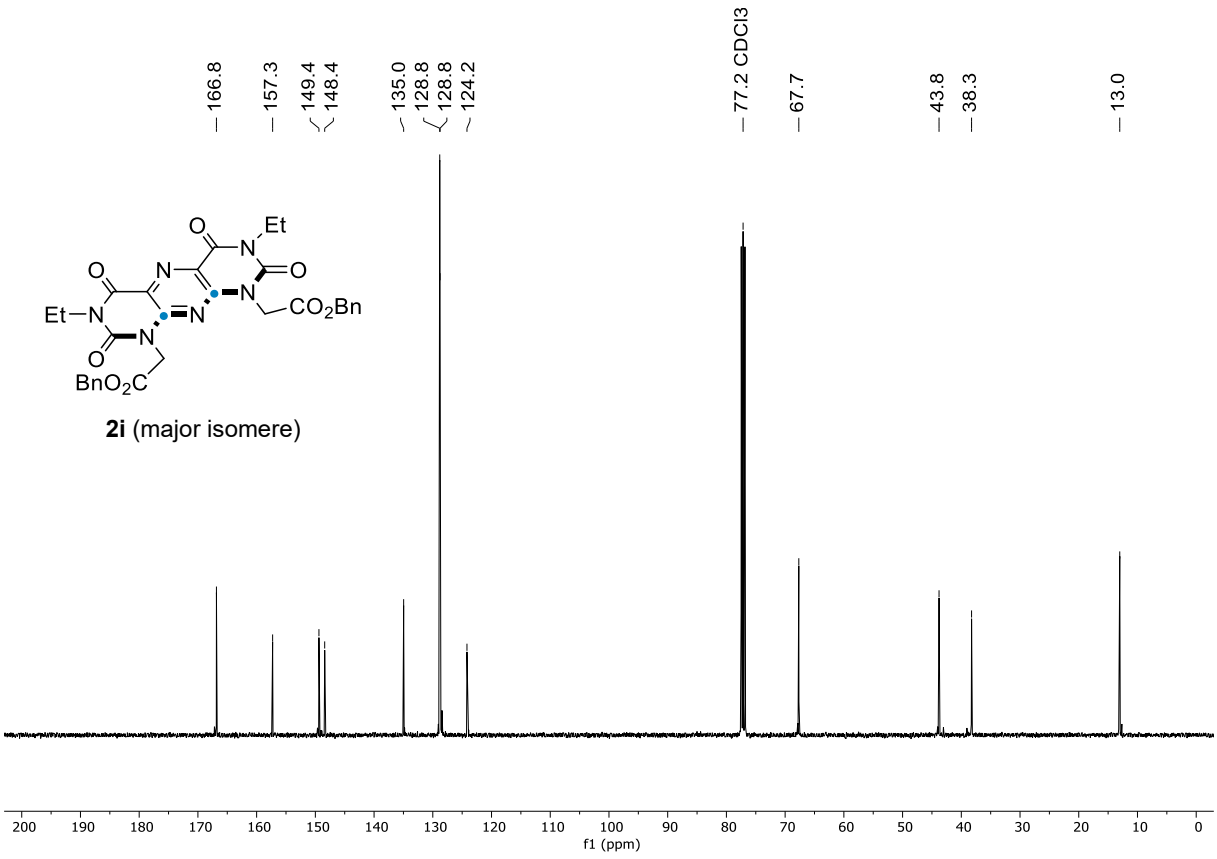
MS (GC-EI): *m*/*z* (%) = 600 (1, $[M]^+$) 403 (13) 281 (29) 253 (12) 207 (100) 191 (11) 133 (10) 91 (32) 73 (15)

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for: $[C_{30}H_{29}N_6O_8]^+$: 601.2042; found: 601.2057 (Error PPM: 2.5).

IR (ATR, neat, ṽ) = 2981 (w), 2965 (w), 2944 (vw), 1749 (m), 1719 (s), 1672 (vs), 1556 (vs), 1435 (s), 1403 (vs), 1378 (vs), 1352 (s), 1299 (s), 1266 (vs), 1189 (vs), 1174 (vs), 1121 (s), 1087 (s), 1058 (s), 1020 (m), 1001 (m), 950 (s), 907 (s), 879 (m), 814 (s), 748 (vs), 695 (vs), 650 (s), 606 (m), 573 (m), 534 (m), 487 (vs), 465 (vs), 434 (s), 406 (vs) cm^{-1}.

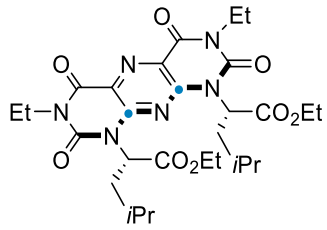


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)(2*S*,2'*S*)-bis(4-methylpentanoate) ((*S*)-2j)



(*S*)-**2j**

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide (*S*)-**1j** (119 mg, 200 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 5:2) afforded the title compound (*S*)-**2j** (86.6 mg, 147 µmol, 74% yield) as a pale orange solid after.

$\mathbf{R}_f$ = 0.18 (*n*-pentane:ethyl acetate = 5:2, Detection)

m.p. = 207-209 °C

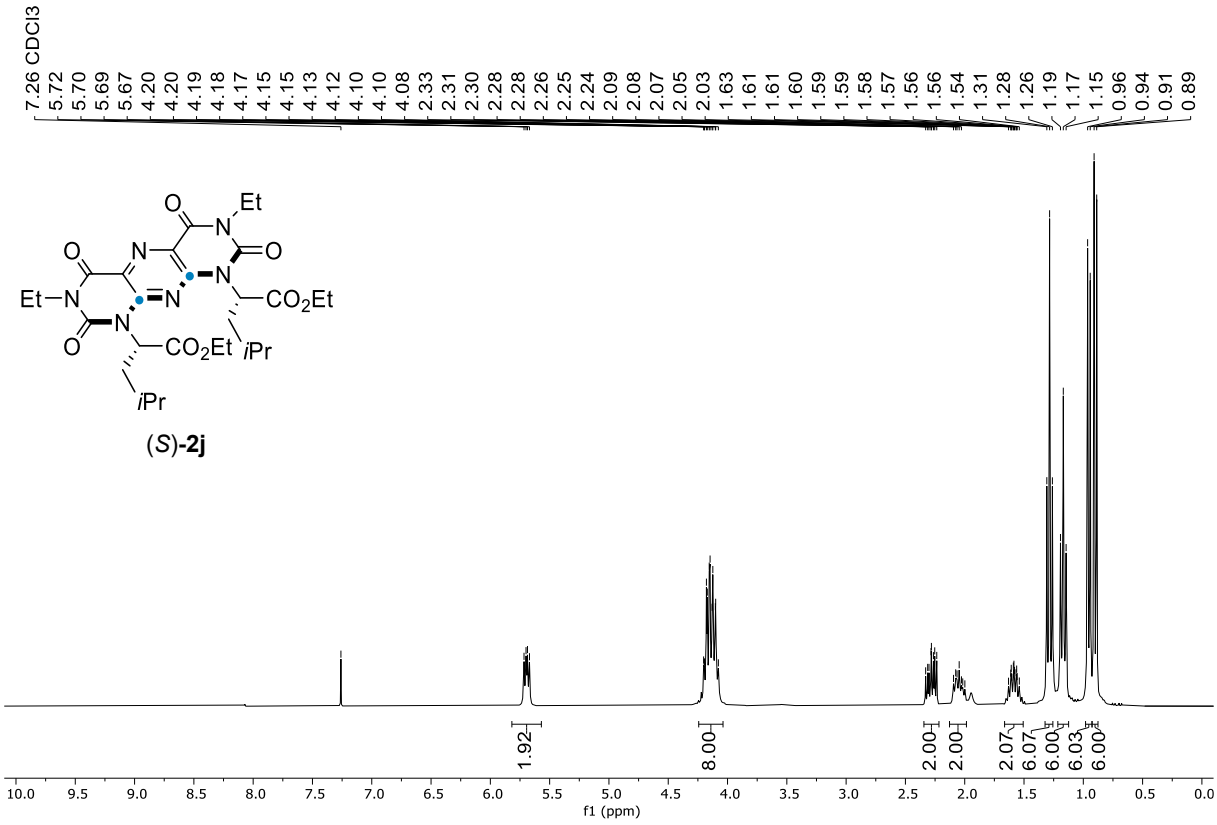
$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{22}}$ = -119.5 (c = 0.5 g/100 ml, DCM)

^{1}H NMR (300 MHz, 323 K, Chloroform-d [7.26 ppm], ppm) δ = 5.69 (dd, *J* = 8.8, 5.1 Hz, 2H), 4.24 – 4.04 (m, 8H), 2.28 (ddd, *J* = 14.5, 8.4, 5.1 Hz, 2H), 2.05 (ddd, *J* = 14.4, 8.7, 5.4 Hz, 2H), 1.66 – 1.51 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 6H), 1.17 (t, *J* = 7.1 Hz, 6H), 0.95 (d, *J* = 6.6 Hz, 6H), 0.90 (d, *J* = 6.6 Hz, 6H).

^{13}C NMR (75 MHz, 323 K, Chloroform-d [77.16 ppm], ppm) δ = 169.2, 157.3, 149.5, 148.7, 124.4, 62.0, 55.3, 38.2, 38.1, 25.9, 23.0, 22.4, 14.1, 13.0.

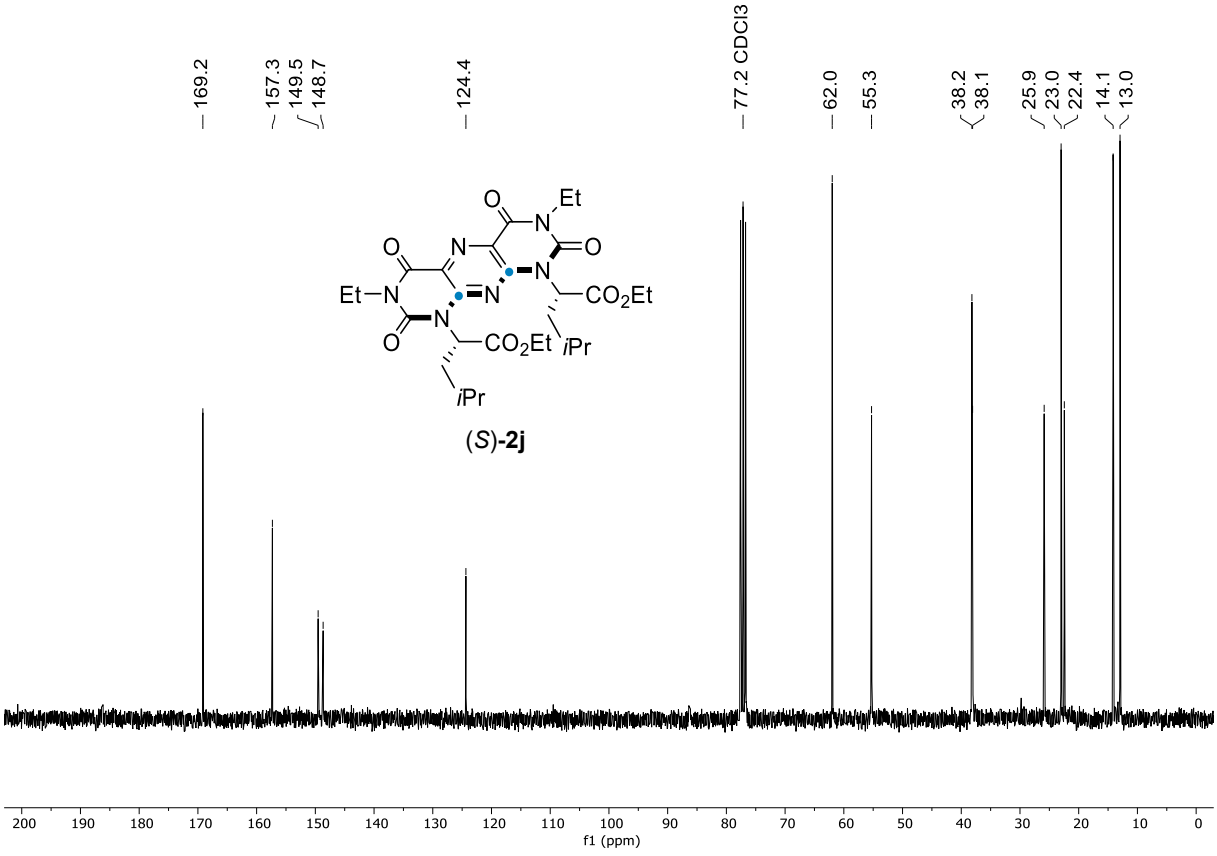
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{28}H_{40}N_6O_8Na]^+$: 611.2800 ; found: 611.2804 (Error PPM: 0.7).

IR (ATR, $\tilde{\nu}$) = 2957 (w), 2940 (w), 1749 (w), 1735 (m), 1723 (s), 1676 (vs), 1558 (vs), 1533 (w), 1452 (w), 1433 (s), 1407 (vs), 1380 (m), 1368 (m), 1346 (m), 1319 (vs), 1293 (w), 1270 (vs), 1248 (m), 1230 (s), 1205 (vs), 1142 (w), 1126 (m), 1087 (m), 1068 (m), 1028 (s), 952 (w), 918 (w), 871 (w), 852 (w), 814 (m), 773 (w), 750 (m), 710 (w), 691 (w), 675 (w), 661 (w), 606 (w), 565 (w), 516 (w), 495 (s), 475 (w), 402 (m) cm^{-1}.



(*S*)-**2j**

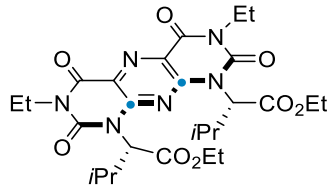
^{1}H NMR (300 MHz, 323 K, Chloroform-d [7.26 ppm], ppm)



(*S*)-**2j**

^{13}C NMR (75 MHz, 323 K,Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)(2*S*,2'*S*)-bis(3-methylbutanoate) ((*S*)-2k)



(*S*)-**2k**

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide (*S*)-**1k** (141 mg, 250 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 2:1) afforded the title compound (*S*)-**2k** (105 mg, 187 µmol, 75% yield) as a pale orange solid.

$\mathbf{R}_f$ = 0.24 (*n*-pentane:ethyl acetate = 2:1, Detection)

m.p. = 218-221 °C

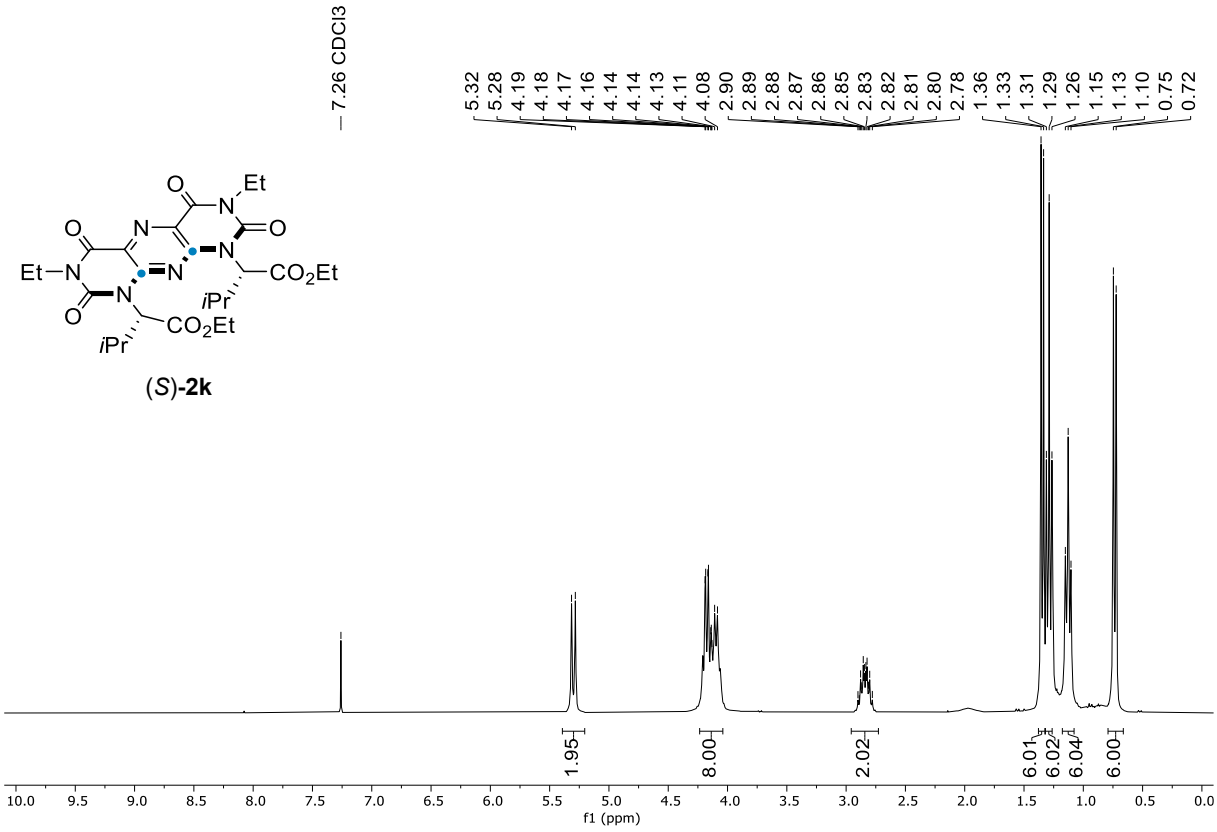
$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{22}}$ = -49.4 (c = 0.47 g/100 ml, DCM)

^{1}H NMR (300 MHz, 323 K, Chloroform-d [7.26 ppm], ppm) δ = 5.30 (d, *J* = 9.6 Hz, 2H), 4.23 – 4.04 (m, 8H), 2.96 – 2.73 (m, 2H), 1.35 (d, *J* = 6.5 Hz, 6H), 1.29 (t, *J* = 7.1 Hz, 6H), 1.13 (t, *J* = 7.1 Hz, 6H), 0.73 (d, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, 323 K, Chloroform-d [77.16 ppm], ppm) δ = 168.6, 157.3, 149.7, 148.7, 124.1, 61.7, 61.6, 38.1, 27.9, 21.8, 19.0, 14.1, 13.0.

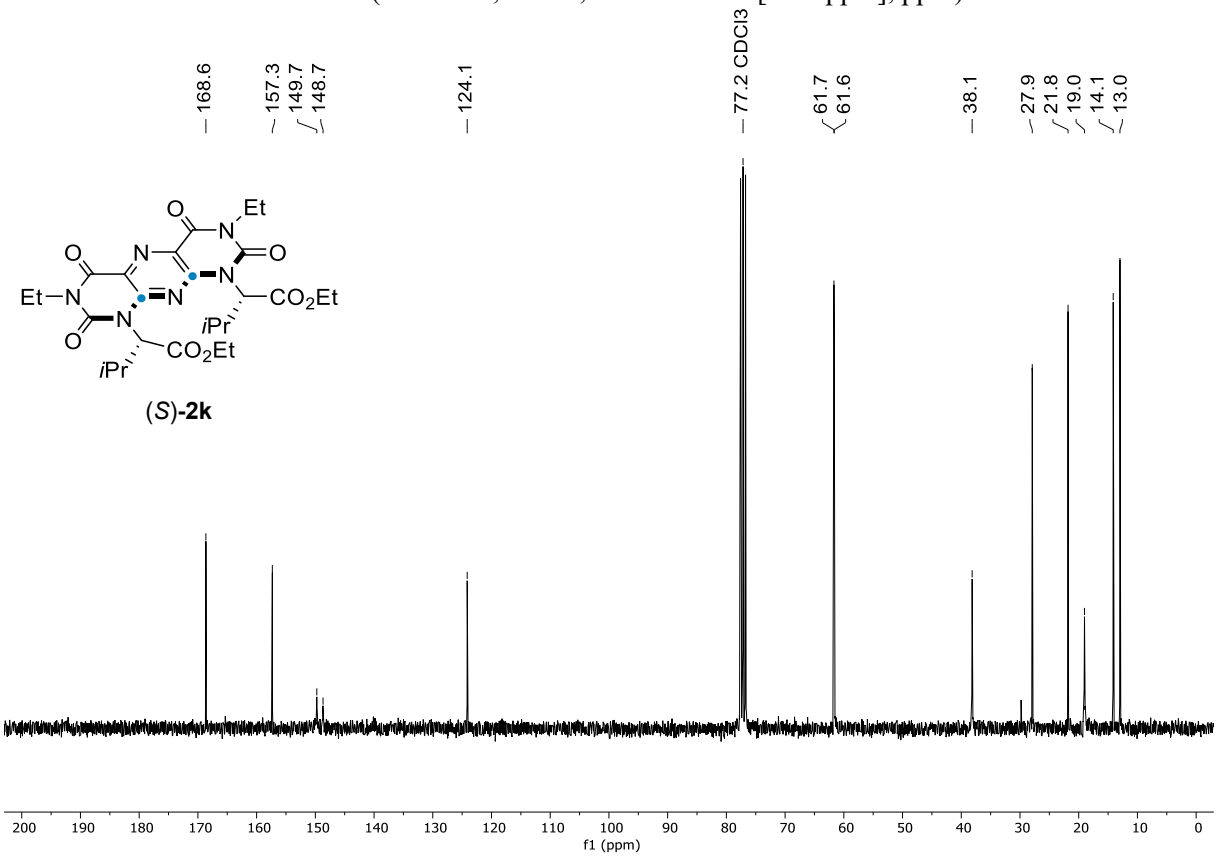
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{26}H_{36}N_6O_8Na]^+$: 583.2487; found: 583.2490 (Error PPM: 0.5).

IR (ATR, neat, $\tilde{\nu}$) = 2983 (w), 2936 (w), 2873 (vw), 1737 (s), 1721 (s), 1678 (vs), 1558 (vs), 1531 (m), 1446 (m), 1431 (s), 1407 (vs), 1389 (s), 1370 (m), 1348 (m), 1323 (vs), 1309 (s), 1266 (vs), 1234 (s), 1207 (vs), 1126 (m), 1089 (s), 1024 (vs), 969 (w), 932 (m), 901 (w), 871 (w), 848 (w), 814 (m), 801 (w), 779 (w), 754 (s), 722 (w), 703 (s), 683 (w), 624 (w), 599 (w), 553 (m), 536 (w), 516 (w), 498 (s), 477 (m), 445 (w), 410 (m) cm^{-1}.



(*S*)-**2k**

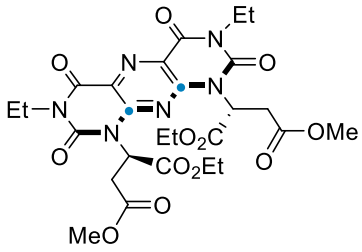
^{1}H NMR (300 MHz, 323 K,Chloroform-d [7.26 ppm], ppm)



(*S*)-**2k**

^{13}C NMR (75 MHz, 323 K, Chloroform-d [77.16 ppm], ppm)

Synthesis of tetramethyl 2,2'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)(2*R*,2'*R*)-disuccinate ((*R*)-2l)



(*R*)-**2l**

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide (*R*)-**1l** (133 mg, 200 µmol, 1.00 equiv.) purification by column chromatography (CH_2Cl_2 to CH_2Cl_2:MeOH = 98.5:1.5) and a subsequent wash with MeOH afforded the title compound (*R*)-**2l** (79.2 mg, 134 µmol, 67% yield) as a colorless solid.

R_f = 0.29 (CH_2Cl_2+ 1.5% MeOH, UV)

m.p. = 257-260 °C

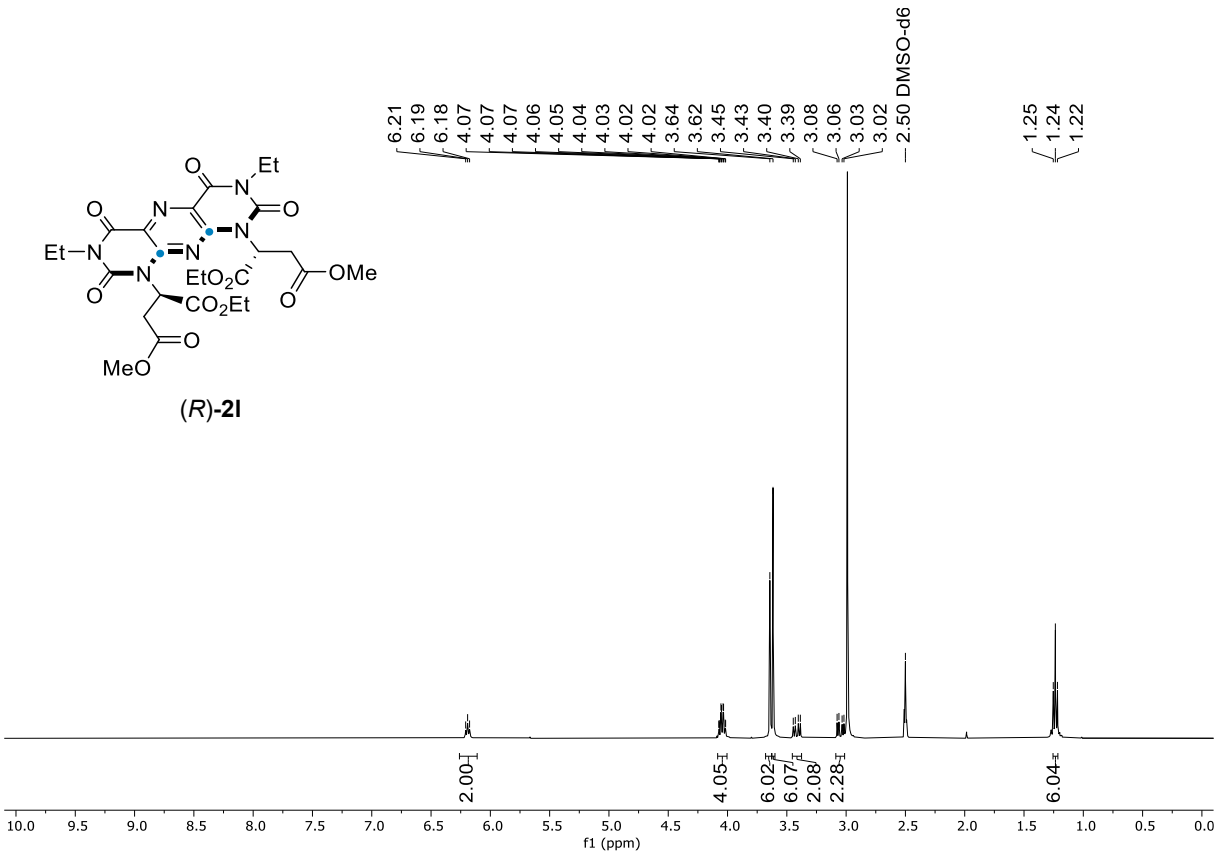
$[\alpha]_D^{23}$ = 82.8 (c = 0.5 g/100 ml, DCM)

^{1}H NMR (400 MHz, 373 K, DMSO-d_6 [2.50 ppm], ppm) δ = 6.19 (t, *J* = 6.6 Hz, 2H), 4.10 – 3.99 (m, 4H), 3.64 (s, 6H), 3.62 (s, 6H), 3.25 (dd, *J* = 147.5, 6.5 Hz, 2H), 3.21 (dd, *J* = 147.5, 6.6 Hz, 2H), 1.24 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, 373 K, DMSO-d_6 [39.5 ppm], ppm) δ = 69.6, 167.9, 156.9, 148.7, 147.2, 123.7, 52.1, 51.3, 51.1, 36.7, 33.0, 11.9.

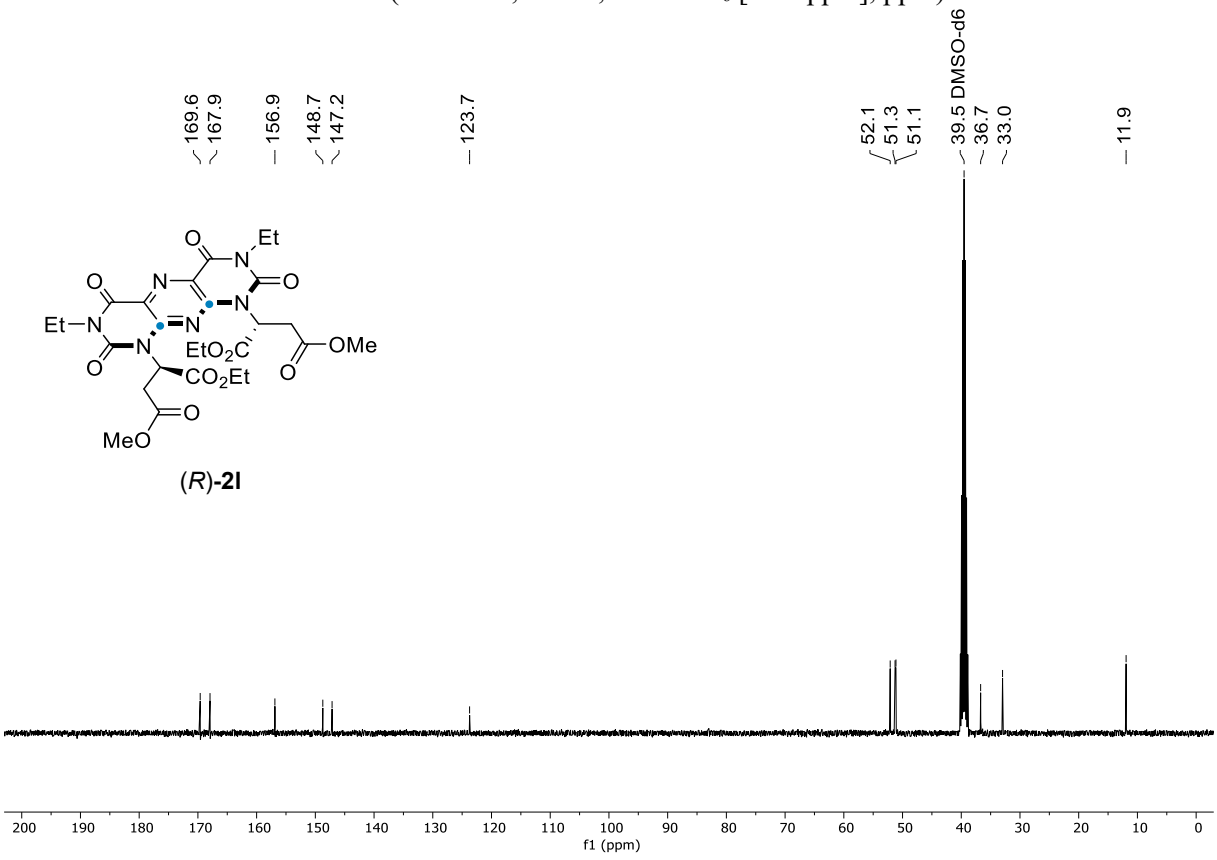
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for: $[C_{24}H_{28}N_6O_{12}Na]^+$: 615.1657; found: 615.1665 (Error PPM: 1.3).

IR (ATR, neat, $\tilde{\nu}$) = 2985 (vw), 2965 (w), 2957 (w), 2920 (vw), 2846 (vw), 1743 (vs), 1713 (s), 1668 (vs), 1564 (vs), 1537 (w), 1513 (w), 1435 (vs), 1411 (vs), 1380 (w), 1348 (m), 1329 (s), 1295 (m), 1272 (vs), 1252 (vs), 1234 (vs), 1197 (m), 1170 (m), 1154 (m), 1130 (w), 1105 (w), 1085 (s), 1050 (m), 1020 (s), 991 (w), 973 (w), 926 (w), 858 (m), 842 (w), 814 (m), 787 (w), 754 (s), 712 (w), 699 (w), 671 (m), 646 (w), 628 (w), 581 (w), 546 (w), 498 (s), 479 (m), 465 (w), 406 (m) cm^{-1}.



(*R*)-**2l**

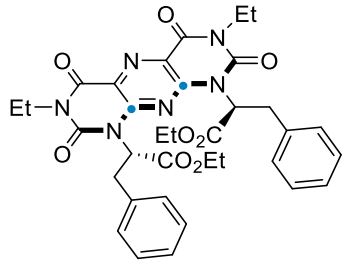
^{1}H NMR (400 MHz, 373 K, DMSO-d_6 [2.50 ppm], ppm)



(*R*)-**2l**

^{13}C NMR (101 MHz, 373 K, DMSO-d_6 [39.5 ppm], ppm)

Synthesis of diethyl 2,2'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)(2*S*,2'*S*)-bis(3-phenylpropanoate) ((*S*)-2n) JM25



(*S*)-**2n**

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide (*S*)-**1n** (165 mg, 250 µmol, 1.00 equiv) afforded the title compound (*S*)-**2n** (108 mg, 165 µmol, 66% yield) as a light orange solid after purification by column chromatography (*n*-pentane:ethyl acetate = 2:1).

R_f = 0.20 (*n*-pentane:ethyl acetate = 2:1, UV)

m.p. = 163-167 °C*

$[\alpha]_D^{22}$ = -118.0 (c = 0.5 g/100 ml, DCM)

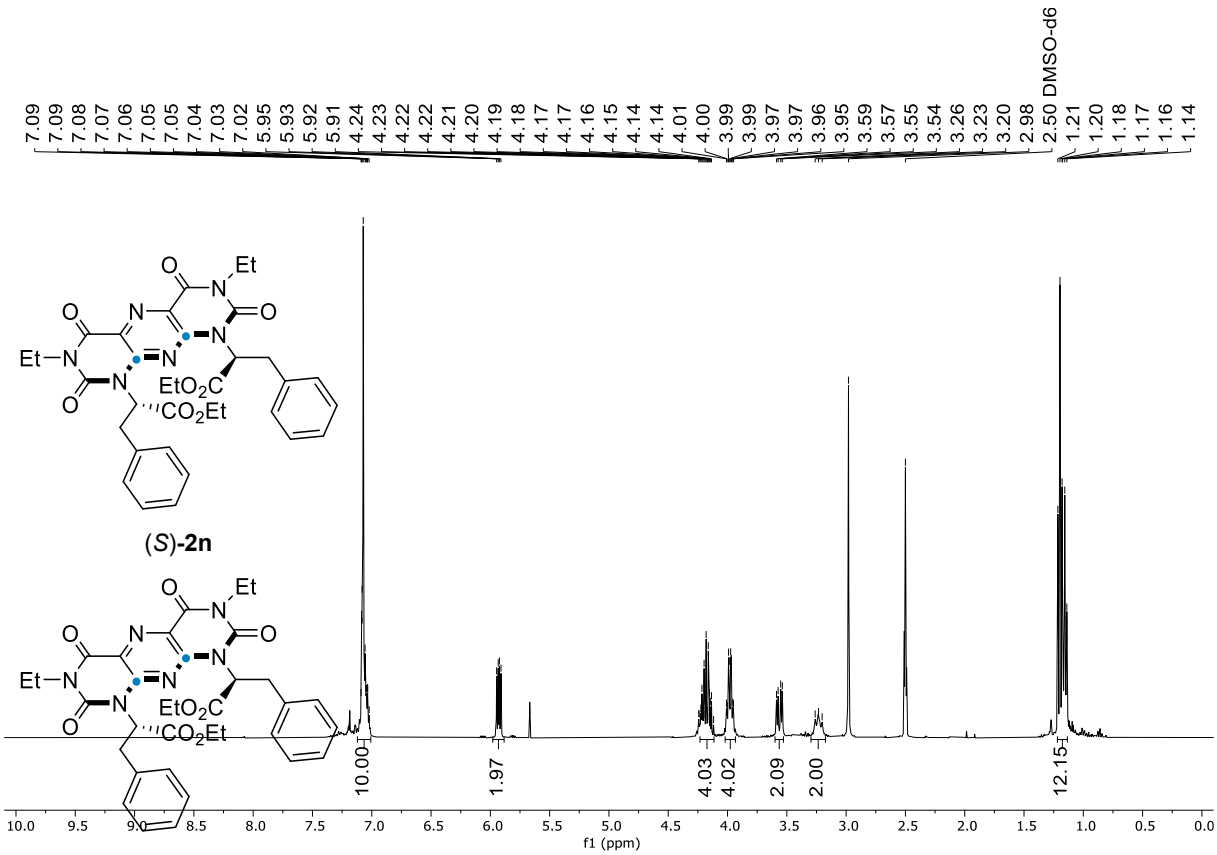
^{1}H NMR (400 MHz, 373 K, DMSO-d_6 [2.50 ppm], ppm) δ = 7.12 – 7.01 (m, 10H), 5.93 (dd, *J* = 9.3, 5.3 Hz, 2H), 4.23 – 4.11 (m, 4H), 4.02 – 3.93 (m, 4H), 3.56 (dd, *J* = 14.2, 5.4 Hz, 2H), 3.30 – 3.17 (m, 2H), 1.20 (t, *J* = 7.1 Hz, 7H), 1.16 (t, *J* = 7.2 Hz, 6H).

^{13}C NMR (101 MHz, 374 K, DMSO-d_6 [39.5 ppm], ppm) δ = 167.7, 156.4, 148.4, 147.2, 136.4, 128.6, 127.6, 126.0, 122.9, 60.8, 56.2, 33.7, 13.3, 11.9.

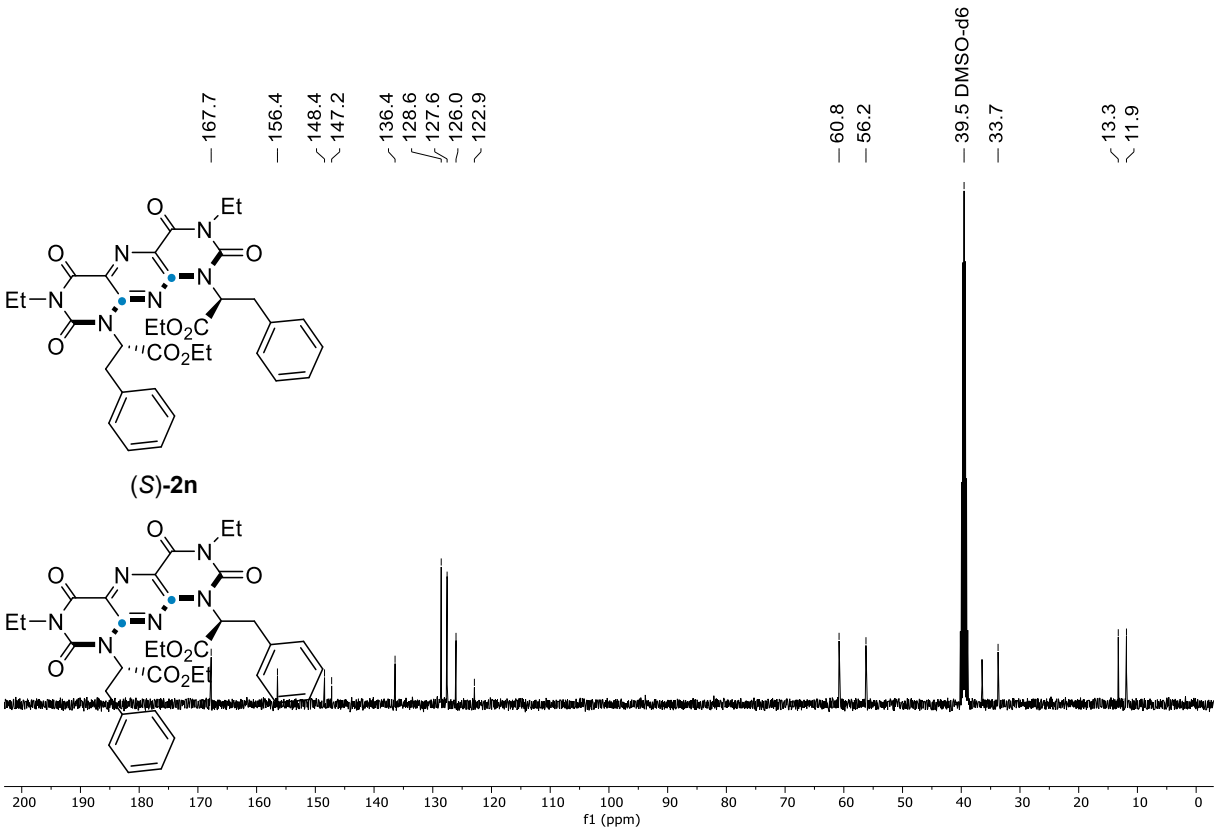
MS (EI): *m/z* (%) = 656 (100, $[M]^+$), 480 (95), 304 (88), 233 (55), 177 (97), 148 (79), 103 (77), 91 (45), 77 (31)

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{34}H_{36}N_6O_8Na]^+$: 679.2487; found: 679.2489 (Error PPM: 0.3).

IR (ATR, neat, ṽ) = 2979 (w), 2938 (w), 1735 (s), 1723 (s), 1678 (vs), 1605 (vw), 1556 (vs), 1497 (w), 1435 (s), 1405 (vs), 1382 (m), 1368 (m), 1346 (m), 1319 (vs), 1264 (vs), 1225 (vs), 1199 (s), 1154 (m), 1117 (w), 1089 (m), 1060 (m), 1026 (s), 948 (m), 860 (w), 812 (m), 791 (w), 750 (vs), 701 (vs), 675 (w), 632 (w), 606 (w), 553 (m), 522 (w), 489 (s), 473 (m), 432 (w), 402 (m) cm^{-1}.

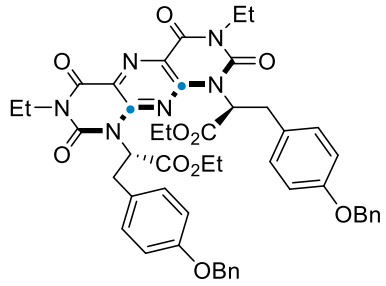


^{1}H NMR (400 MHz, 373.1 K, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, 373.1 K, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)(2*S*,2'*S*)-bis(3-(4-(benzyloxy)phenyl)propanoate) ((*S*)-2m)



(*S*)-**2m**

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide (*S*)-**1m** (218 mg, 250 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 2:1 to 3:2) afforded the title compound (*S*)-**2m** (119 mg, 137 µmol, 55% yield) as a light orange solid.

$\mathbf{R}_f$ = 0.37 (*n*-pentane:ethyl acetate = 3:2, Detection)

m.p. = broad starting at 80 °C

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23}}$ = -141.4 (c = 0.5 g/100 ml, DCM)

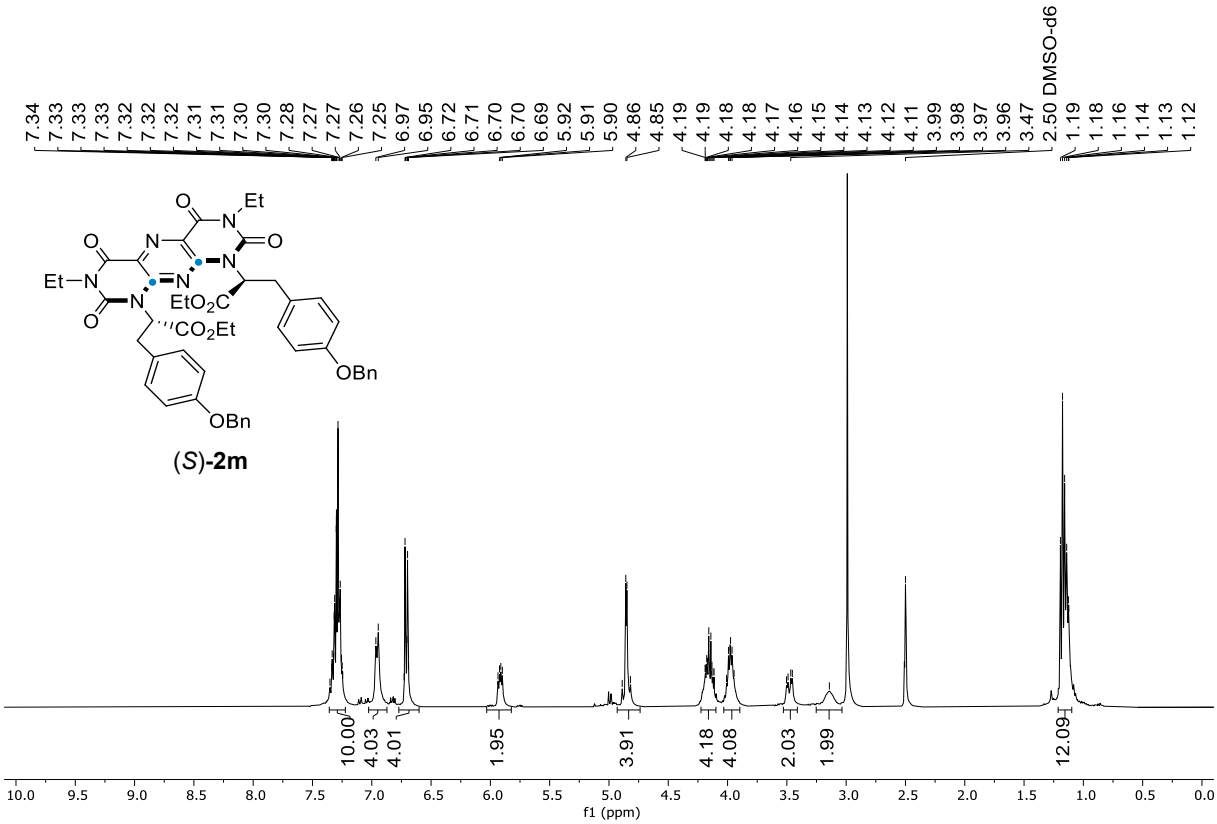
^{1}H NMR (400 MHz, 373.1 K, Chloroform-d [7.26 ppm], ppm) δ = 7.36 – 7.22 (m, 10H), 7.03 – 6.87 (m, 4H), 6.77 – 6.60 (m, 4H), 6.03 – 5.82 (m, 2H), 4.93 – 4.74 (m, 4H), 4.22 – 4.10 (m, 4H), 4.03 – 3.90 (m, 4H), 3.53 – 3.41 (m, 2H), 3.25 – 3.04 (m, 2H), 1.21 – 1.10 (m, 12H).

^{13}C NMR (101 MHz, 373.1 K, Chloroform-d [77.16 ppm], ppm) δ = 167.8, 156.9, 156.5, 148.4, 147.2, 136.6, 129.7, 128.6, 127.7, 127.7, 127.1, 126.7, 123.0, 114.3, 69.1, 60.8, 56.4, 36.5, 32.8, 13.3, 11.9.

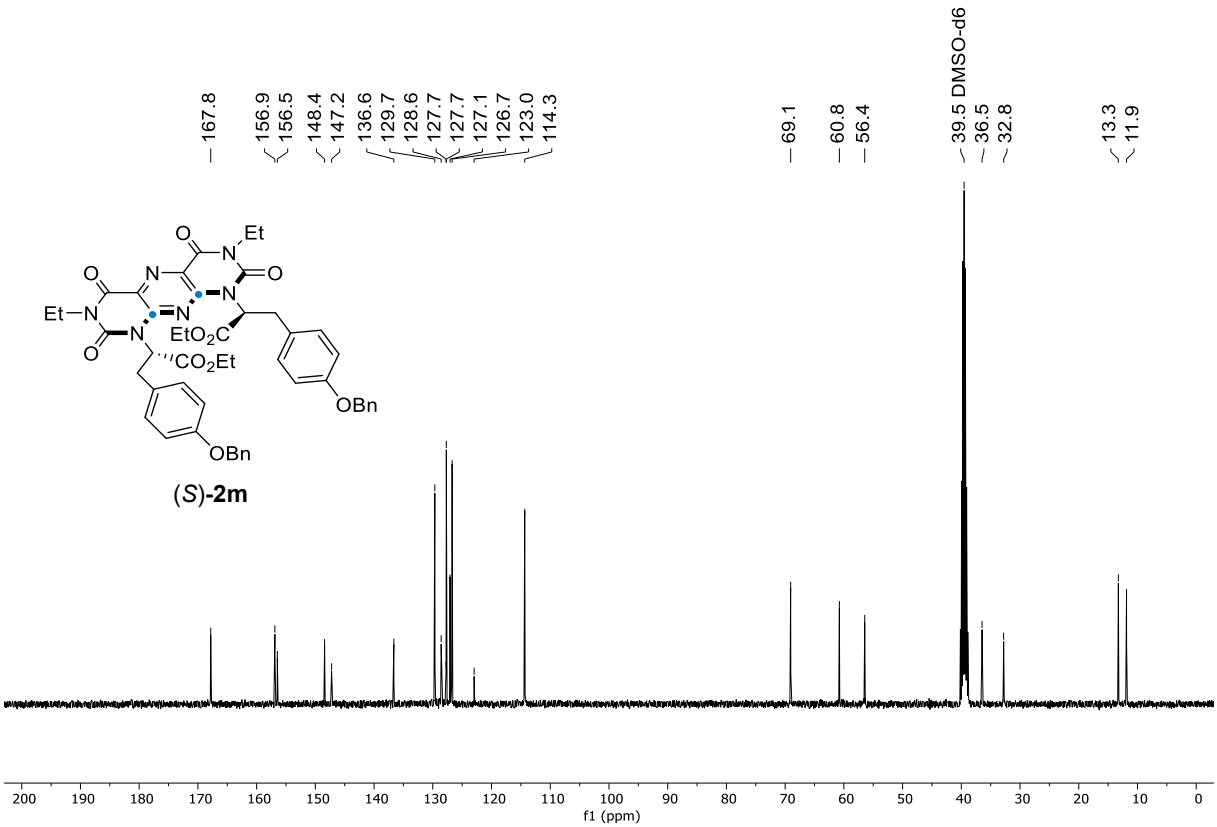
MS (EI): *m/z* (%) = 868 (47), 677 (19). 586 (49), 497 (44), 395 (47), 305 (71), 192 (56), 147 (62), 91 (100).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{48}H_{49}N_6O_{10}]^+$: 869.3505; found: 869.3505 (Error PPM: 0.0).

IR (ATR, neat, $\tilde{\nu}$) = 2979 (w), 2936 (w), 2873 (vw), 1735 (m), 1723 (s), 1678 (vs), 1611 (w), 1556 (vs), 1509 (s), 1452 (m), 1435 (s), 1403 (vs), 1380 (m), 1368 (m), 1344 (m), 1319 (vs), 1264 (vs), 1223 (vs), 1174 (vs), 1158 (s), 1111 (m), 1091 (m), 1064 (m), 1024 (vs), 948 (m), 920 (w), 863 (m), 844 (m), 812 (s), 736 (vs), 695 (s), 675 (w), 640 (w), 610 (m), 555 (m), 493 (s), 459 (m), 432 (m) cm^{-1}.

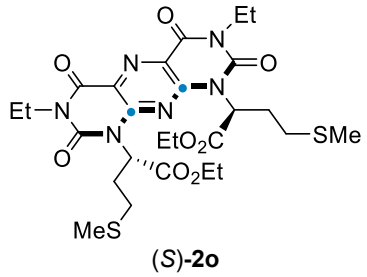


^{1}H NMR (400 MHz, 373.1 K, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, 373.1 K, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)(2*S*,2'*S*)-bis(4-(methylthio)butanoate) ((*S*)-2o)



(*S*)-**2o**

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide (*S*)-**1o** (126 mg, 200 µmol, 1.00 equiv.) after purification by column chromatography (*n*-pentane:ethyl acetate = 2:1 to 1:1) afforded the title compound (*S*)-**2o** (41.2 mg, 65.9 µmol, 33% yield) as a pale yellow solid together with minor amounts of an unknown impurity. This reaction was repeated two more times with similar results.

R_f = 0.3 (*n*-pentane:ethyl acetate = 1:1, UV)

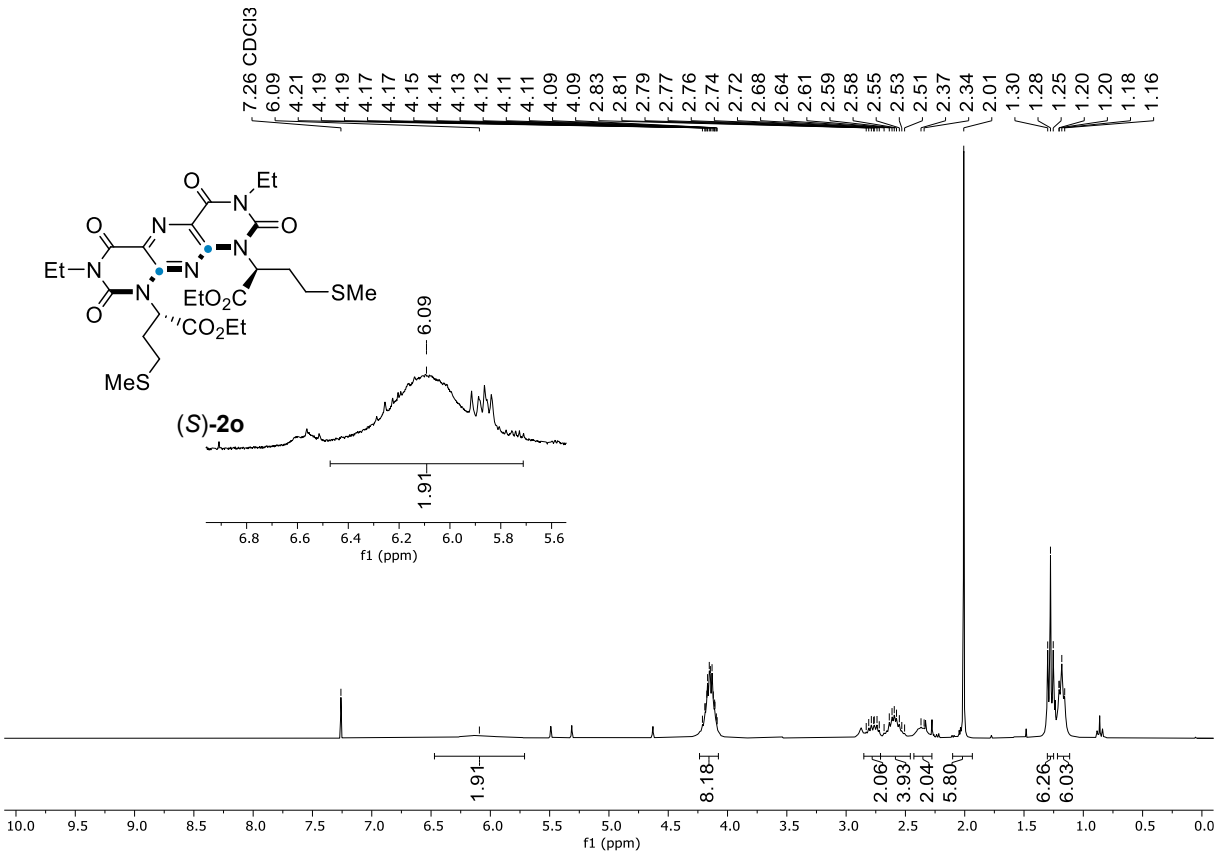
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 6.09 (s, 2H), 4.24 – 4.08 (m, 8H), 2.85 – 2.71 (m, 2H), 2.71 – 2.46 (m, 4H), 2.43 – 2.28 (m, 2H), 2.01 (s, 6H), 1.28 (t, *J* = 7.0 Hz, 6H), 1.18 (t, *J* = 6.8 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 168.8, 157.3, 149.5, 148.6, 124.3, 62.1, 55.1, 38.0, 31.3, 28.0, 15.5, 14.2, 13.0.

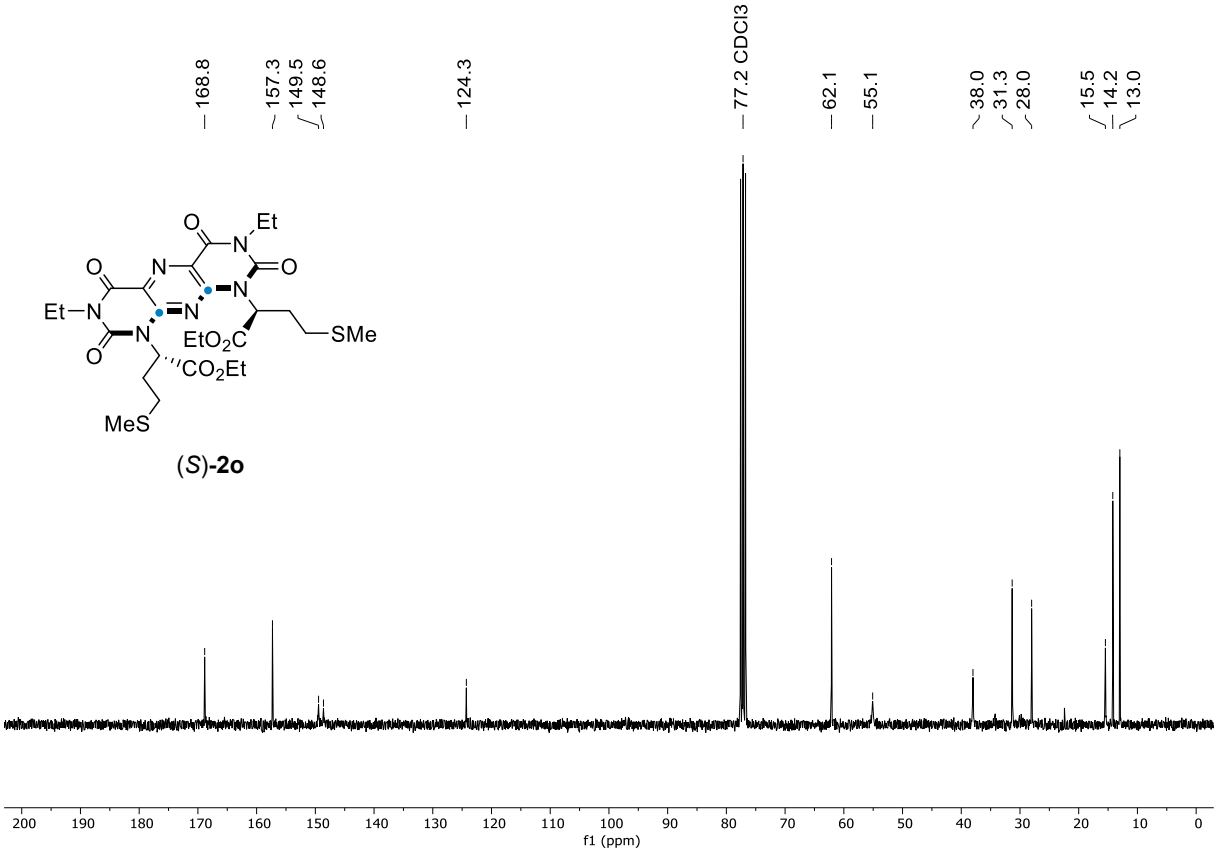
MS (GC-EI): *m/z* (%) = 624 (1, $[M]^+$) 430 (13) 281 (30) 253 (13) 207 (100) 191 (12) 73 (21).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{26}H_{37}N_6O_8S_2]^+$: 625.2109; found: 625.2101 (Error PPM: -1.3).

IR (ATR, neat, $\tilde{\nu}$) = 2981 (w), 2936 (w), 1733 (m), 1719 (s), 1674 (vs), 1556 (vs), 1435 (s), 1405 (vs), 1368 (m), 1321 (vs), 1268 (vs), 1225 (vs), 1152 (m), 1119 (w), 1091 (m), 1073 (m), 1024 (s), 944 (m), 860 (w), 812 (m), 752 (s), 705 (w), 669 (w), 602 (w), 548 (w), 495 (s), 402 (m) cm^{-1}.

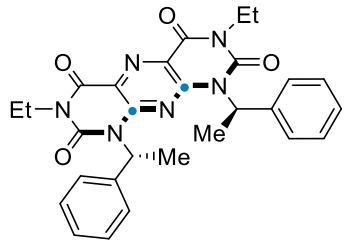


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of 3,7-Diethyl-1,9-bis((*S*)-1-phenylethyl)pyrimido[5,4-*g*]pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetraone ((*S*)-2p)



(*S*)-**2p**

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide (*S*)-**1p** (129 mg, 249 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:1) afforded the title compound (*S*)-**2p** (60.1 mg, 117 µmol, 47% yield) as a pale red solid.

R$_f$ = 0.23 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = 269-271 °C

$[\alpha]_D^{22}$ = -284.5 (c = 1.0 g/100 ml, DCM)

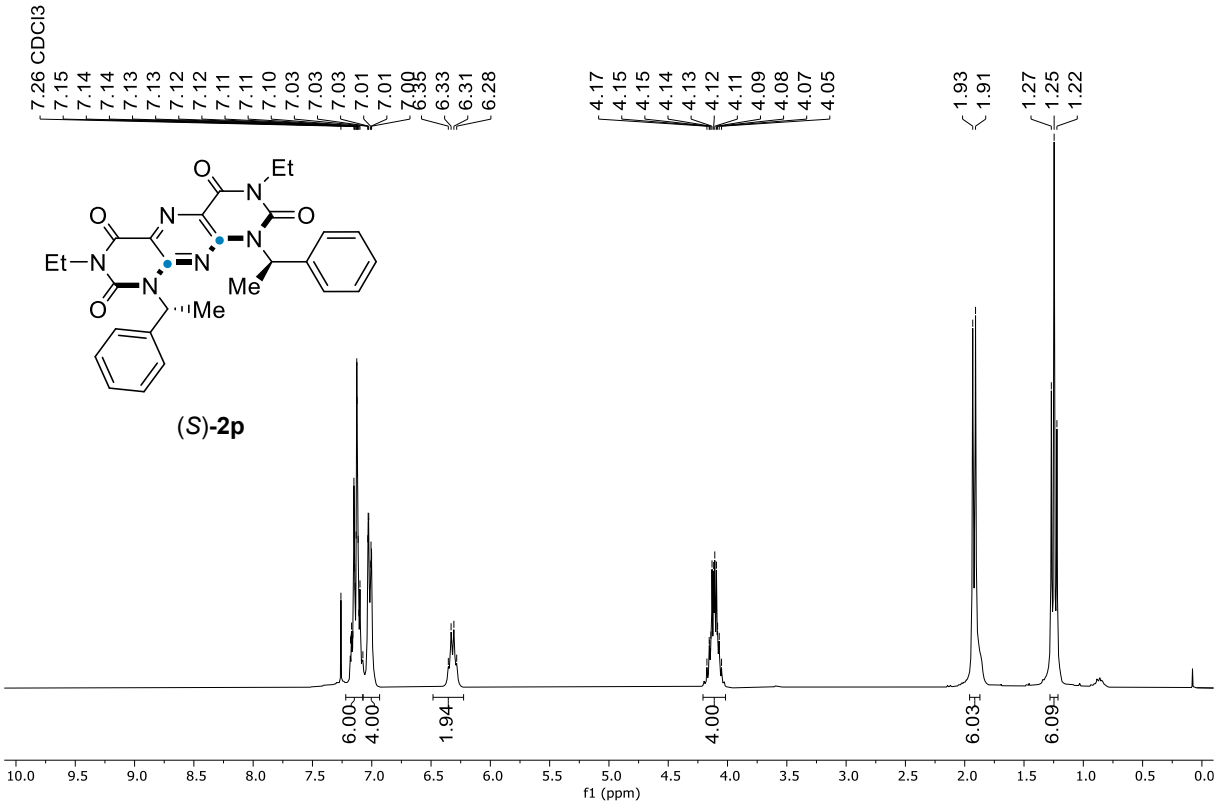
^{1}H NMR (300 MHz, 323K, Chloroform-d [7.26 ppm], ppm) δ = 7.20 – 7.08 (m, 6H), 7.07 – 6.97 (m, 4H), 6.32 (q, *J* = 7.1 Hz, 2H), 4.32 – 3.95 (m, 4H), 1.92 (d, *J* = 7.1 Hz, 6H), 1.25 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, 323K, Chloroform-d [77.16 ppm], ppm) δ = 157.6, 149.5, 148.8, 139.0, 128.6, 127.5, 126.1, 124.2, 53.4, 37.9, 16.1, 13.0.

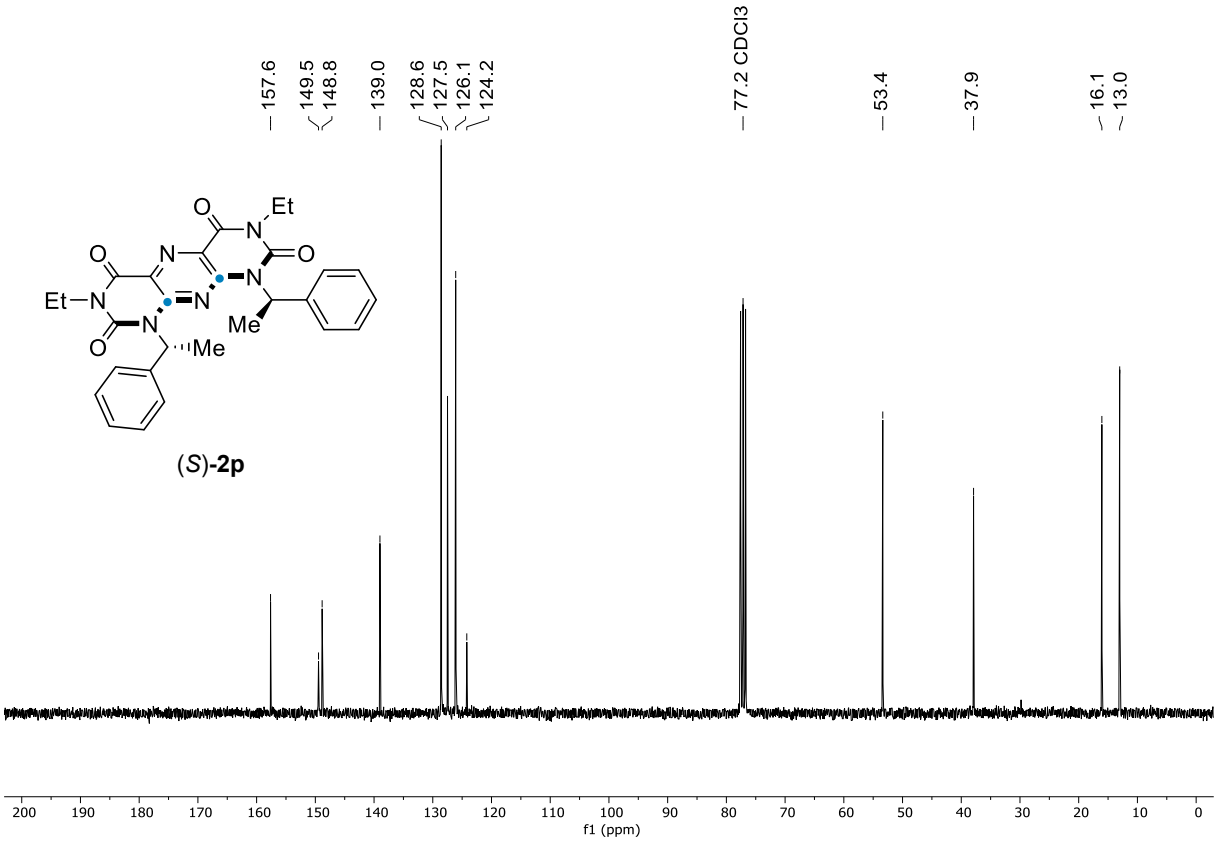
MS (EI): *m/z* (%) = 512 (62, $[M]^+$), 408 (100), 308 (14), 105 (89).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{28}H_{28}N_6O_4Na]^+$: 535.2064; found: 535.2065 (Error PPM: 0.2).

IR (ATR, neat, $\tilde{\nu}$) = 2983 (w), 2934 (w), 1713 (m), 1670 (vs), 1556 (vs), 1531 (m), 1495 (m), 1431 (s), 1407 (vs), 1368 (s), 1354 (s), 1325 (vs), 1270 (vs), 1230 (vs), 1150 (m), 1073 (s), 1054 (s), 1028 (m), 1011 (m), 960 (m), 913 (w), 871 (w), 846 (w), 814 (s), 783 (m), 752 (vs), 695 (vs), 642 (s), 608 (s), 551 (s), 522 (s), 500 (vs), 477 (s), 467 (s), 420 (s), 408 (s) cm^{-1}.

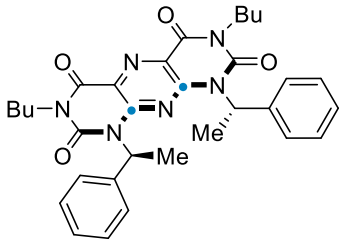


^{1}H NMR (300 MHz, 323K, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, 323K, Chloroform-d [77.16 ppm], ppm)

Synthesis of 3,7-dibutyl-1,9-bis((*S*)-1-phenylethyl)pyrimido[5,4-*g*]pteridine-2,4,6,8 (1*H*,3*H*,7*H*,9*H*)-tetraone ((*S*)-2q) JM16



(*S*)-**2q**

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide (*S*)-**1q** (143 mg, 250 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:1) afforded the title compound (*S*)-**2q** (64.5 mg, 113 µmol, 45% yield) as a pale red solid.

$\mathbf{R}_f$ = 0.33 (*n*-pentane:ethyl acetate = 2:1, UV)

m.p. = 204 °C

$[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{23}}$ = -260.0 (c = 1.0 g/100 ml, DCM)

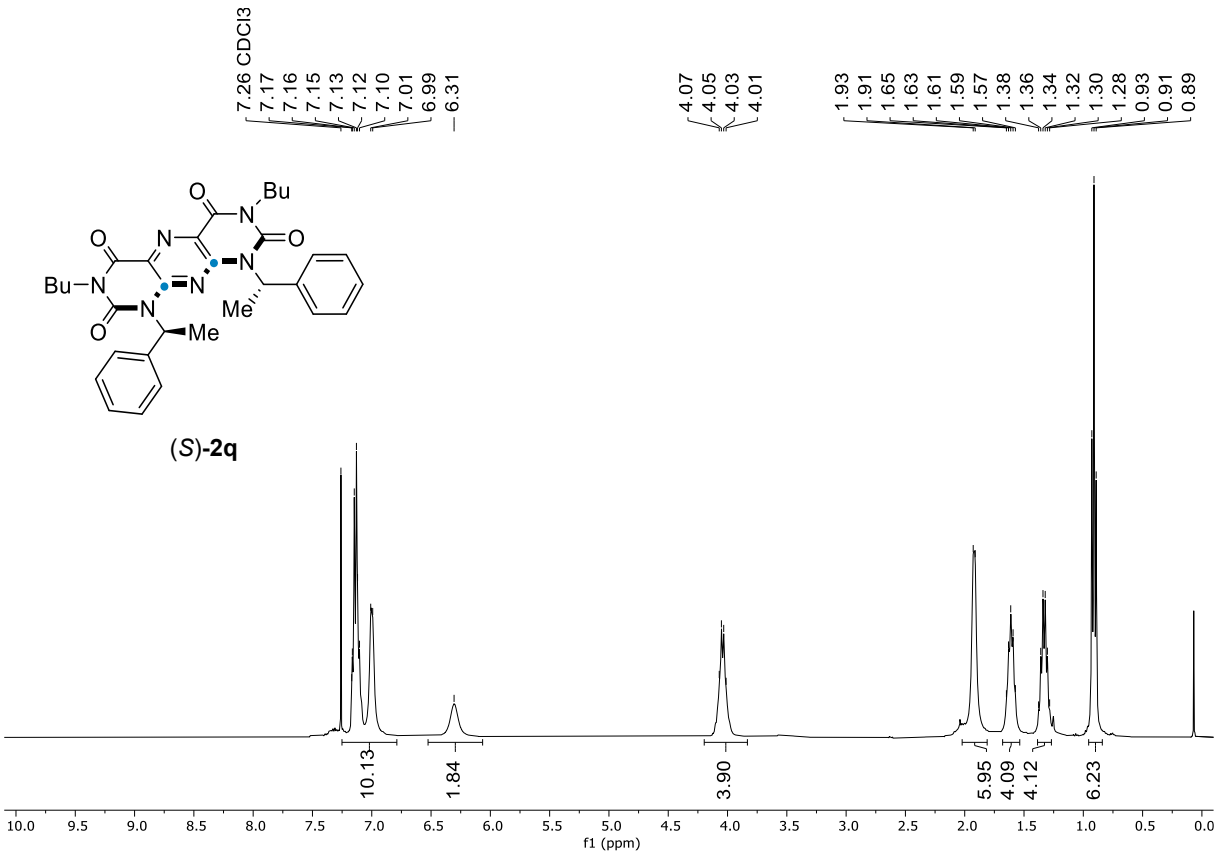
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.19 – 6.90 (m, 10H), 6.31 (bs, 2H), 4.12 – 3.99 (m, 4H), 1.92 (d, *J* = 6.7 Hz, 6H), 1.67 – 1.55 (m, 4H), 1.42 – 1.26 (m, 4H), 0.91 (t, *J* = 7.3 Hz, 6H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 157.8, 149.5, 148.7, 138.9, 128.6, 127.5, 126.0, 12101.1, 53.2, 42.5, 29.8, 20.2, 16.0, 13.9.

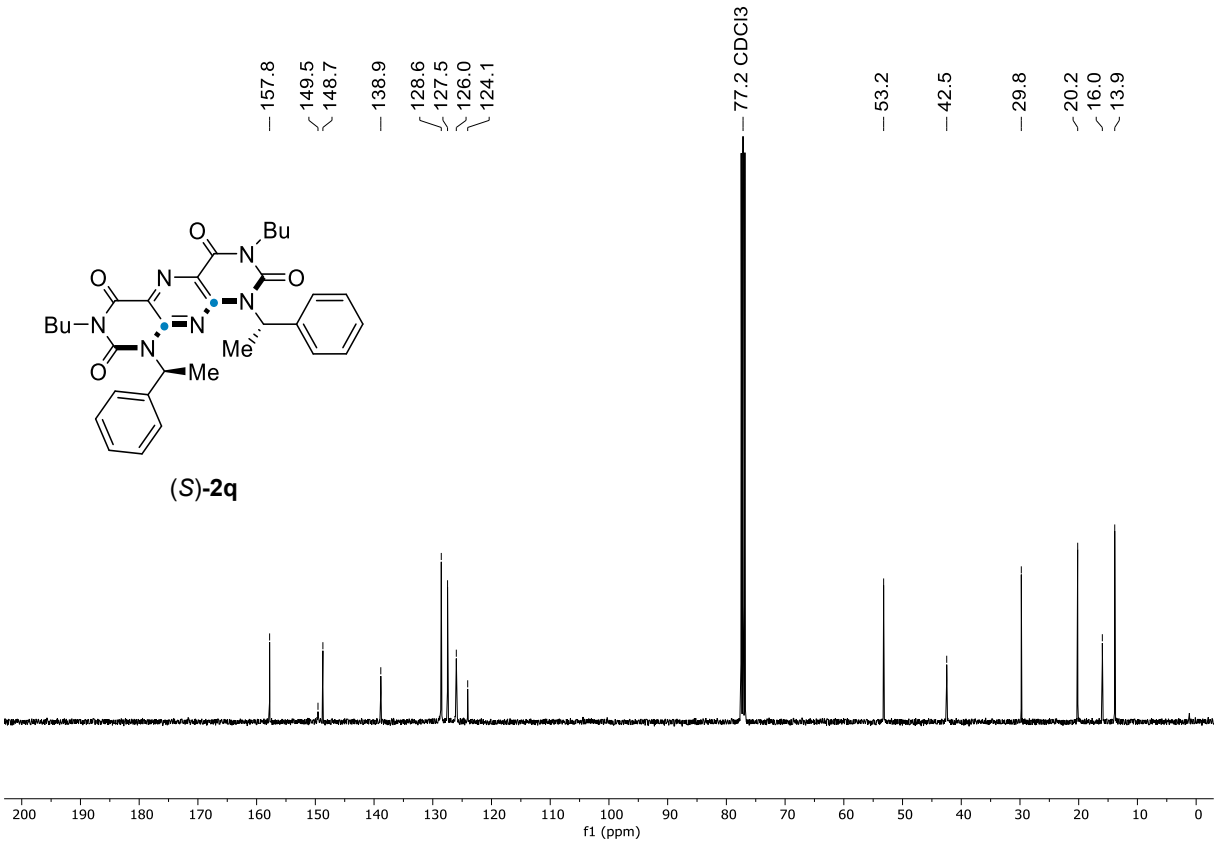
MS (EI): *m/z* (%) = 568 (36, $[M]^+$), 464 (64), 360 (36), 105 (100).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{32}H_{36}N_6O_4Na]^+$: 591.2690; found: 591.2687 (Error PPM: -0.5).

IR (ATR, neat, $\tilde{\nu}$) = 2959 (w), 2934 (w), 2873 (w), 1715 (s), 1672 (vs), 1560 (vs), 1497 (w), 1464 (w), 1429 (vs), 1409 (vs), 1376 (m), 1362 (s), 1327 (s), 1274 (vs), 1232 (vs), 1195 (w), 1150 (w), 1115 (w), 1101 (w), 1081 (w), 1056 (m), 1030 (w), 1009 (w), 960 (w), 946 (w), 909 (w), 840 (vw), 814 (m), 787 (w), 775 (w), 752 (vs), 693 (vs), 652 (w), 638 (w), 620 (w), 606 (w), 553 (s), 522 (m), 506 (s), 483 (m), 471 (m), 434 (m), 404 (w) cm^{-1}.

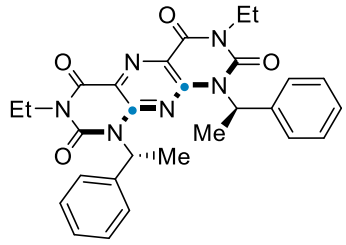


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of 3,7-Diethyl-1,9-bis((*R*)-1-phenylethyl)pyrimido[5,4-*g*]pteridine-2,4,6,8-(1*H*,3*H*,7*H*,9*H*)-tetraone ((*R*)-2r)



(*R*)-**2r**

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide (*R*)-**1r** (103 mg, 200 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:1) afforded the title compound (*R*)-**2r** (45.1 mg, 88.0 µmol, 44% yield) as a pale red solid.

R_f = 0.23 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = 270-273 °C

$[\alpha]_D^{23}$ = +282.0 (c = 1.0 g/100 ml, DCM)

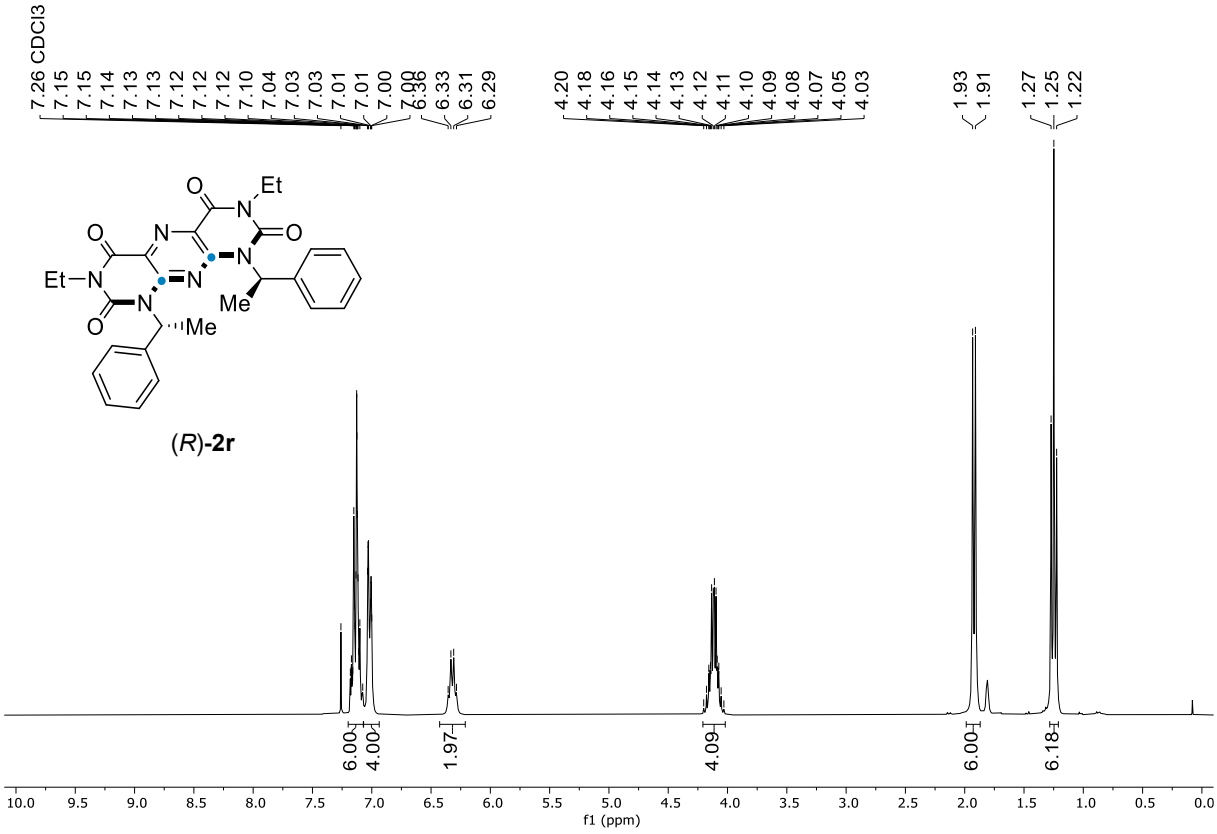
^{1}H NMR (300 MHz, 323K, Chloroform-d [7.26 ppm], ppm) δ = 7.20 – 7.07 (m, 6H), 7.07 – 6.94 (m, 4H), 6.32 (q, *J* = 7.0 Hz, 2H), 4.21 – 4.02 (m, 4H), 1.92 (d, *J* = 7.0 Hz, 6H), 1.25 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, 323K, Chloroform-d [77.16 ppm], ppm) δ = 157.6, 149.5, 148.9, 139.0, 128.6, 127.5, 126.1, 124.2, 53.4, 37.9, 16.1, 13.0.

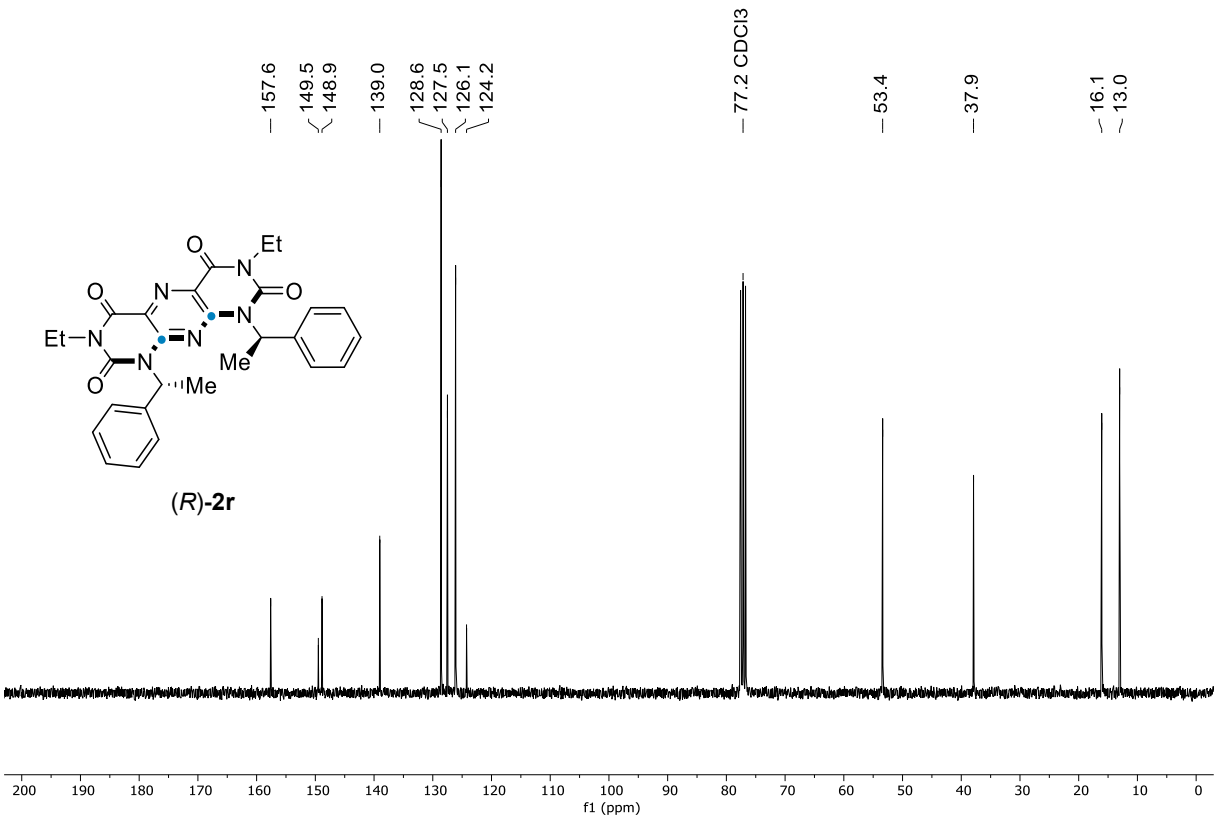
MS (EI): *m/z* (%) = 512 (41, $[M]^+$), 408 (100), 308 (16), 105 (98).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{28}H_{29}N_6O_4]^+$: 513.2245; found: 513.2247 (Error PPM: 0.4).

IR (ATR, neat, $\tilde{\nu}$) = 2977 (w), 2938 (w), 1713 (s), 1672 (vs), 1560 (vs), 1497 (m), 1433 (s), 1407 (vs), 1368 (vs), 1323 (vs), 1272 (vs), 1232 (vs), 1195 (s), 1150 (m), 1121 (w), 1079 (m), 1056 (m), 1040 (w), 1028 (m), 1013 (m), 956 (m), 916 (w), 873 (w), 848 (w), 814 (m), 783 (w), 752 (vs), 695 (vs), 642 (m), 608 (m), 551 (m), 526 (m), 516 (m), 500 (s), 479 (m), 467 (m), 445 (m), 424 (w) cm^{-1}.

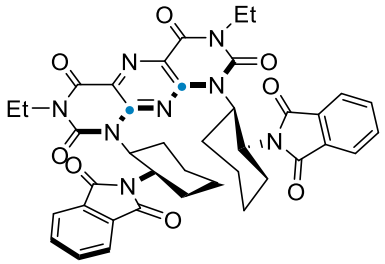


^{1}H NMR (300 MHz, 323K, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, 323K, Chloroform-d [77.16 ppm], ppm)

Synthesis of 1,9-bis((1*R*,2*R*)-2-(1,3-dioxoisoindolin-2-yl)cyclohexyl)-3,7-diethylpyrimido[5,4-*g*]pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetraone ((1*R*,2*R*)-2s)



(1*R*,2*R*)-**2s**

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide (1*R,*2*R*)-**1s** (190 mg, 250 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:1) afforded the title compound (1*R,*2*R*)-**2s** (110 mg, 145 µmol, 58% yield, *ir*: 3.5:1.0) as an off white solid.

R$_f$ = 0.20 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = >350 °C*

$[\alpha]_D^{22}$ = +95,4 (c = 0.5 g/100 ml, DCM)

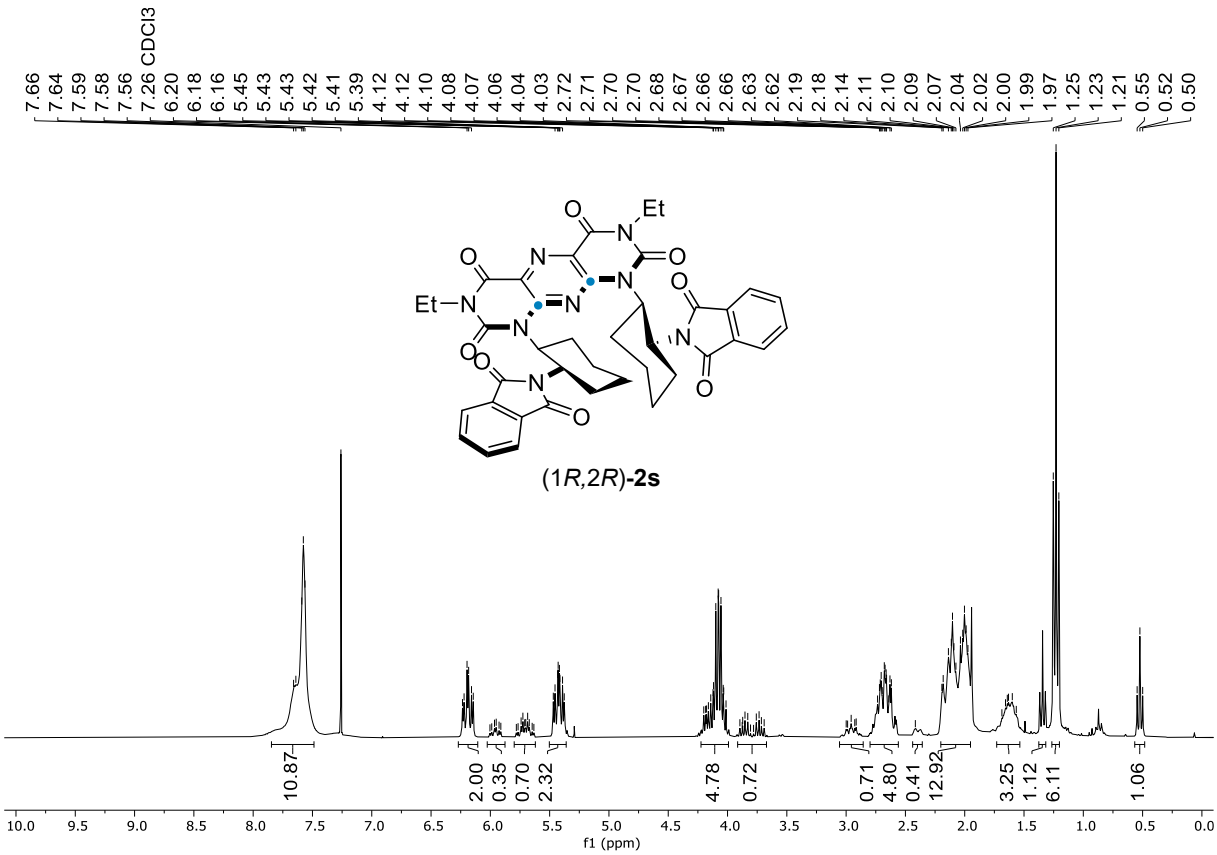
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.85 – 7.49 (m, 11H), 6.19 (td, *J* = 11.6, 4.2 Hz, 2H), 5.71 (dtd, *J* = 15.8, 11.8, 4.3 Hz, 1H), 5.42 (ddd, *J* = 12.2, 11.1, 4.3 Hz, 2H), 4.22 – 3.99 (m, 5H), 3.91 – 3.67 (m, 1H), 3.06 – 2.86 (m, 1H), 2.80 – 2.56 (m, 5H), 2.20 – 1.95 (m, 13H), 1.73 – 1.54 (m, 3H), 1.34 (t, *J* = 7.1 Hz, 1H), 1.23 (t, *J* = 7.0 Hz, 6H), 0.52 (t, *J* = 7.0 Hz, 1H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 157.4, 149.0, 148.8, 134.2, 123.4, 56.5, 50.1, 37.5, 29.6, 28.9, 25.4, 25.2, 12.7.

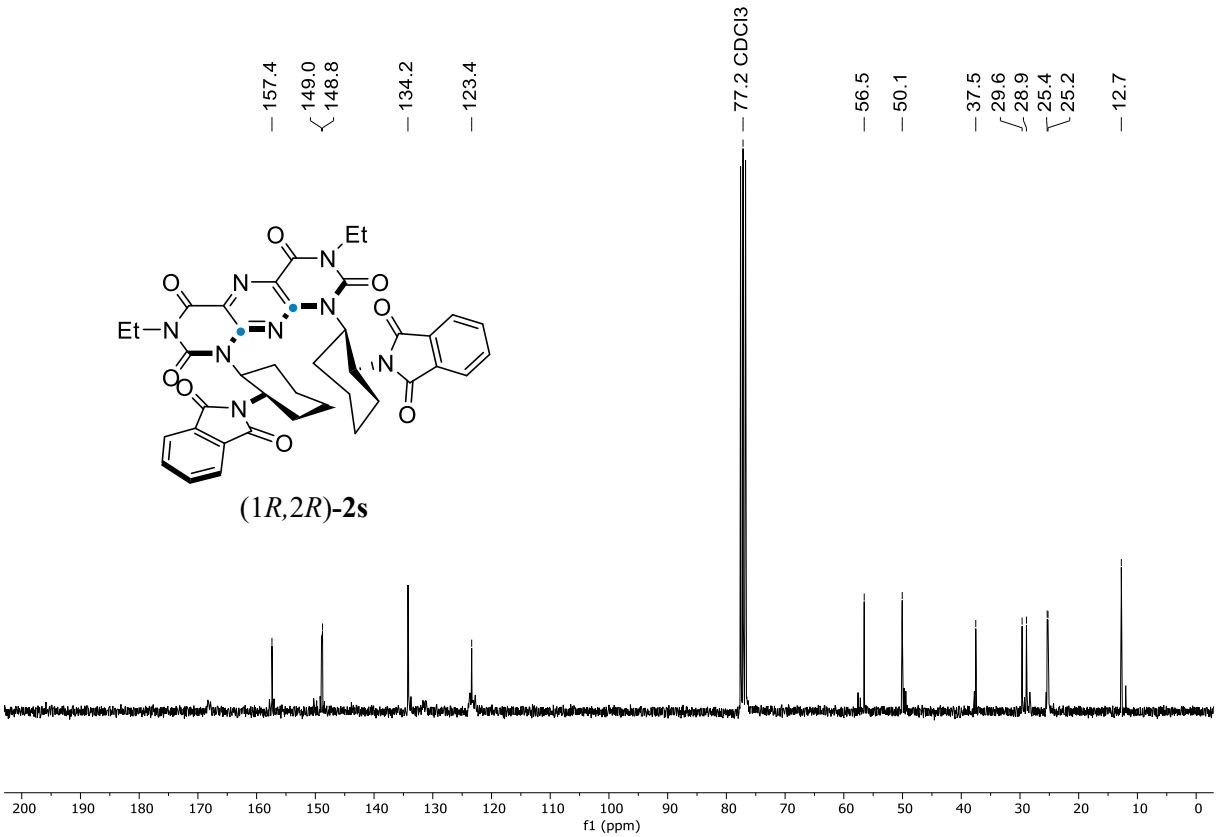
MS (EI): *m/z* (%) = 758 (9, $[M]^+$), 611 (6), 532 (100), 195 (30), 226 (33), 148 (42).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{40}H_{38}N_8O_8Na]^+$: 781.2704; found: 781.2706 (Error PPM: 0.3).

IR (ATR, neat, ṽ) = 2977 (vw), 2936 (vw), 2859 (vw), 1772 (vw), 1719 (vs), 1678 (vs), 1613 (vw), 1560 (vs), 1517 (w), 1454 (w), 1433 (w), 1409 (s), 1372 (s), 1321 (s), 1274 (m), 1232 (w), 1191 (w), 1174 (w), 1150 (w), 1075 (w), 1022 (w), 956 (w), 946 (w), 911 (w), 871 (w), 814 (w), 791 (w), 756 (w), 718 (vs), 699 (w), 652 (w), 636 (w), 569 (vw), 530 (w), 500 (w), 481 (w), 455 (w), 406 (w) cm^{-1}.

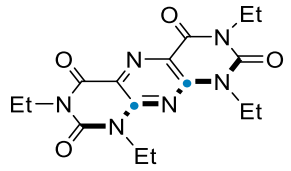


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of 1,3,7,9-Tetraethylpyrimido[5,4-*g*]pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetraone (2u) JM23



2u

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **1u** (91.4 mg, 251 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:2) and a subsequent wash with Et_2O afforded the title compound **2u** (18.1 mg, 50.2 µmol, 20% yield) as a pale brown solid.

R_f = 0.22 (*n*-pentane:ethyl acetate = 1:2, UV)

m.p. = 236-237 °C

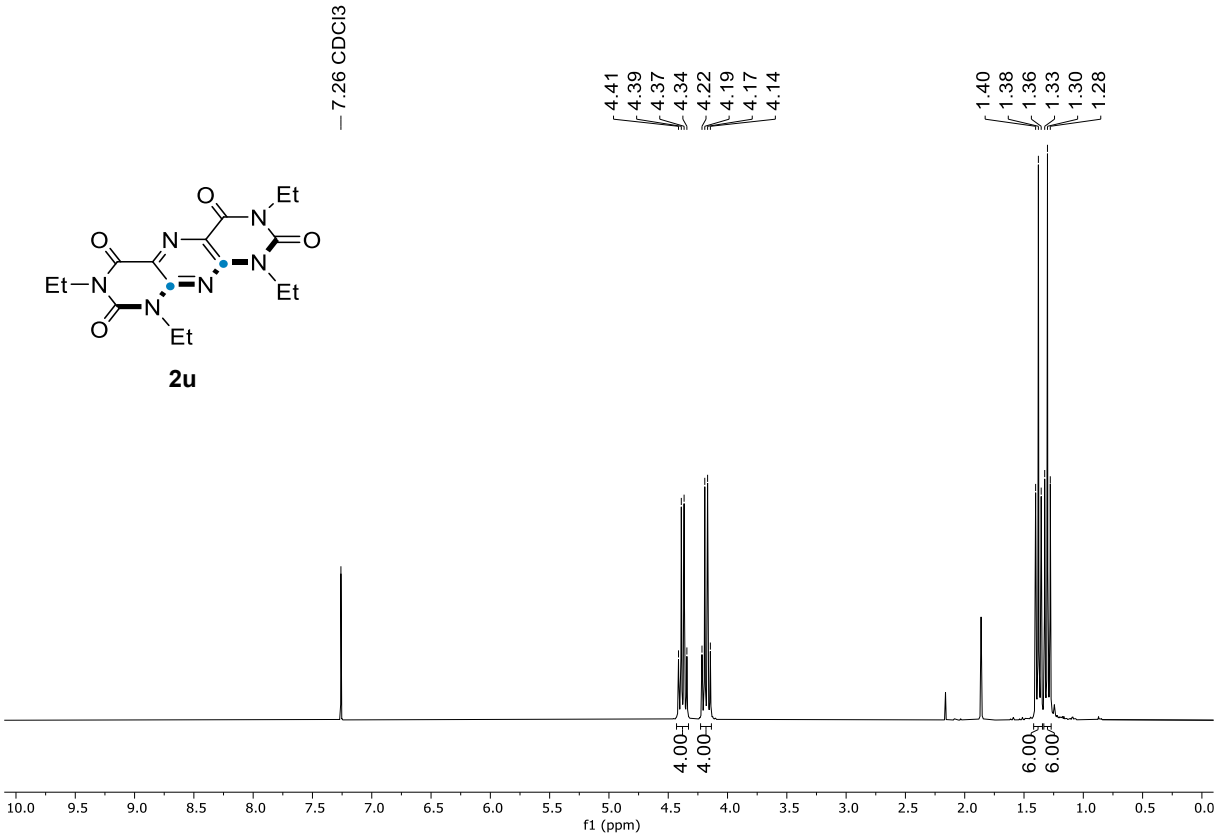
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.38 (q, *J* = 7.1 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 1H), 1.38 (t, *J* = 7.0 Hz, 1H), 1.30 (t, *J* = 7.1 Hz, 1H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 157.7, 149.6, 149.2, 123.9, 38.8, 37.9, 13.1, 13.1.

MS (GC-EI): *m/z* (%) = 360 (100, $[M]^+$), 331 (16), 304 (11), 274 (13), 93 (10).

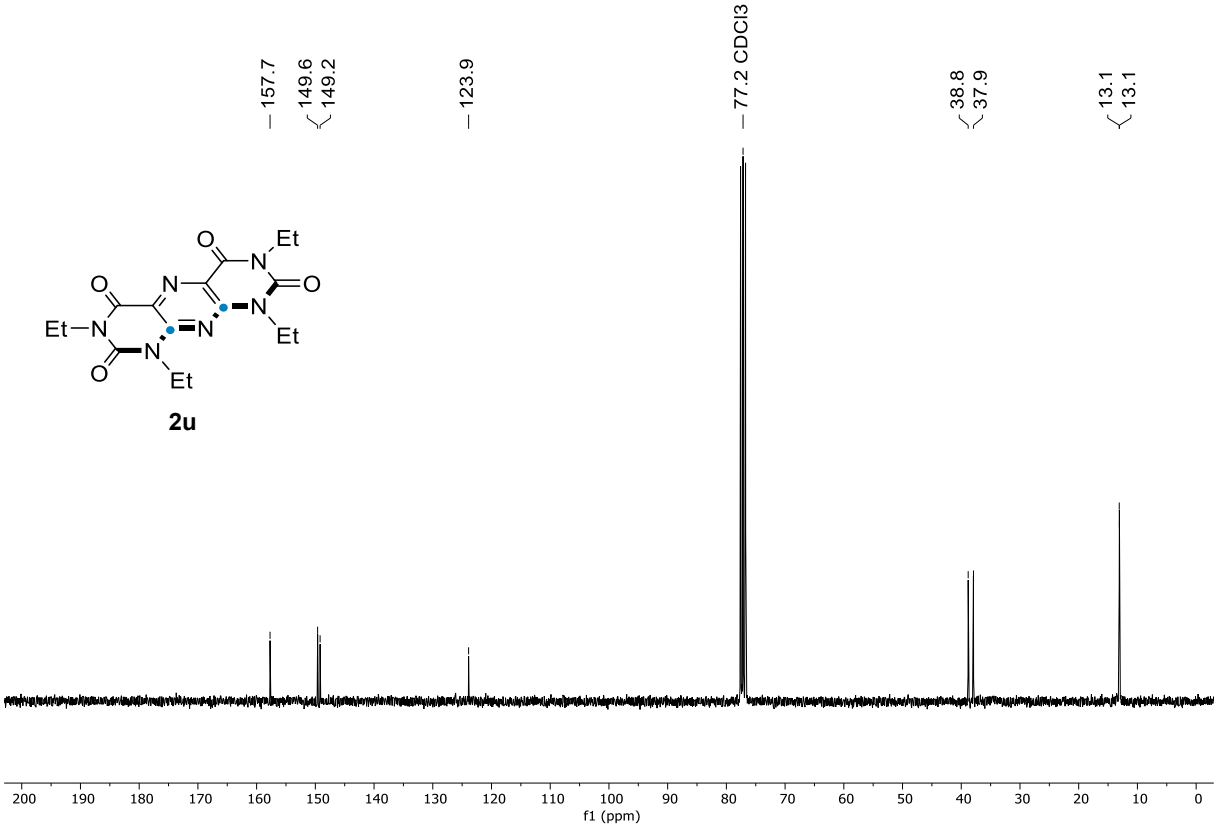
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{16}H_{20}N_6O_4Na]^+$: 383.1438; found: 383.1438 (Error PPM: 0.0).

IR (ATR, neat, ṽ) = 2985 (w), 2961 (w), 2938 (w), 1713 (m), 1672 (vs), 1562 (vs), 1507 (w), 1472 (w), 1452 (s), 1433 (s), 1415 (vs), 1374 (s), 1358 (m), 1340 (m), 1313 (s), 1274 (s), 1238 (vs), 1221 (s), 1119 (m), 1077 (s), 1032 (m), 950 (m), 873 (w), 846 (w), 814 (m), 781 (w), 752 (vs), 712 (w), 622 (w), 571 (w), 528 (w), 495 (vs), 459 (s), 410 (s) cm^{-1}.



2u

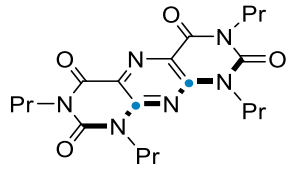
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



2u

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of 1,3,7,9-Tetrapropylpyrimido[5,4-*g*]pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetraone (2v) JM24



2v

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **1v** (105 mg, 250 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:2) and a subsequent wash with Et_2O afforded the title compound **1v** (18.8 mg, 45.1 µmol, 18% yield) as a light brown solid.

R_f = 0.16 (*n*-pentane:ethyl acetate = 1:2, UV)

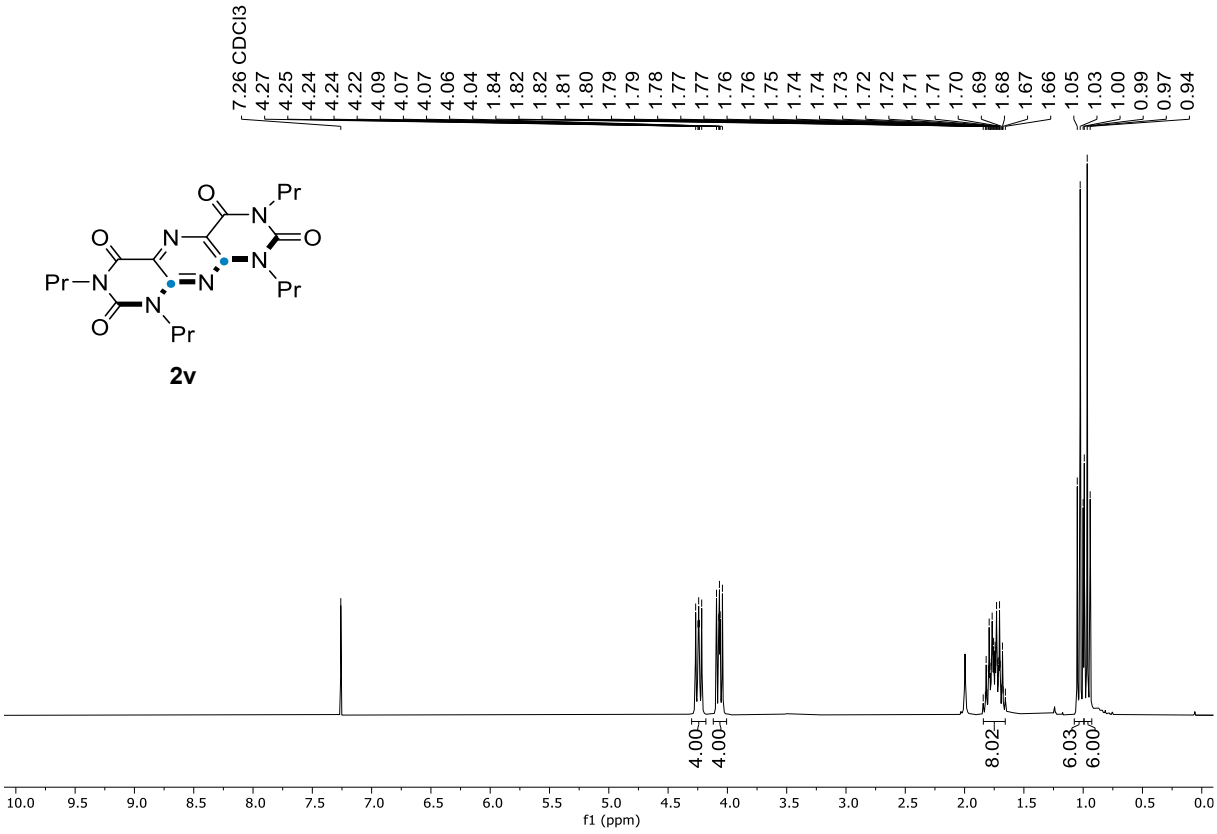
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.42 – 4.14 (m, 4H), 4.14 – 3.92 (m, 4H), 1.87 – 1.63 (m, 7H), 1.02 (t, *J* = 7.5 Hz, 6H), 0.97 (t, *J* = 7.5 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 157.9, 150.0, 149.3, 123.8, 45.1, 44.1, 21.2, 21.1, 11.4, 11.4.

MS (GC-EI): *m/z* (%) = 416 (100, $[M]^+$), 375 (11), 333 (9), 302 (20), 290 (12)

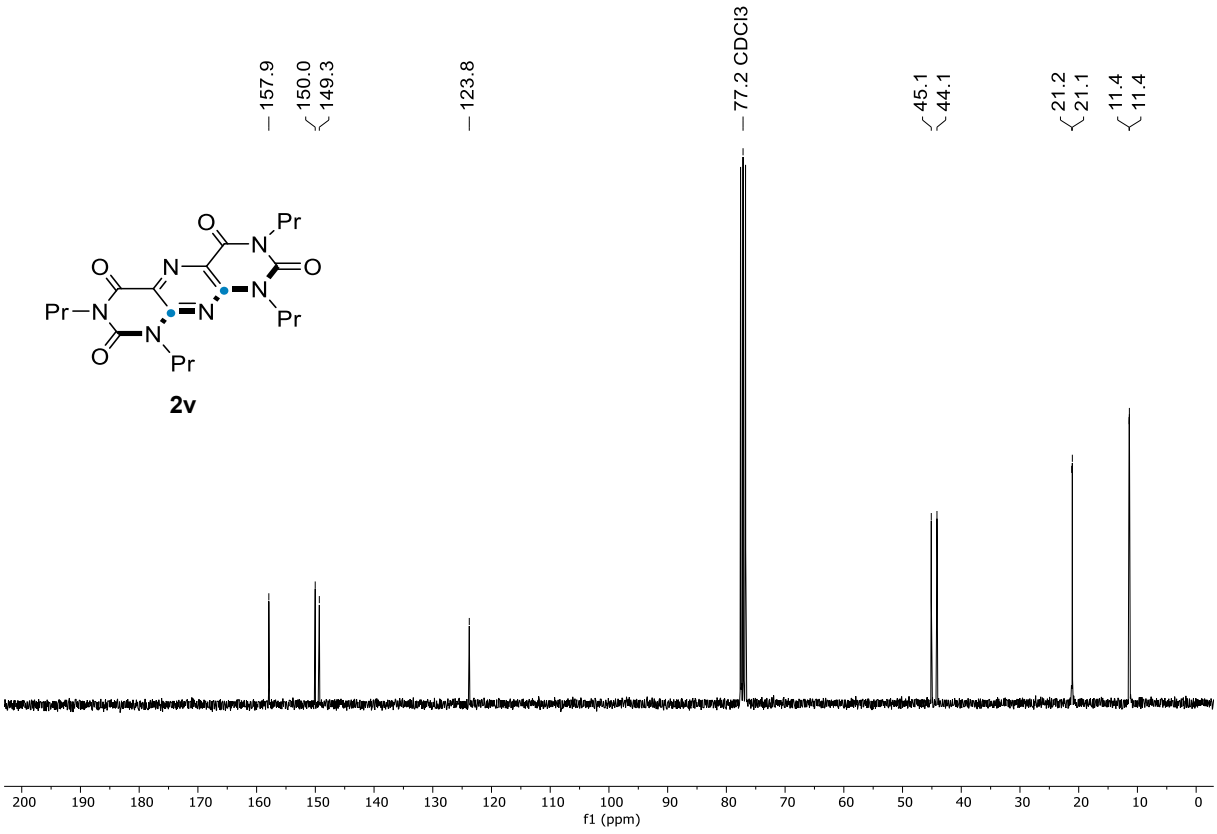
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{20}H_{28}N_6O_4Na]^+$: 439.2064; found: 439.2069 (Error PPM: 1.1).

The analytic data agrees with those reported in literature.[10]



2v

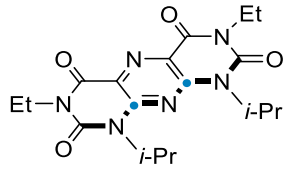
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



2v

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of 3,7-Diethyl-1,9-diisopropylpyrimido[5,4-*g*]pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetraone (2w)



2w

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **1w** (97.9 mg, 249 µmol, 1.00 equiv.) purification by column chromatography (methylene chloride:ethyl acetate = 9:1) afforded the title compound **2w** (17.9 mg, 46.1 µmol, 18% yield) as an off white solid.

R_f = 0.23 (methylene chloride:ethyl acetate = 9:1, UV)

m.p. = 221-225 °C

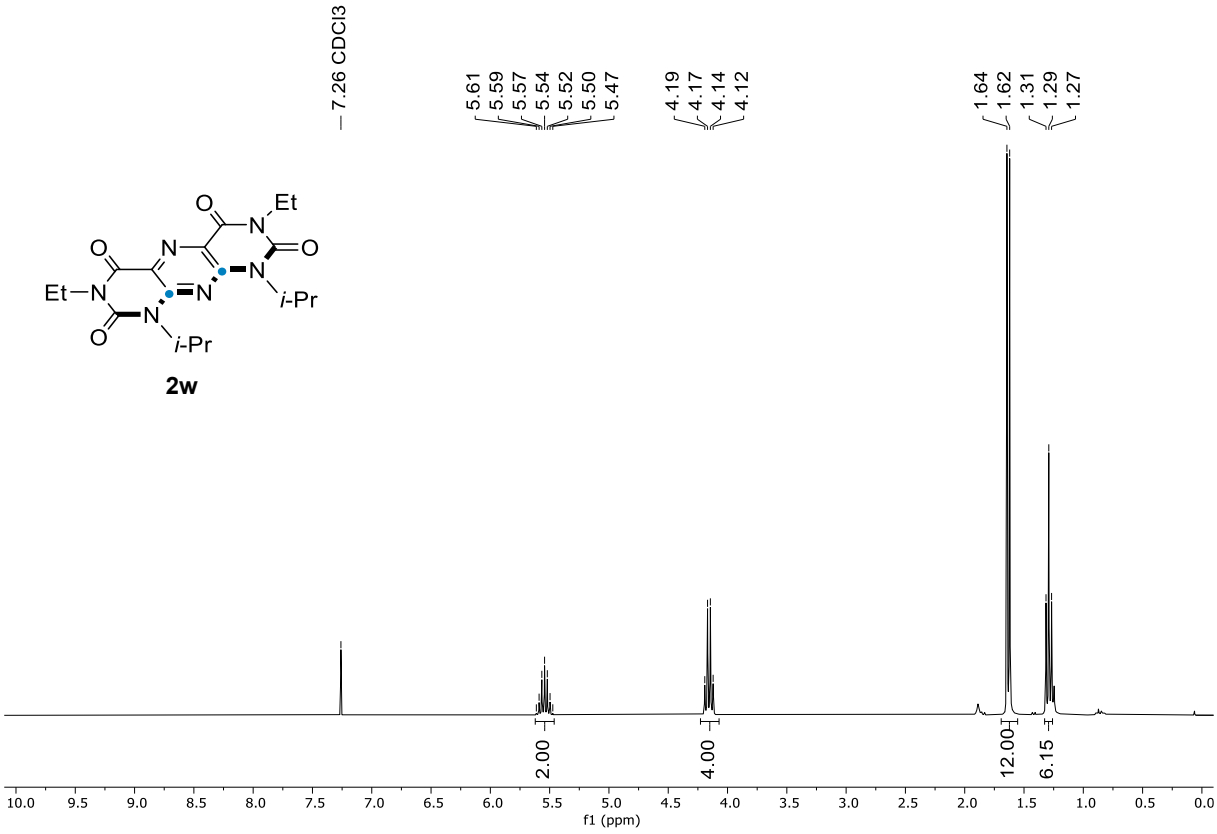
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 5.54 (hept, *J* = 7.0 Hz, 2H), 4.16 (q, *J* = 7.0 Hz, 4H), 1.63 (d, *J* = 7.0 Hz, 12H), 1.29 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 157.7, 149.4, 149.0, 123.8, 48.8, 37.8, 19.7, 13.1.

MS (GC-EI): *m/z* (%) = 388 ([M]$^+$,100), 346 (31), 304 (78), 276 (10), 260 (21), 246 (13), 232 (17), 161 (12).

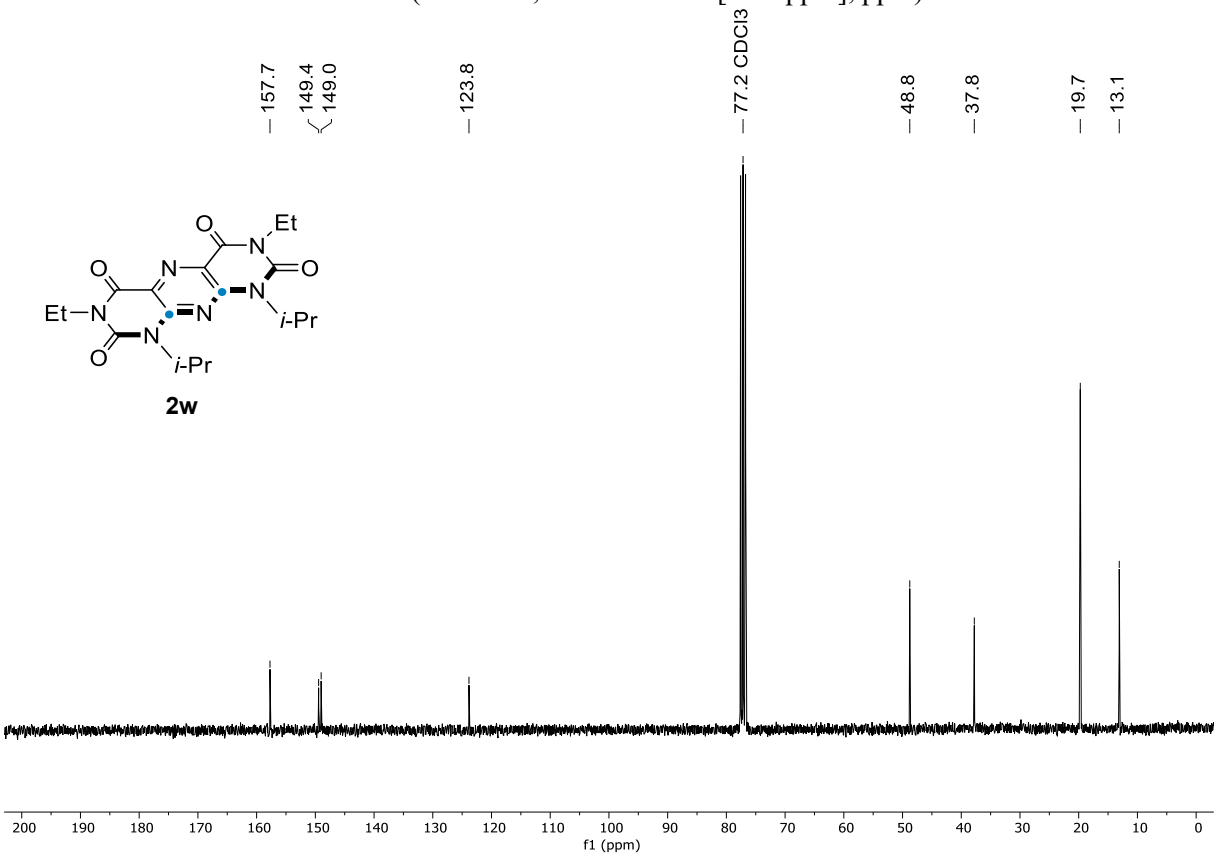
HRMS (ESI-TOF): *m/z* [M+Na]$^+$ calcd. for [$C_{18}H_{24}N_6O_4Na$]$^+$: 411.1751; found: 411.1750 (Error PPM: -0.2).

IR (ATR, neat, ṽ) = 2975 (w), 2930 (w), 2885 (vw), 1715 (s), 1674 (vs), 1552 (vs), 1433 (s), 1407 (vs), 1368 (vs), 1323 (vs), 1266 (vs), 1232 (vs), 1170 (w), 1140 (m), 1121 (w), 1079 (s), 1064 (s), 964 (m), 907 (w), 828 (w), 814 (m), 756 (vs), 710 (w), 599 (w), 493 (s), 447 (s), 406 (m) cm^{-1}.



2w

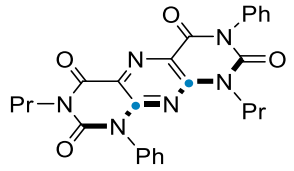
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



2w

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of 1,7-Diphenyl-3,9-dipropylpyrimido[5,4-*g*]pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetraone (2t)



2t

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **1t** (122 mg, 250 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 1:1) afforded the title compound **2t** (77.9 mg, 161 µmol, 64%) as a pale brown solid.

R_f = 0.3 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = 256-260 °C*

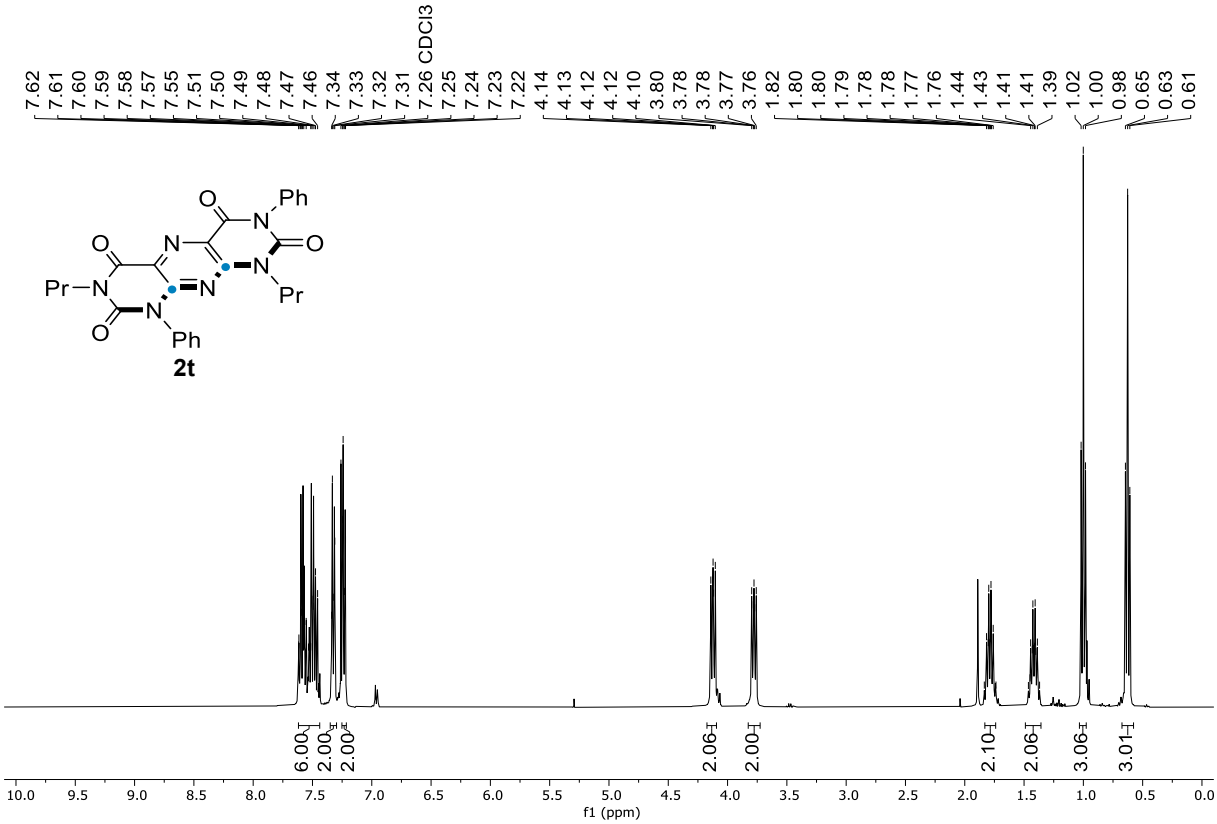
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.62 – 7.44 (m, 6H), 7.35 – 7.30 (m, 2H), 7.25 – 7.21 (m, 2H), 4.17 – 4.09 (m, 2H), 3.83 – 3.73 (m, 2H), 1.83 – 1.74 (m, 2H), 1.49 – 1.36 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H), 0.63 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 157.9, 157.7, 150.3, 150.0, 149.4, 134.7, 134.5, 129.9, 129.7, 129.6, 129.3, 128.6, 128.3, 124.8, 123.5, 45.3, 44.4, 21.1, 20.6, 11.4, 10.9.

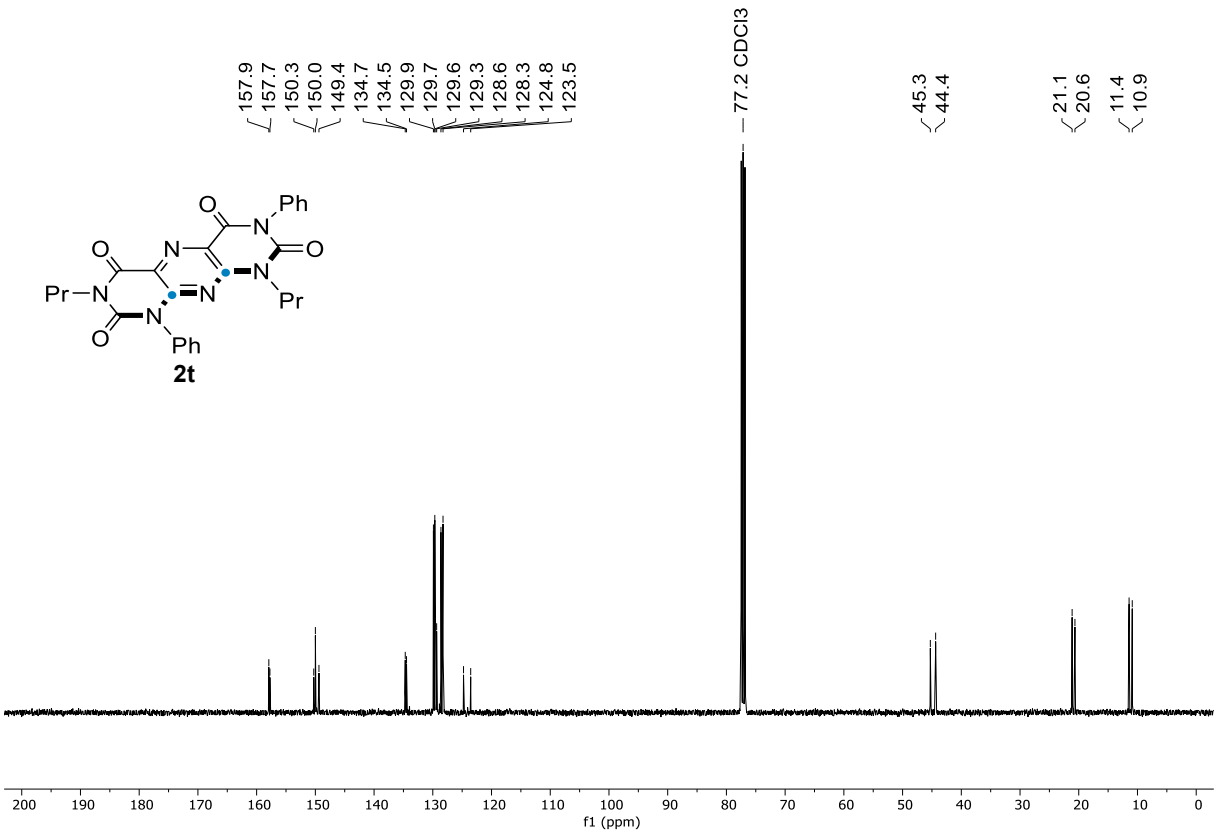
MS (EI): *m/z* (%) = 484 (100, $[M]^+$), 443 (8), 399 (13), 357 (12), 77 (6).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{26}H_{24}N_6O_4Na]^+$: 507.1751; found: 507.1754 (Error PPM: 0.6).

IR (ATR, neat, $\tilde{\nu}$) = 2963 (w), 2934 (w), 2875 (w), 1723 (s), 1676 (vs), 1597 (w), 1550 (vs), 1491 (m), 1454 (m), 1429 (s), 1413 (s), 1380 (vs), 1317 (vs), 1266 (vs), 1219 (vs), 1187 (s), 1154 (s), 1087 (s), 1070 (s), 1003 (m), 901 (w), 875 (w), 840 (w), 812 (s), 791 (w), 748 (vs), 714 (m), 691 (vs), 661 (m), 646 (m), 618 (w), 569 (m), 520 (vs), 485 (vs), 463 (s), 426 (m) cm^{-1}.

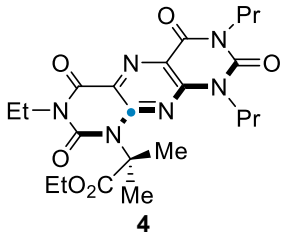


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl 2-(3-ethyl-2,4,6,8-tetraoxo-7,9-dipropyl-3,4,6,7,8,9-hexahydropyrimido[5,4-*g*]pteridin-1(2*H*)-yl)-2-methylpropanoate (4)



4

Following the general procedure GP3, but using 2.25 equiv. of AgF_2 using the corresponding tetrahydropteridine-6-carboxamide **3** (95.0 mg, 250 µmol, 1.00 equiv) purification by column chromatography (*n*-pentane:ethyl acetate = 1:1) afforded the title compound **4** (66.9 mg, 141 µmol, 73% yield) as a colorless solid.

R_f = 0.32 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = 230-231 °C

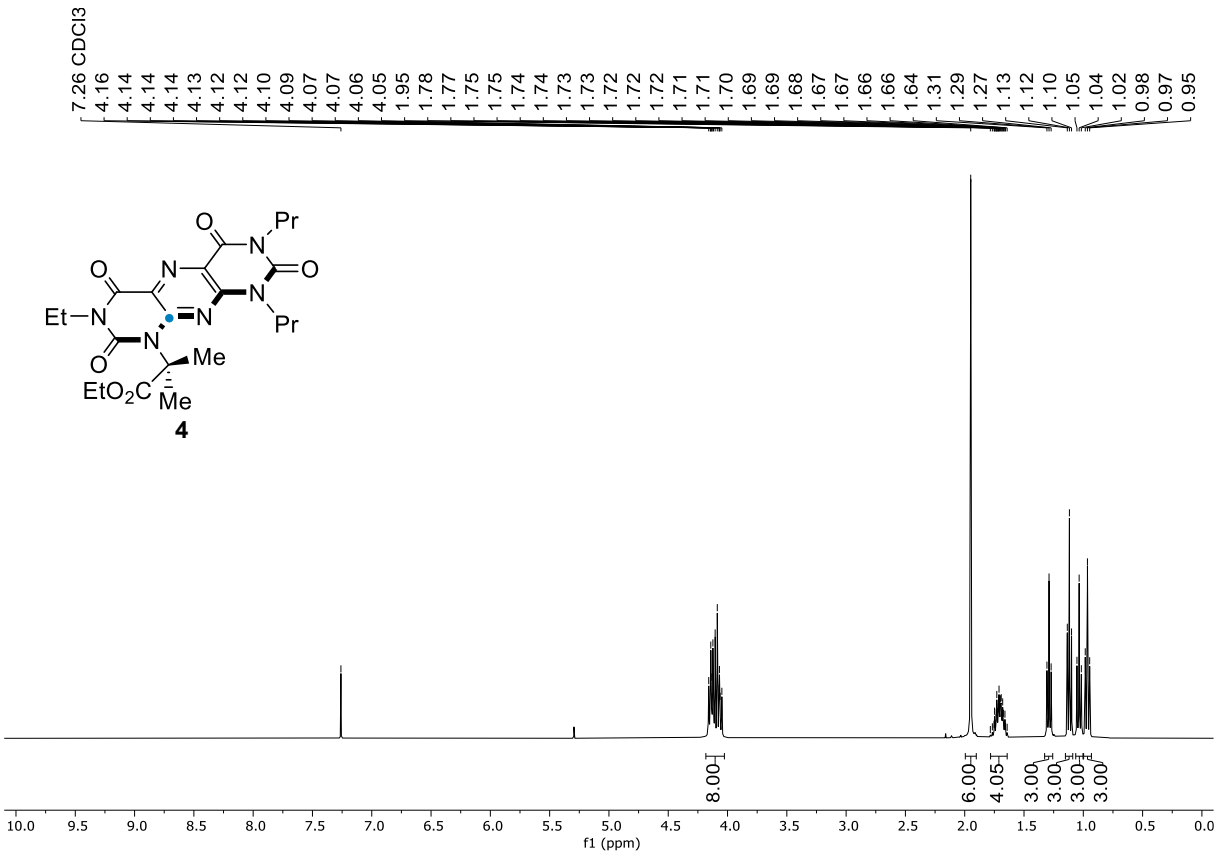
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.18 – 4.03 (m, 8H), 1.95 (s, 6H), 1.78 – 1.64 (m, 4H), 1.29 (t, *J* = 7.0 Hz, 3H), 1.12 (t, *J* = 7.1 Hz, 3H), 1.04 (t, *J* = 7.4 Hz, 3H), 0.97 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 172.6, 157.7, 157.6, 149.9, 149.8, 149.5, 148.1, 124.5, 123.8, 65.8, 61.8, 45.1, 44.2, 38.2, 25.0, 21.4, 21.1, 14.1, 13.1, 11.4, 11.1.

MS (GC-EI): *m/z* (%) = 474 (53, $[M]^+$), 401 (16), 360 (40), 330 (100).

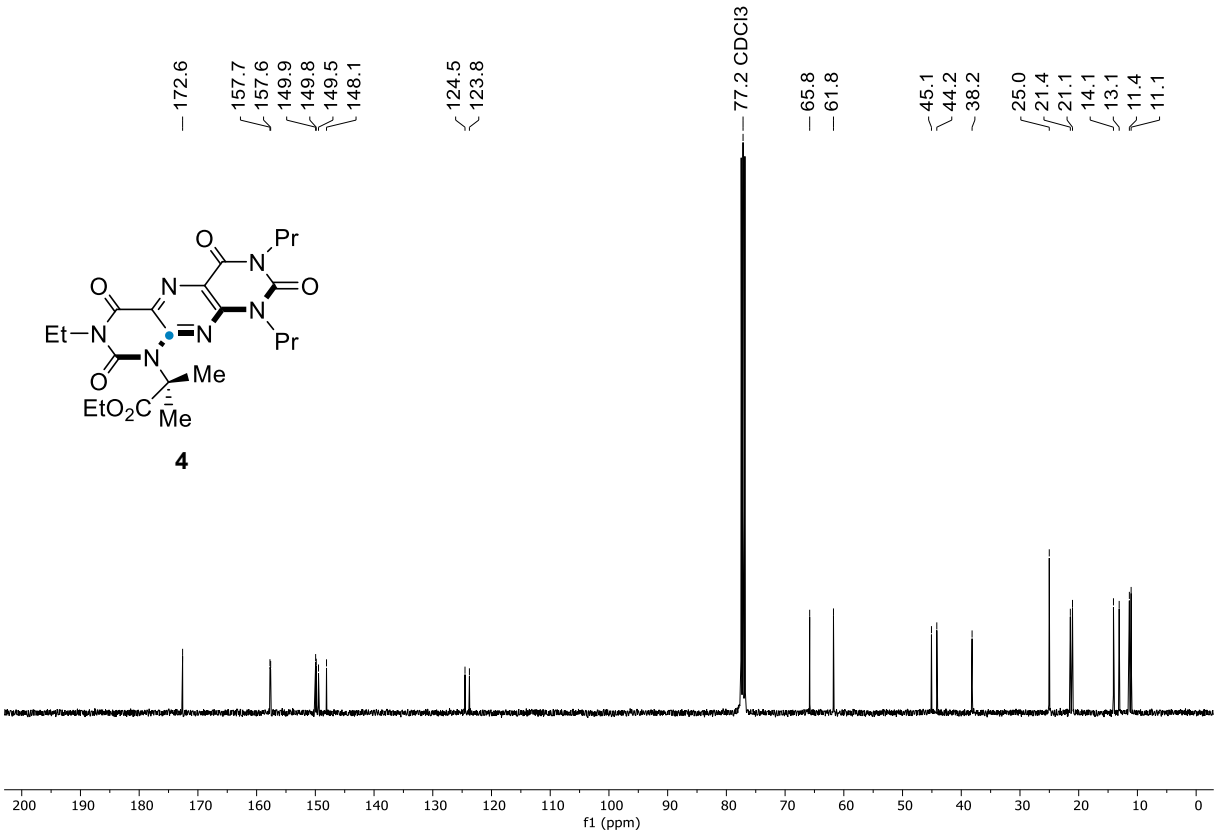
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{22}H_{30}N_6O_6Na]^+$: 497.2119; found: 497.2122 (Error PPM: 0.6).

IR (ATR, neat, $\tilde{\nu}$) = 2967 (w), 2940 (w), 2879 (w), 1737 (m), 1717 (s), 1674 (vs), 1558 (vs), 1509 (w), 1458 (w), 1429 (s), 1417 (s), 1403 (vs), 1376 (vs), 1323 (s), 1303 (w), 1272 (vs), 1242 (vs), 1219 (m), 1207 (w), 1177 (m), 1154 (s), 1126 (m), 1105 (m), 1089 (m), 1073 (m), 1028 (m), 983 (w), 964 (w), 948 (w), 920 (w), 901 (w), 889 (w), 865 (w), 834 (w), 816 (m), 765 (m), 752 (vs), 736 (w), 712 (w), 689 (w), 665 (w), 553 (w), 534 (w), 485 (s), 455 (w), 420 (m) cm^{-1}.



4

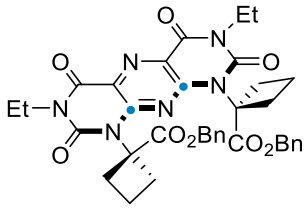
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



4

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of dibenzyl 1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2,6*H*)-diyl)bis(cyclobutane-1-carboxylate) (2y)



2y

Following the general procedure GP3, using the corresponding 2,6-dicarboxamide **1y** (136 mg, 199 µmol, 1.00 equiv.) purification by column chromatography (dichloromethane → dichloromethane:MeOH = 98.5:1.5) afforded the title compound **2y** (109 mg, 160 µmol, 80% yield) as an ofwhite solid.

R_f = 0.18 (dichloromethane + 1.5% MeOH, UV)

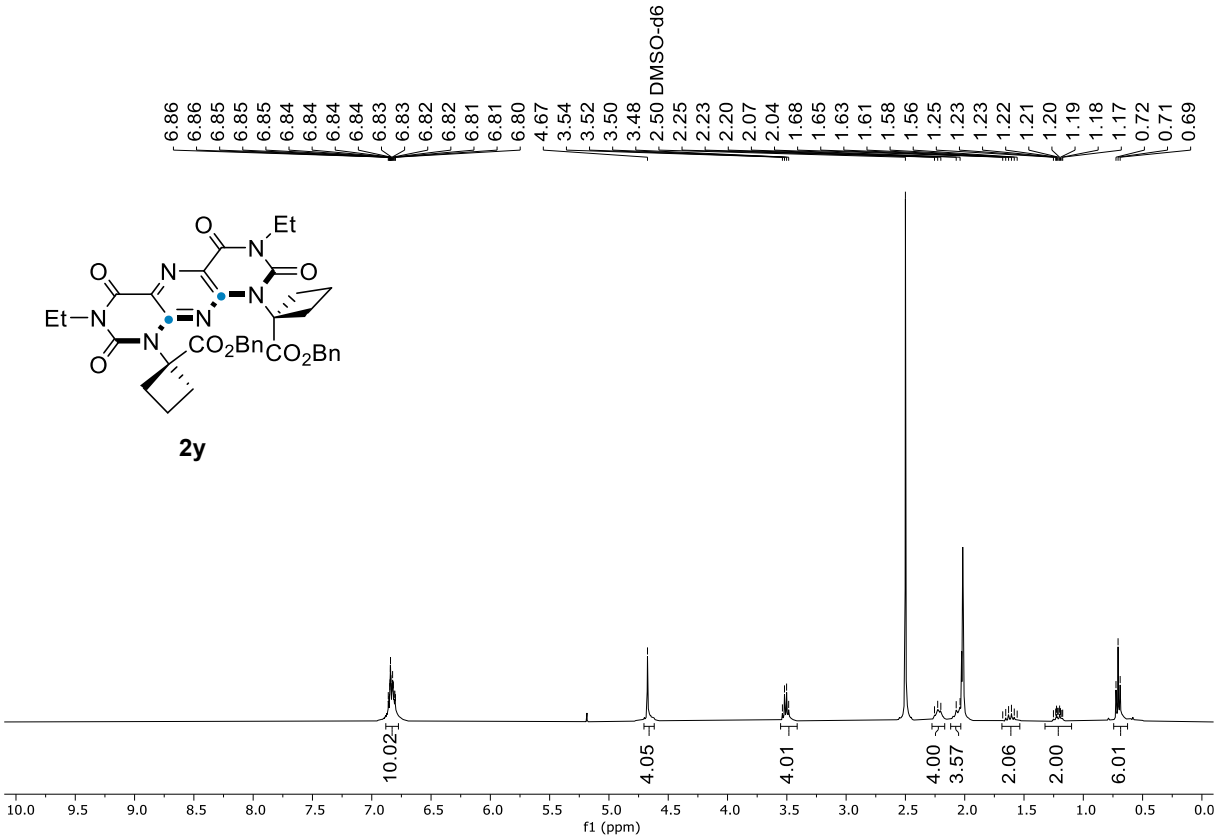
m.p. = 247-249 °C

^{1}H NMR (400 MHz, 373 K, DMSO-d_6 [2.50 ppm], ppm) δ = 7.35 – 7.25 (m, 10H), 5.15 (s, 4H), 3.98 (q, *J* = 7.0 Hz, 4H), 2.75 – 2.64 (m, 4H), 2.59 – 2.51 (m, 4H), 2.16 – 2.02 (m, 2H), 1.80 – 1.57 (m, 2H), 1.18 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, 373 K , DMSO-d_6 [39.5 ppm], ppm) δ = 170.7, 157.4, 148.0, 147.0, 135.0, 127.9, 127.6, 126.9, 66.2, 63.7, 36.2, 32.5, 15.2, 12.0.

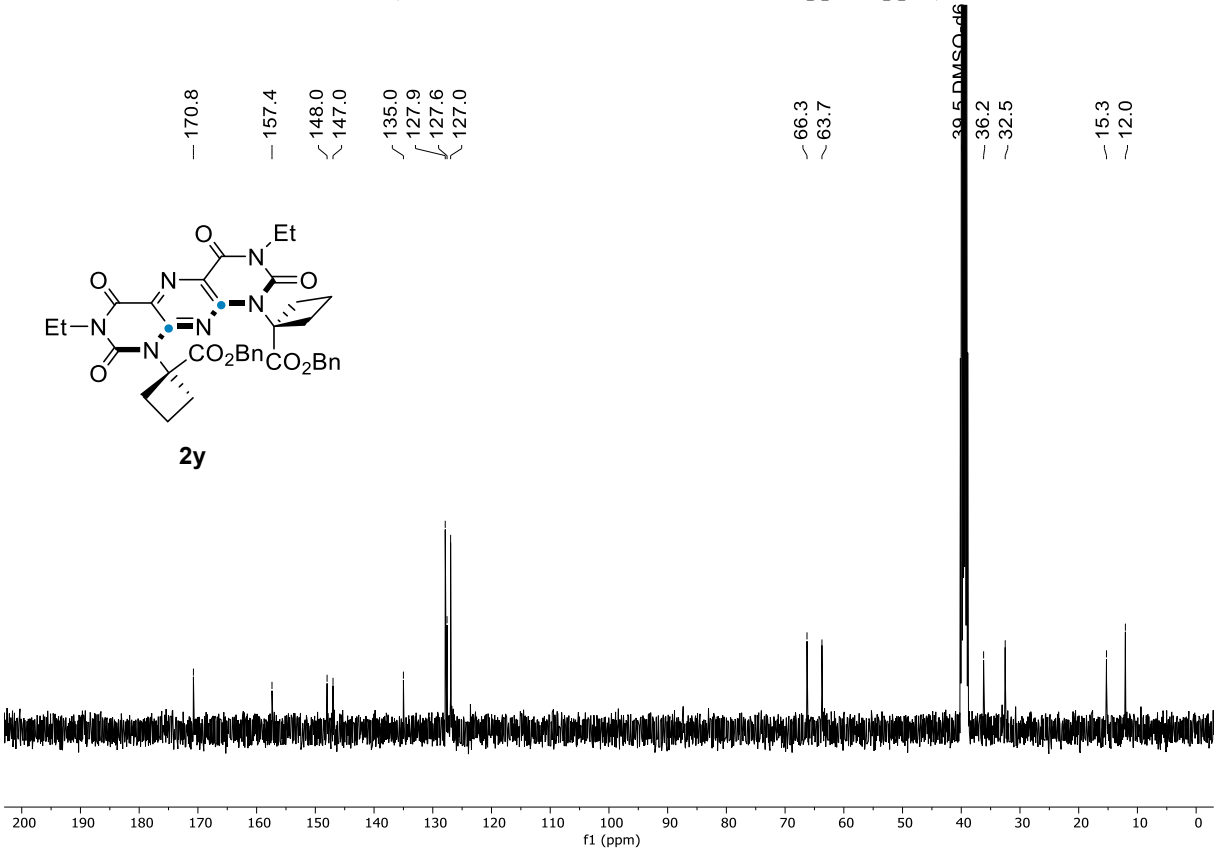
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{36}H_{36}N_6O_8Na]^+$: 703.2487; found: 703.2493 (Error PPM: 0.9).

IR (ATR, neat, $\tilde{\nu}$) = 2987 (w), 2961 (w), 2936 (w), 1727 (vs), 1692 (vs), 1678 (vs), 1548 (vs), 1499 (w), 1433 (vs), 1417 (s), 1395 (vs), 1374 (vs), 1331 (vs), 1321 (vs), 1303 (vs), 1268 (vs), 1232 (s), 1215 (vs), 1205 (vs), 1146 (vs), 1119 (vs), 1107 (vs), 1081 (s), 1064 (s), 1048 (s), 989 (s), 975 (s), 942 (m), 932 (m), 918 (m), 907 (w), 885 (w), 844 (w), 809 (s), 759 (vs), 742 (vs), 728 (vs), 716 (vs), 693 (vs), 620 (w), 597 (w), 577 (w), 553 (w), 502 (vs), 457 (s), 440 (m), 430 (m), 410 (s) cm^{-1}.



2y

^{1}H NMR (400 MHz, 373 K, DMSO-d_6 [2.50 ppm], ppm)

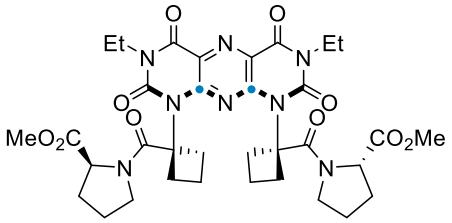


2y

^{13}C NMR (101 MHz, 373 K , DMSO-d_6 [39.5 ppm], ppm)

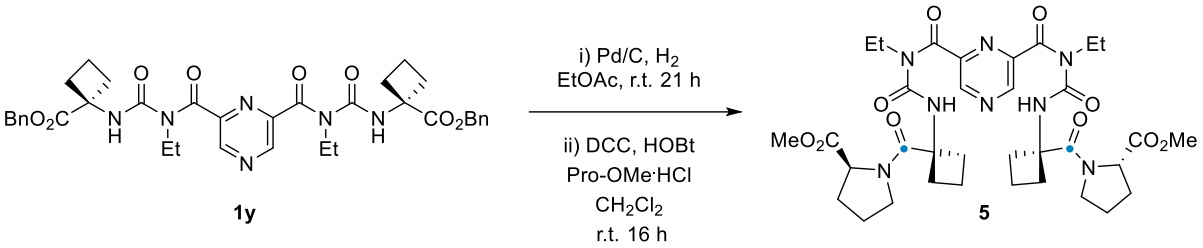
4.4 Synthesis of pyrimidopteridinetetraone derivatives

Synthesis of dimethyl (1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(cyclobutane-1,1-diyl-1-carbonyl))(*S*)-di-*L*-prolinate (6)



6

Synthesis from uncyclized precursor 1y:



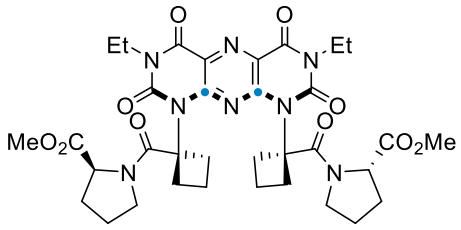
1y **5**

Pd/C (159 mg, 37.2 µmol, 0.05 equiv.) was added to a stirred suspension of benzyl ester **1y** (510 mg, 745 µmol, 1.00 equiv.) in EtOAc (8.7 mL) under ambient conditions. The atmosphere was then replaced by bubbling H_2 (balloon) though the solution for 10 min. Subsequently, the reaction mixture was stirred at ambient temperature under H_2 for 20 h. Afterwards, the mixture was filtered through a plug of Celite and washed with MeOH (3 × 10 mL). Evaporation of the solvent yields the corresponding crude carboxylic acid (375 mg, 743 µmol, quant.), which was used without further purification.

The obtained carboxylic acid (240 mg, 475.7 µmol, 1.00 equiv.) was dissolved in dichloromethane (1.00 mL). HOBt monohydrate (129 mg, 940.6 µmol, 1.98 equiv.) was added at 0 °C and the reaction was stirred for 5 min. Subsequently, DCC (223 mg, 1.08 mmol, 2.27 equiv.) was added and the reaction was further stirred at 0 °C for 30 min. Then Pro-OMe HCl (179 mg, 1.08 mmol, 2.27 equiv.) and TEA (182 mg, 1.80 mmol, 3.78 equiv.) were added, the reaction was allowed to warm to room temperature and stirred overnight. Afterwards, the solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate) to yield compound **5** (189.9 mg, 0.26 mmol, 55%) as an off-white solid.

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **5** (75.0 mg, 103 µmol, 1.00 equiv) at 0 °C with purification by column chromatography (pure ethyl acetate), afforded the title compound **6** (38.0 mg, 52.6 µmol, 51%) as an off white solid.

Characterization of dimethyl (1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(cyclobutane-1,1-diyl-1-carbonyl))(*S*)-di-*L*-prolinate (6)



6

m.p. = 209-214 °C

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.52 (s, 2H), 4.18 – 4.03 (m, 4H), 3.70 – 3.63 (m, 10H), 3.07 (s, 4H), 2.74 (s, 4H), 2.32 – 1.76 (m, 12H), 1.27 (t, *J* = 7.0 Hz, 6H).

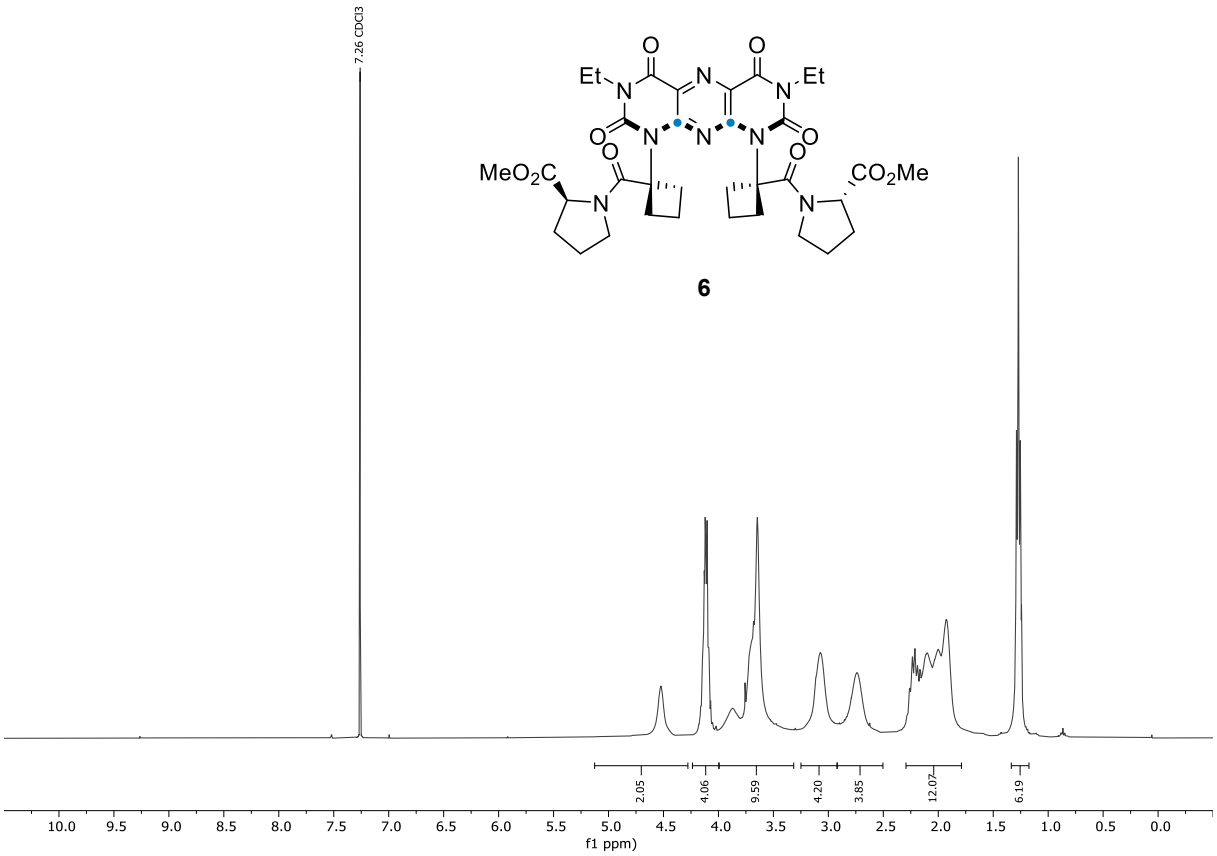
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 172.9, 169.7, 157.9, 149.3, 148.5, 124.5, 67.1, 61.7, 52.2, 46.6, 32.3, 28.0, 26.0, 15.8, 13.1.

HPLC (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI): tR = 20.2 min

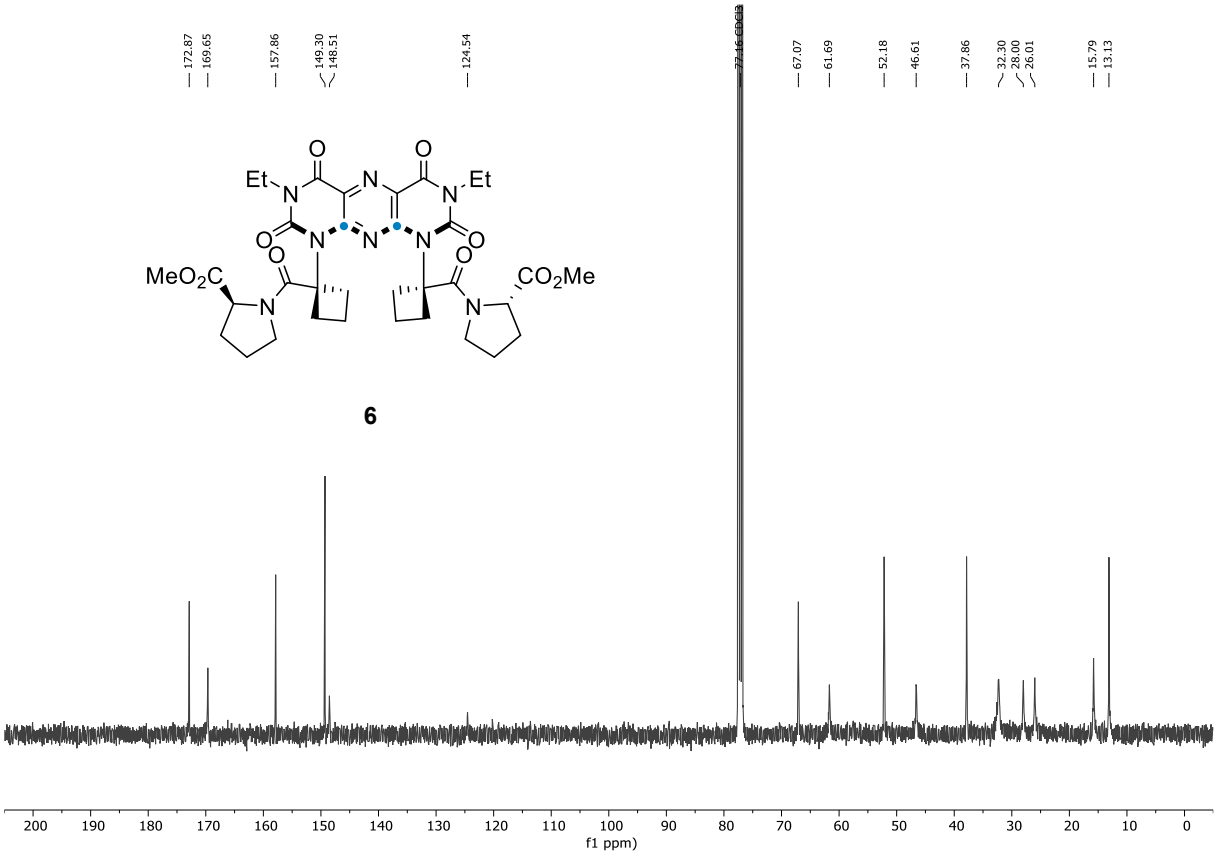
MS (ESI): *m/z* (%) = 745 (15, $[M+Na]^+$), 723 (100, $[M+H]^+$), 594 (89).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{34}H_{42}N_8O_{10}Na]^+$: 745.2916; found: 745.2906 (Error PPM: 1.3).

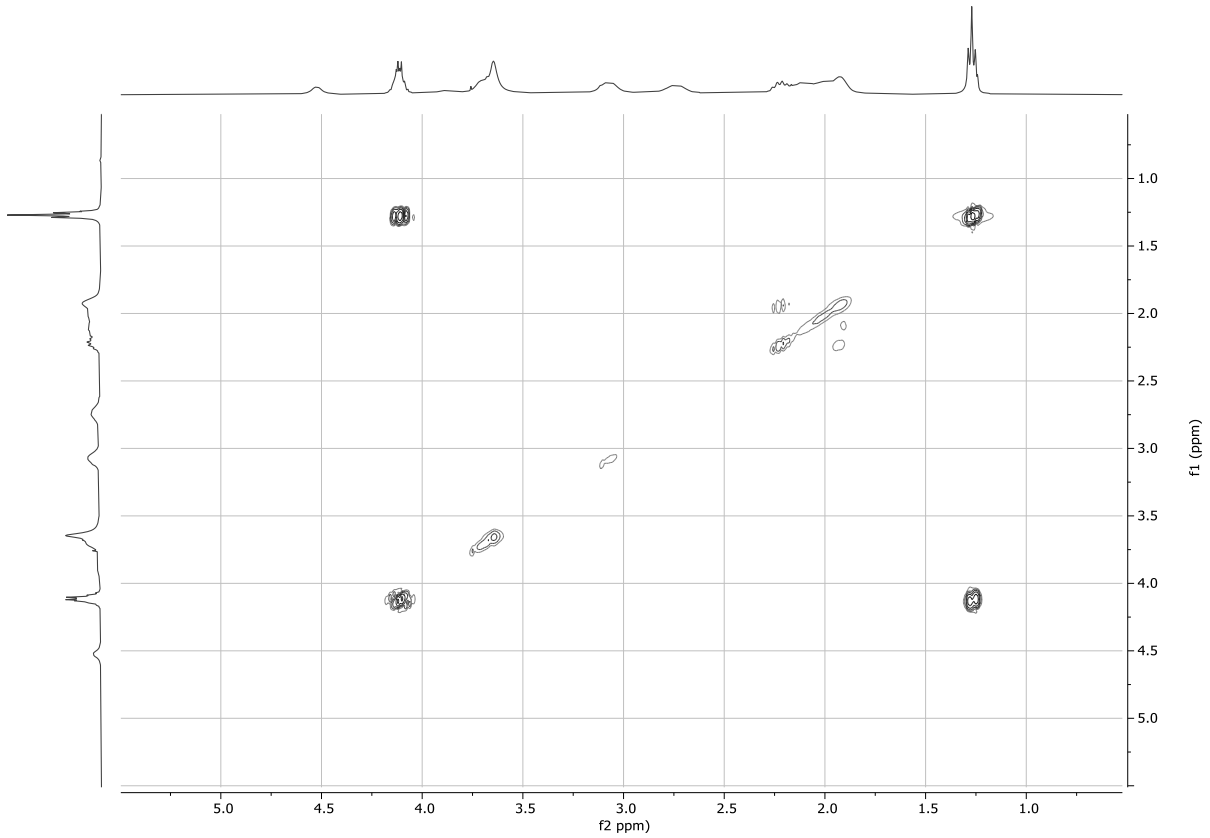
IR (ATR, neat, $\tilde{\nu}$) = 3328 (m), 2934 (w), 1715 (m), 1654 (vs), 1554 (vs), 1433 (s), 1399 (vs), 1378 (vs), 1334 (vs), 1272 (vs), 1240 (vs), 1166 (m), 1130 (m), 1077 (vs), 1048 (vs), 975 (m), 940 (m), 879 (m), 812 (s), 761 (s), 720 (s), 708 (s), 689 (s), 581 (vs), 557 (vs), 493 (vs) cm^{-1}.



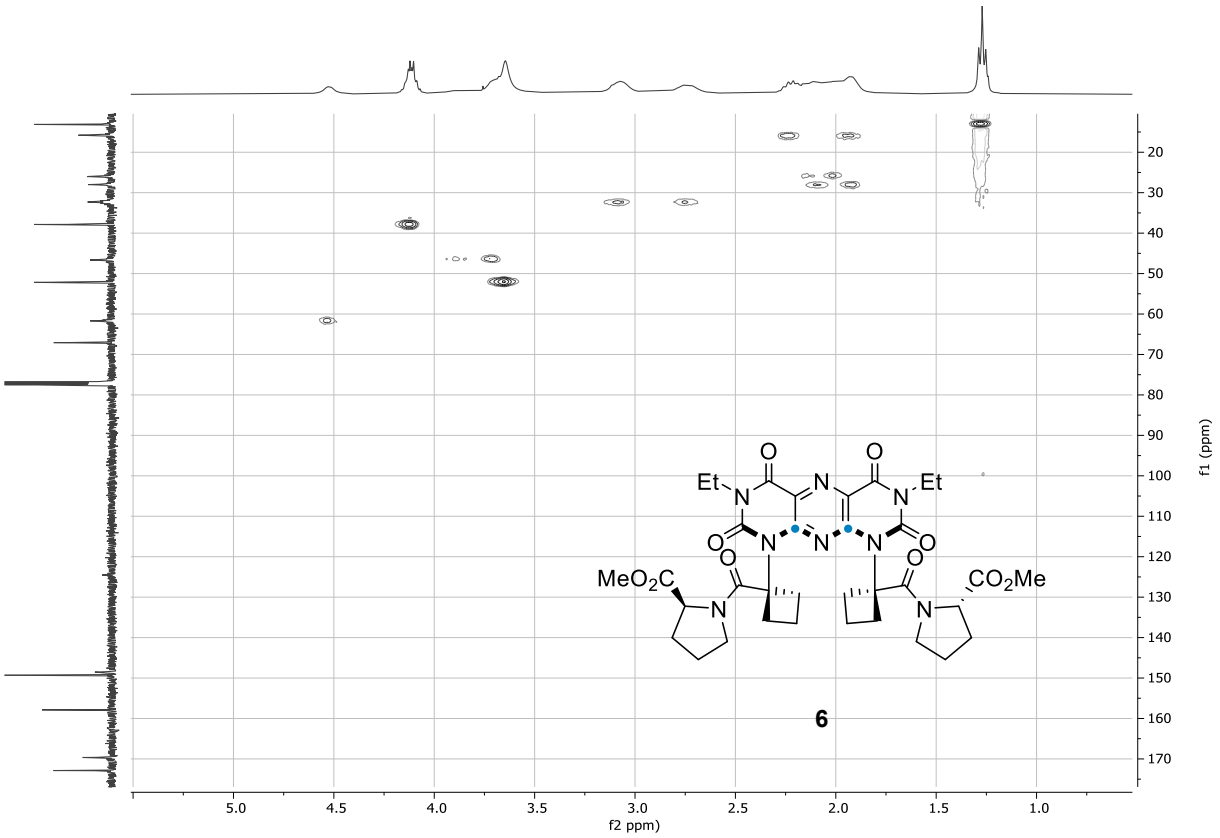
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



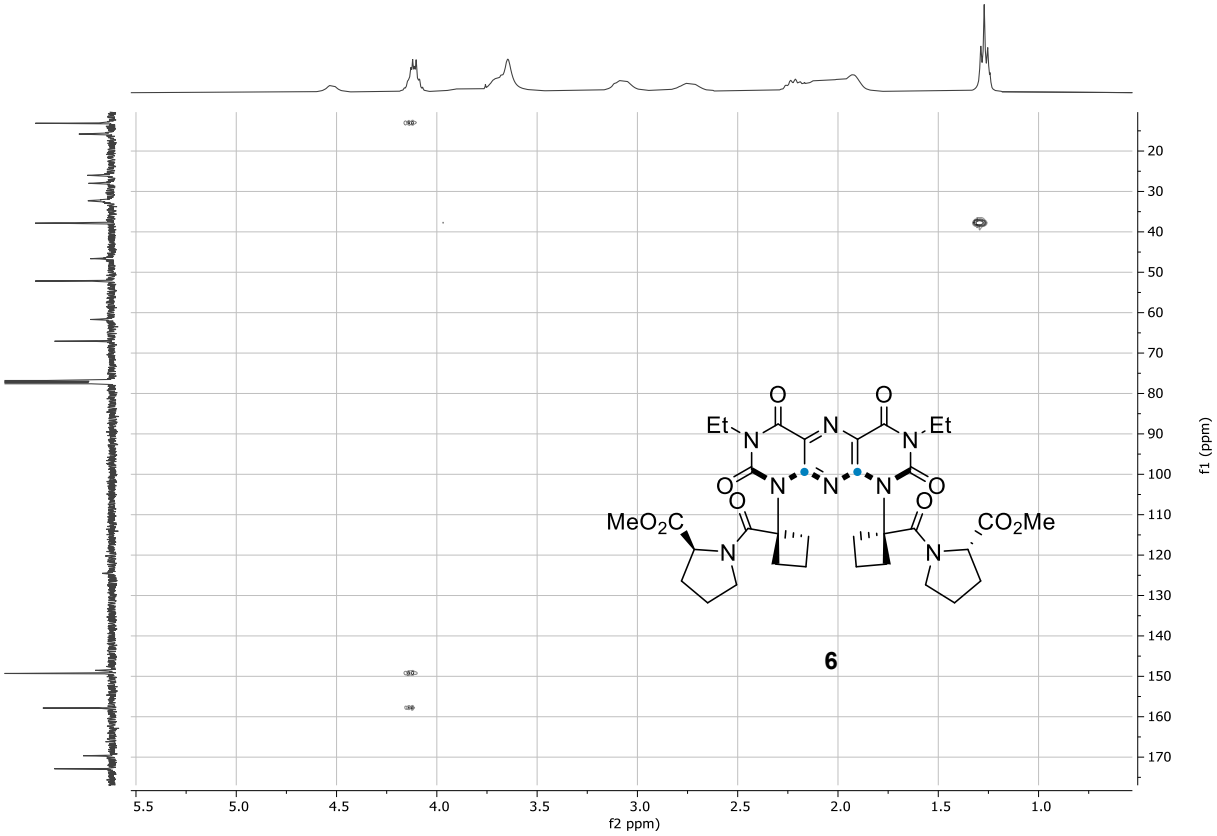
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)



^{1}H,^{1}H COSY-45 of compound **6**.



^{1}H,^{13}C HSQC of compound **6**.



^{1}H,^{13}C HMBC of compound **6**.

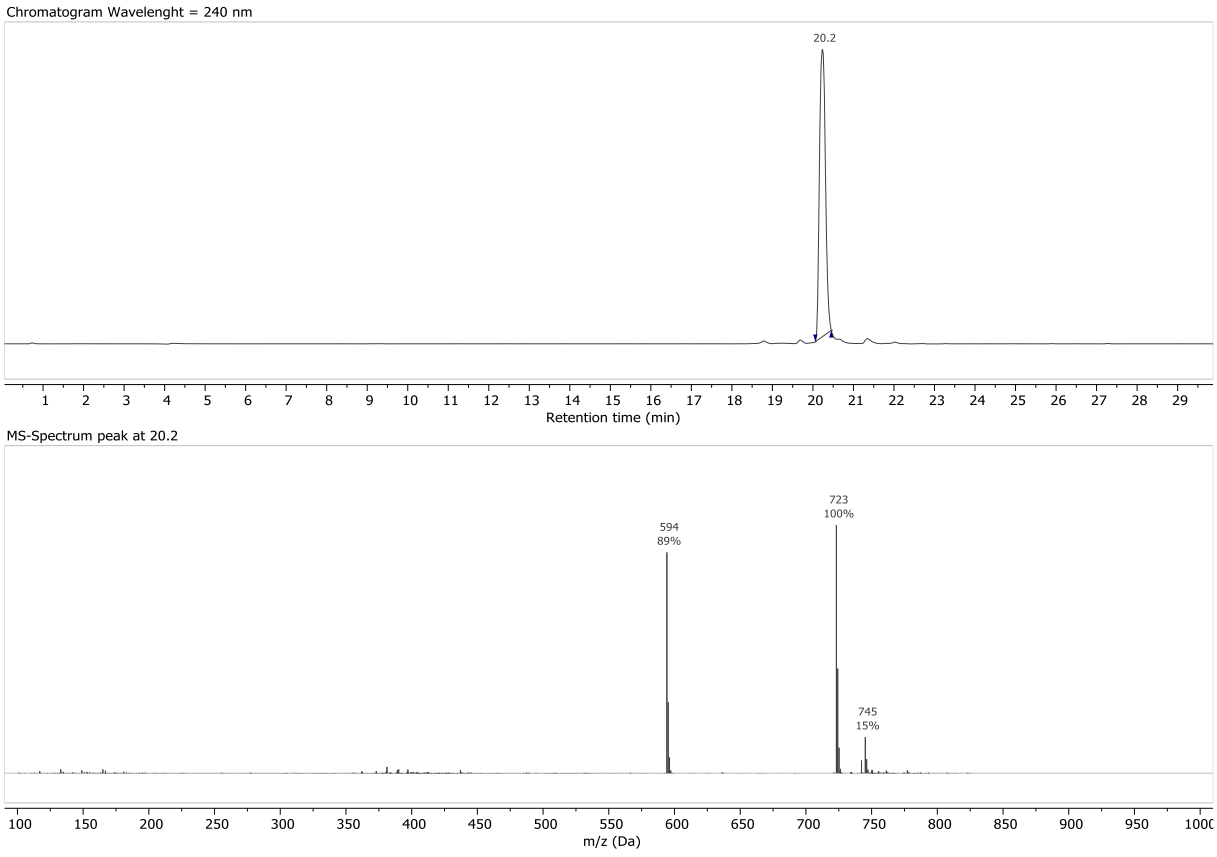
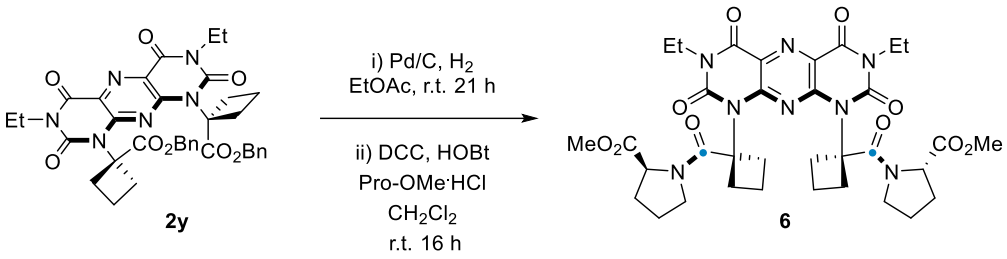


Figure S 4. Chromatogram and corresponding mass spectrum of isolated compound **6**; (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI).

Synthesis from PPT carboxylic acid 7:

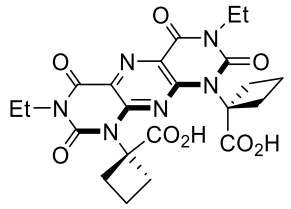


To a stirred suspension of PPT **2y** (298 mg, 435 µmol, 1.00 equiv)) in EtOAc (8.7 mL) under argon at rt Pd/C (5% Pd content, 92.6 mg, 43.5 µmol, 10 mol%) was added. The argon atmosphere was replaced by bubbling H_2 (balloon) through the solution for 10 min. The reaction mixture was then stirred at rt under H_2 for another 44 h. Afterwards, the H_2 atmosphere was replaced by argon and the flask was opened. The suspension was filtered through Celite and thoroughly washed with MeOH (3 × 10 mL, IMPORTANT: Keep Pd/C wetted at all times!). The filtrate was concentrated under reduced pressure to afford the title compound **7** (216 mg, 432 µmol, 99%) as a pale-yellow solid.

Carboxylic acid **7** (50.0 mg, 0.10 mmol, 1.00 equiv.), methyl (2*S*)-pyrrolidin-1-ium-2-carboxylate chloride (66.2 mg, 400 µmol, 3.97 equiv.) and HATU (155 mg, 407 µmol, 4.05 equiv) were added in a flask. The flask was cooled down to 0 °C using an ice bath. Subsequently DMF (2 mL) and triethylamine (38.5 mg, 380 µmol, 3.78 equiv.) was added. The reaction mixture was allowed to warm to ambient temperature and was stirred for 16 h. The reaction was monitored with HPLC-MS revealing a uncomplete conversion. An additional portion HATU (70.0 mg, 184 µmol, 1.83 equiv.) and *N*,*N*-diethylethanamine (38.4 mg, 379 µmol, 3.77 equiv.) was added to the flask and the reaction was stirred additional 24 h. The crude mixture was then concentrated *in vacuo* and directly purified by reversed-phase column chromatography (water:methanol = 95:5 to 20:80). Evaporation of the solvent furnished the title compound **8** (57.0 mg, 775 µmol, 77%) as an off-white solid.

The analytic date is in accordance with those reported vide supra.

Characterization of 1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(cyclobutane-1-carboxylic acid) (7) 267



7

R_f = 0.43 (methylene chloride:methanol= 1:1, UV)

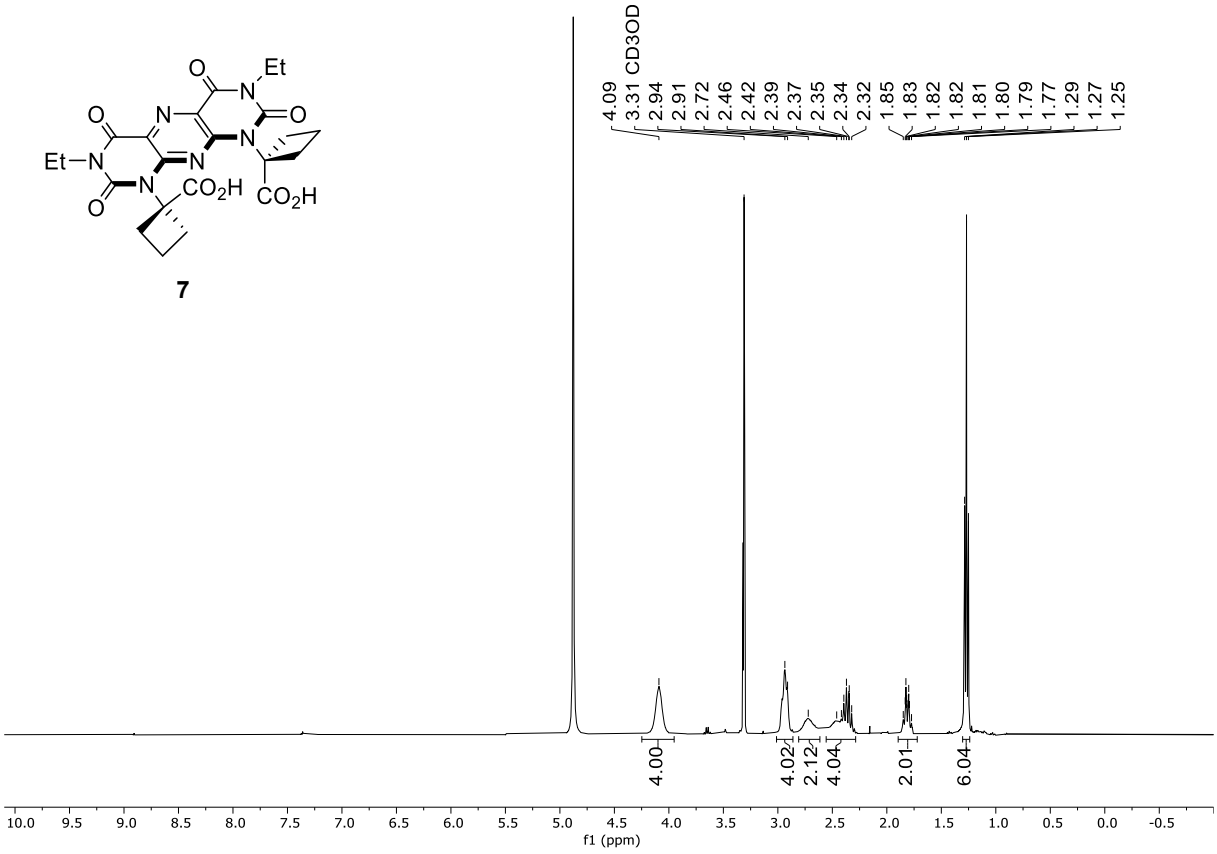
m.p. = degradation starting at 300 °C

^{1}H NMR (400 MHz, MeOD-d_4 [3.31 ppm], ppm) δ = 4.09 (s, 4H), 3.01 – 2.86 (m, 4H), 2.81 – 2.61 (m, 2H), 2.56 – 2.29 (m, 4H), 1.90 – 1.72 (m, 2H), 1.27 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (101 MHz, MeOD-d_4 [49.0 ppm], ppm) δ = 175.6, 160.4, 150.3, 149.6, 125.5, 65.9, 38.4, 35.3, 34.3, 17.1, 13.1.

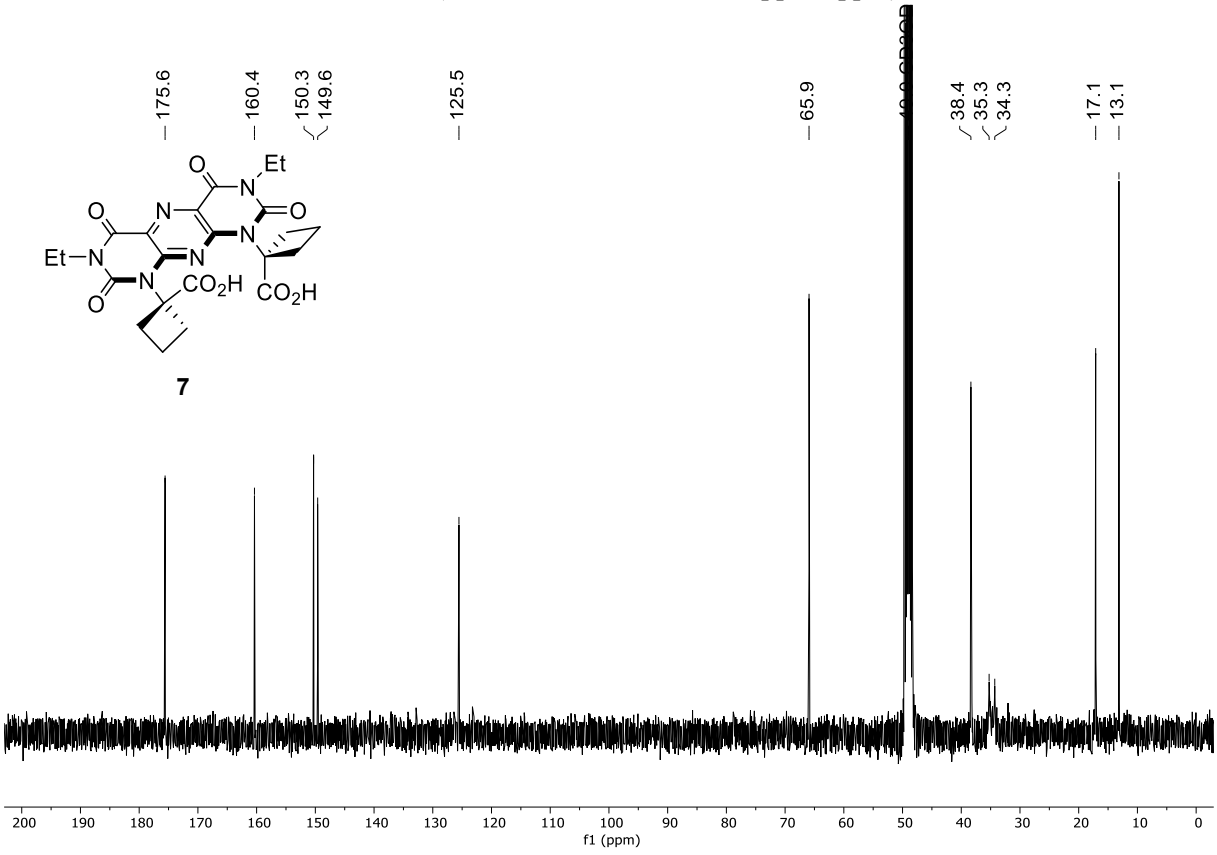
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{22}H_{24}N_6O_8Na]^+$: 523.1548; found: 523.1540 (Error PPM: -1.5).

IR (ATR, neat, ṽ) = 2971 (w), 1733 (m), 1682 (vs), 1548 (vs), 1435 (s), 1393 (vs), 1374 (vs), 1336 (vs), 1268 (vs), 1250 (s), 1225 (vs), 1158 (s), 1126 (m), 1079 (m), 1064 (m), 1048 (m), 1003 (w), 975 (w), 936 (w), 897 (w), 848 (w), 812 (m), 799 (w), 789 (w), 756 (vs), 714 (s), 708 (s), 687 (w), 630 (m), 577 (w), 555 (w), 502 (vs), 477 (m), 459 (m), 424 (m), 402 (m) cm^{-1}.



7

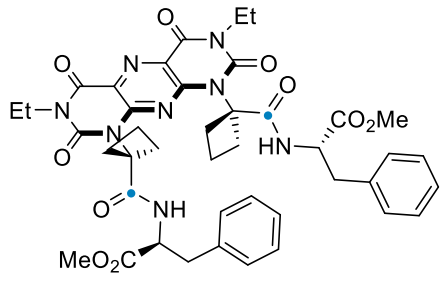
^{1}H NMR (400 MHz, MeOD-d_4 [3.31 ppm], ppm)



7

^{13}C NMR (101 MHz, MeOD-d_4 [49.0 ppm], ppm)

Synthesis of dimethyl 2,2'-((1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(cyclobutane-1,1-diyl-1-carbonyl))bis(azanediyl))(2*S*,2'*S*)-bis(3-phenylpropanoate) (8)



8

Carboxylic acid **7** (50 mg, 100.0 µmol, 1.00 equiv), methyl (*S*)-phenylalanate hydrochloride (47.4 mg, 219.8 µmol, 2.20 equiv) and HATU (82 mg, 215.7 µmol, 2.16 equiv) were dissolved in DMF (2.00 mL). Subsequently, TEA (30.3 mg, 299.7 µmol, 3.00 equiv) was added at 0°C and the reaction mixture was stirred overnight. Afterwards, the solvent was evaporated and the residue was purified by reversed-phase column chromatography (water:methanol = 95:5 to 0:100) to yield the title compound (65 mg, 79 mmol, 79%) as a colorless solid.

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.50 – 6.78 (m, 12H), 4.87 (dt, *J* = 7.8, 6.2 Hz, 2H), 4.25 – 3.92 (m, 4H), 3.87 – 3.53 (m, 6H), 3.34 – 2.87 (m, 6H), 2.85 – 2.44 (m, 3H), 2.12 – 1.59 (m, 7H), 1.28 (t, *J* = 7.0 Hz, 6H).

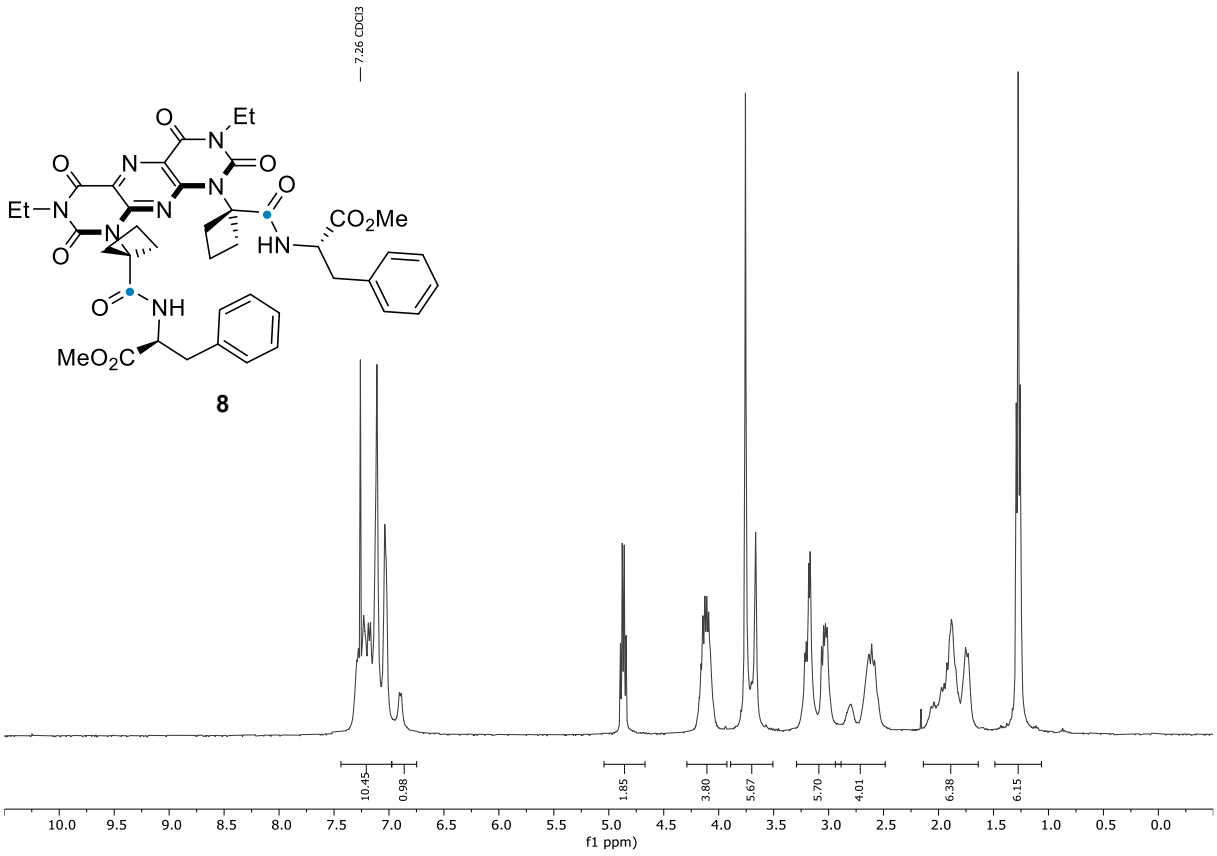
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 172.3, 170.6, 157.7, 149.4, 147.8, 136.1, 129.2, 128.5, 127.2, 124.4, 65.7, 53.6, 52.6, 37.9, 34.8, 31.6, 16.2, 13.1.

HPLC (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI)**:** tR = 25.2 min

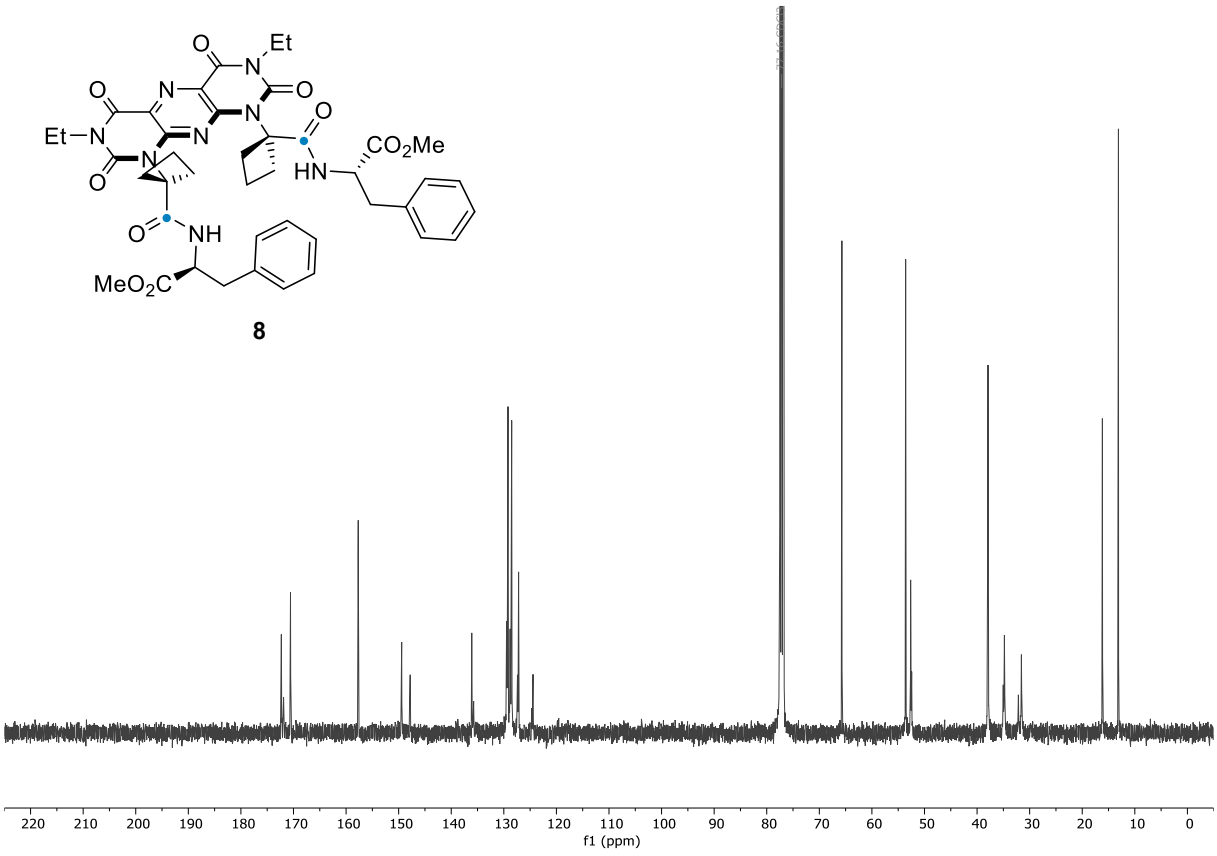
MS (ESI): *m/z* (%) = 823 (100, $[M+H]^+$).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{42}H_{46}N_8O_{10}+Na]^+$: 823.3410; found: 823.3426 (Error PPM: 1.9).

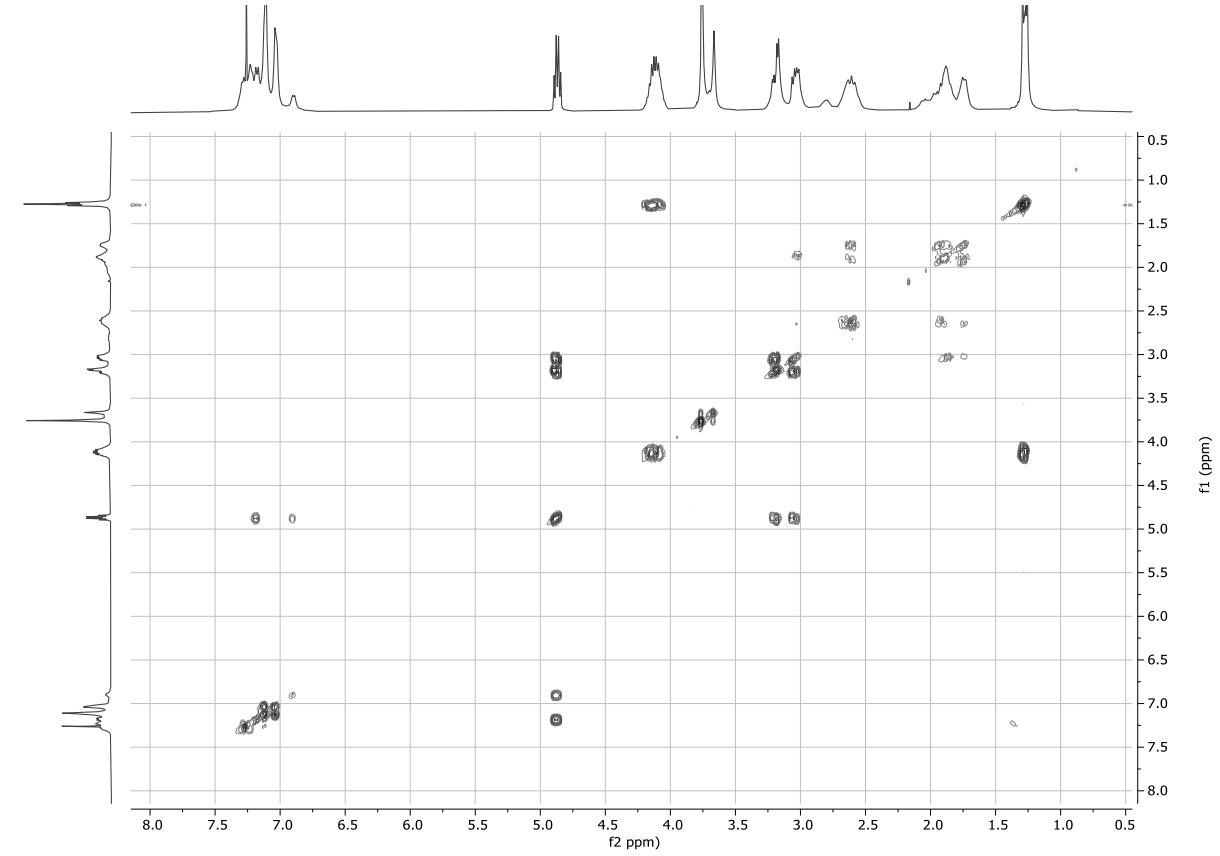
IR (ATR, neat, ṽ) = 3350 (w), 2948 (w), 1719 (s), 1670 (vs), 1552 (vs), 1519 (vs), 1433 (vs), 1395 (vs), 1376 (vs), 1334 (vs), 1266 (vs), 1236 (vs), 1179 (s), 1164 (s), 1132 (s), 1030 (vs), 973 (s), 942 (s), 899 (m), 879 (m), 812 (s), 791 (m), 759 (vs), 748 (s), 720 (s), 701 (vs), 673 (s), 659 (s), 622 (s), 599 (s), 591 (s), 579 (s), 567 (s), 555 (vs), 551 (vs), 538 (vs), 516 (vs), 491 (vs), 463 (vs), 455 (vs), 434 (vs), 420 (vs) cm^{-1}.



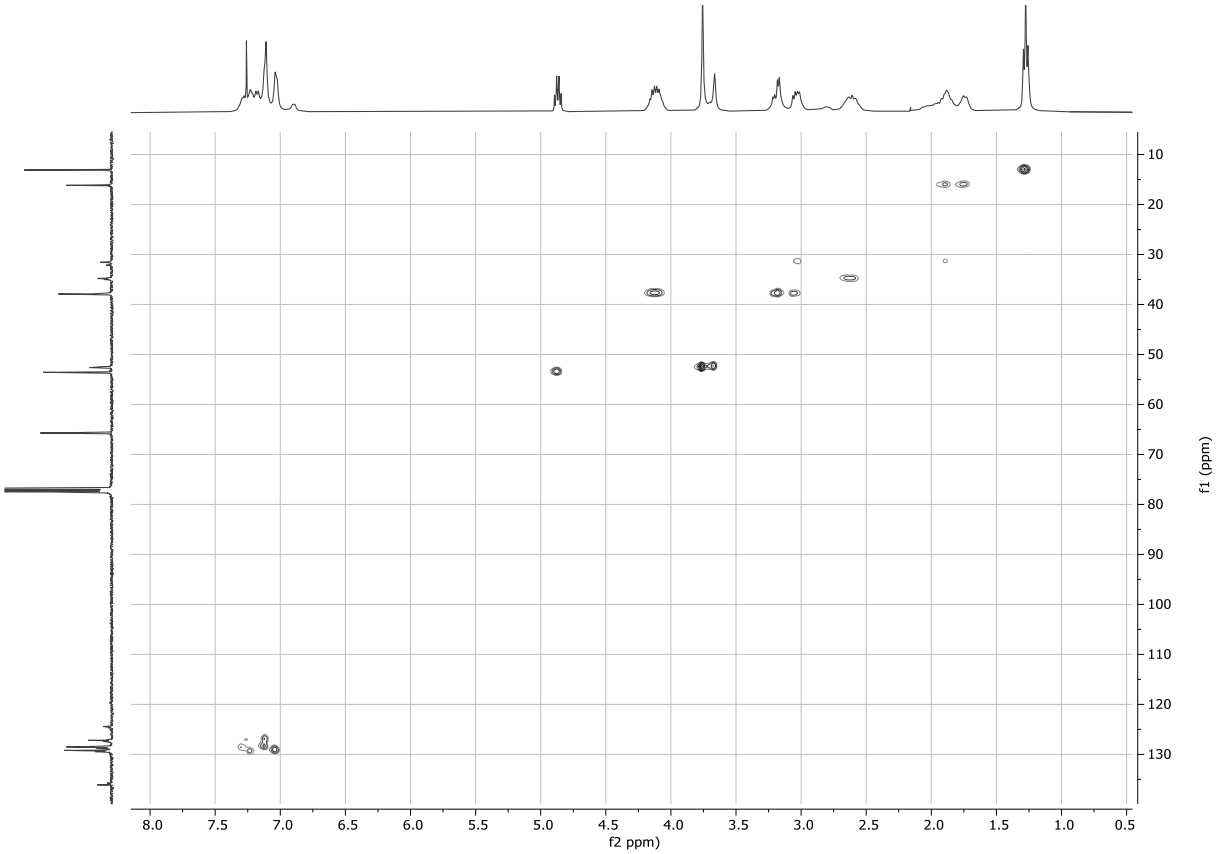
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



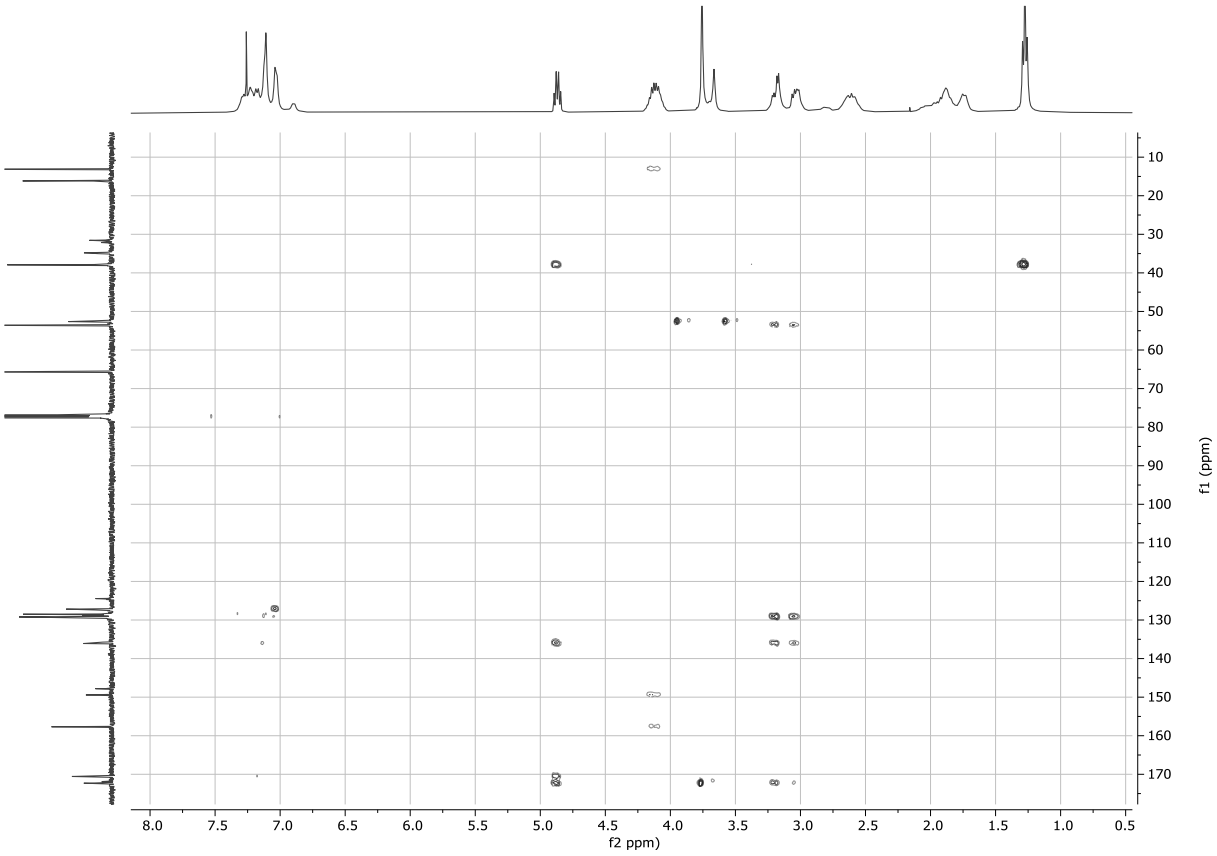
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)



^{1}H,^{1}H COSY-45 of compound **8**.



^{1}H,^{13}C HSQC of compound **8**.



^{1}H,^{13}C HMBC of compound **8**.

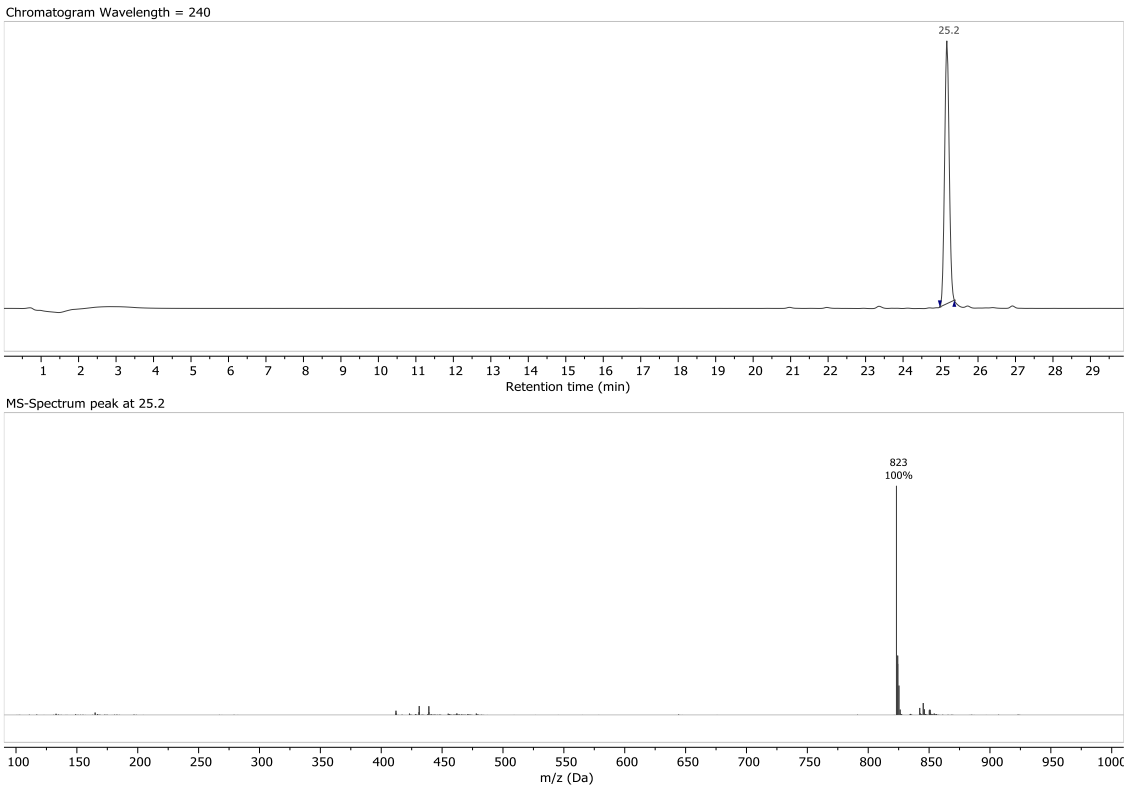
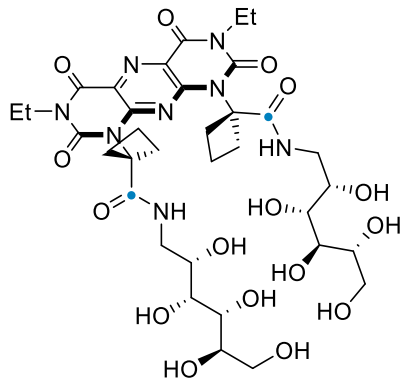


Figure S 5. Chromatogram and corresponding mass spectrum of isolated compound **8**; (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI).

Synthesis of 1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(N-((2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl)cyclobutane-1-carboxamide) (9)



9

Acid **7** (50.1 mg, 100 µmol, 1.00 equiv), glucamine (54.4 mg, 300 µmol, 3.00 equiv), 1-hydroxybenzotriazole;hydrate (38.3 mg, 250 µmol, 2.50 equiv) and EDC HCl (47.9 mg, 250 µmol, 2.50 equiv) were added in a flask. The flask wa s cooled down to 0 °C using an ice bath an DMF (2 mL) was added. After 5 min the reaction mixture was allowed to warm to ambient temperature and was stirred for 16 h. The crude mixture was then concentrated *in vacuo* and direct purification by reversed-phase column chromatography (water:methanol = 95:5 to 50:50) yielded the title compound **9** (47.0 mg, 56.8 µmol, 57% yield) as an off white solid.

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 4.11 (m, 4H), 3.84 – 3.76 (m, 2H), 3.75 – 3.68 (m, 4H), 3.67 – 3.54 (m, 7H), 3.43 – 3.36 (m, 1H), 3.30 – 3.23 (m, 1H), 3.15 – 3.02 (m, 1H), 2.96 – 2.78 (m, 4H), 2.74 – 2.55 (m, 2H), 2.47 – 2.17 (m, 4H), 1.86 – 1.73 (m, 2H), 1.30 (t, *J* = 7.1 Hz, 6H).

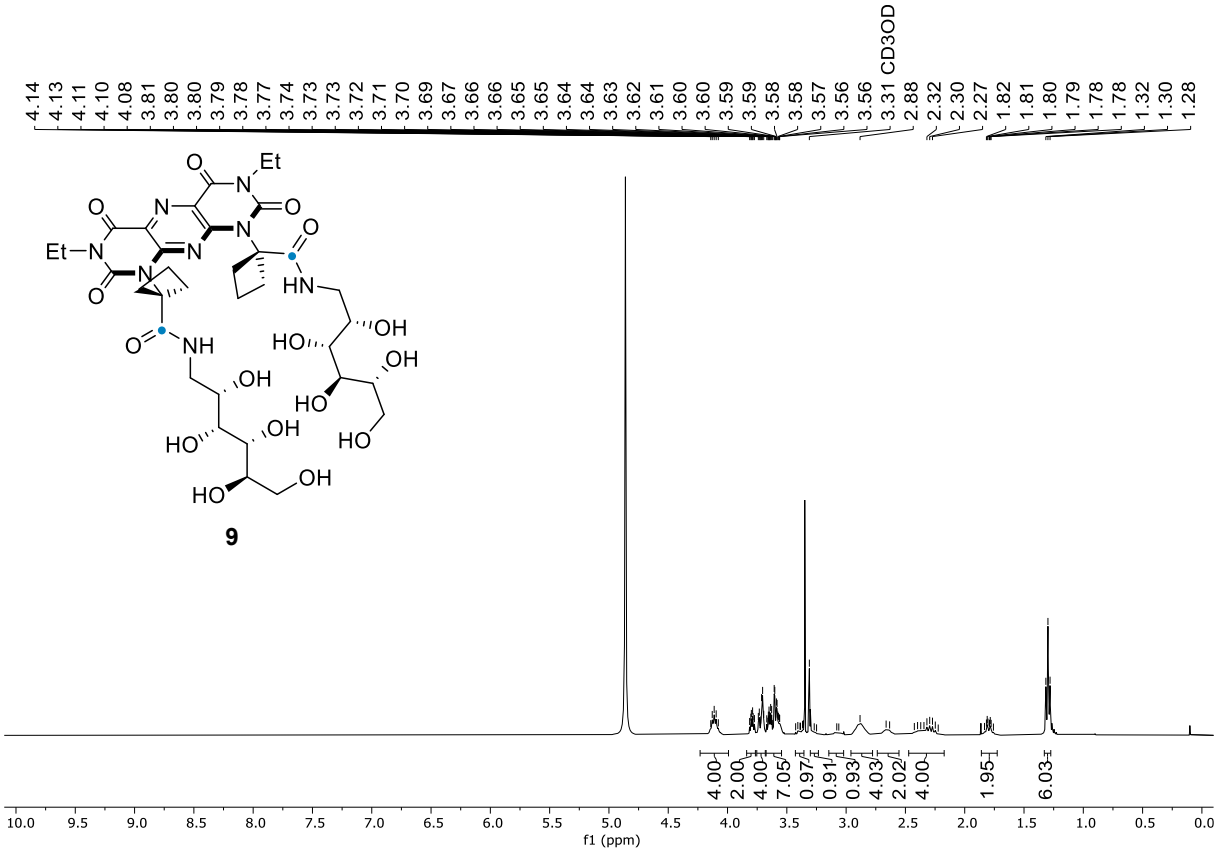
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.6, 160.5, 150.5, 149.6, 126.1, 73.7, 73.0, 71.4, 67.3, 64.6, 44.0, 38.6, 35.9, 34.6, 17.1, 13.2.

HPLC (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI): tR = 9.3

MS (ESI): *m/z* (%) = 849 (69, $[M+Na]^+$), 827 (31, $[M+H]^+$), 441 (100), 433 (38).

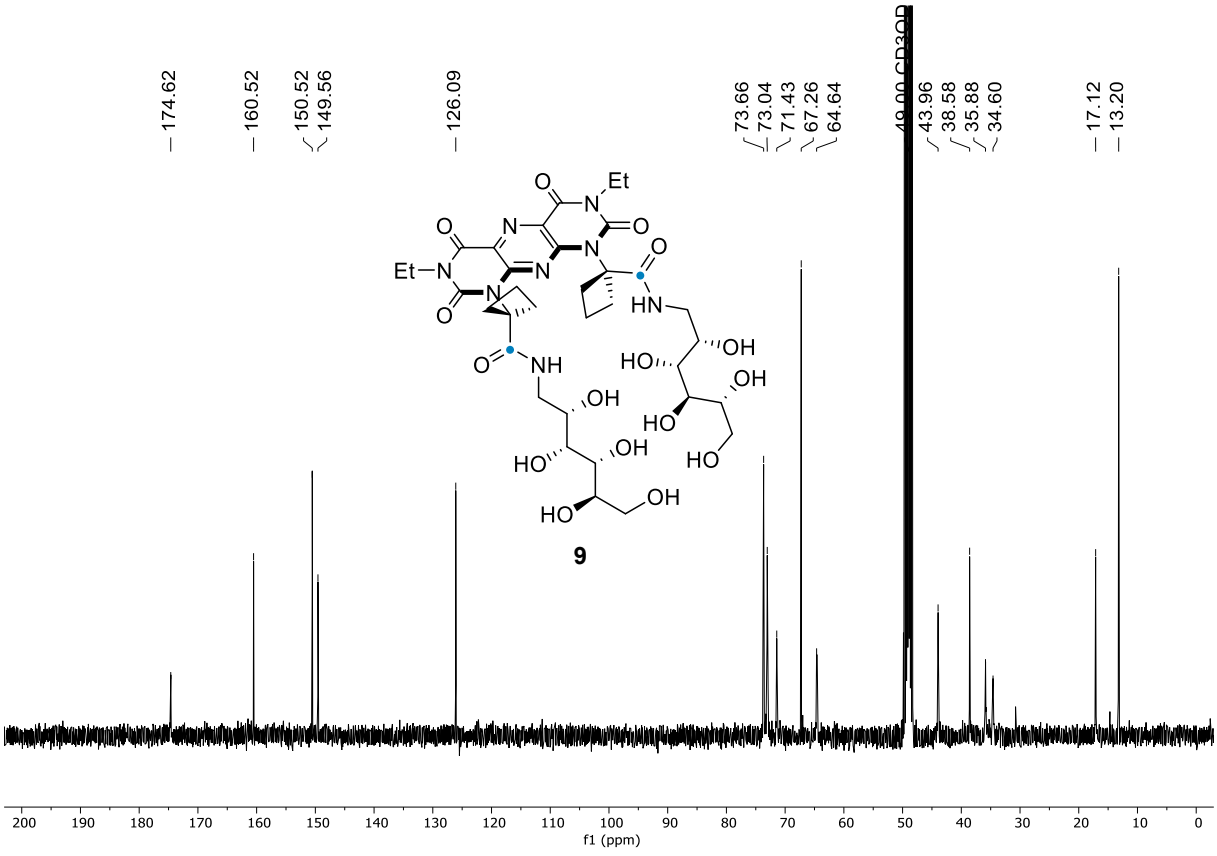
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{34}H_{50}N_8O_{16}Na]^+$: 849.3237; found: 849.3237 (Error PPM: 0.0).

IR (ATR, neat, ṽ) = 3328 (m), 2934 (w), 1715 (m), 1654 (vs), 1554 (vs), 1433 (s), 1399 (vs), 1378 (vs), 1334 (vs), 1272 (vs), 1240 (vs), 1166 (m), 1130 (m), 1077 (vs), 1048 (vs), 975 (m), 940 (m), 879 (m), 812 (s), 761 (s), 720 (s), 708 (s), 689 (s), 581 (vs), 557 (vs), 493 (vs) cm^{-1}.



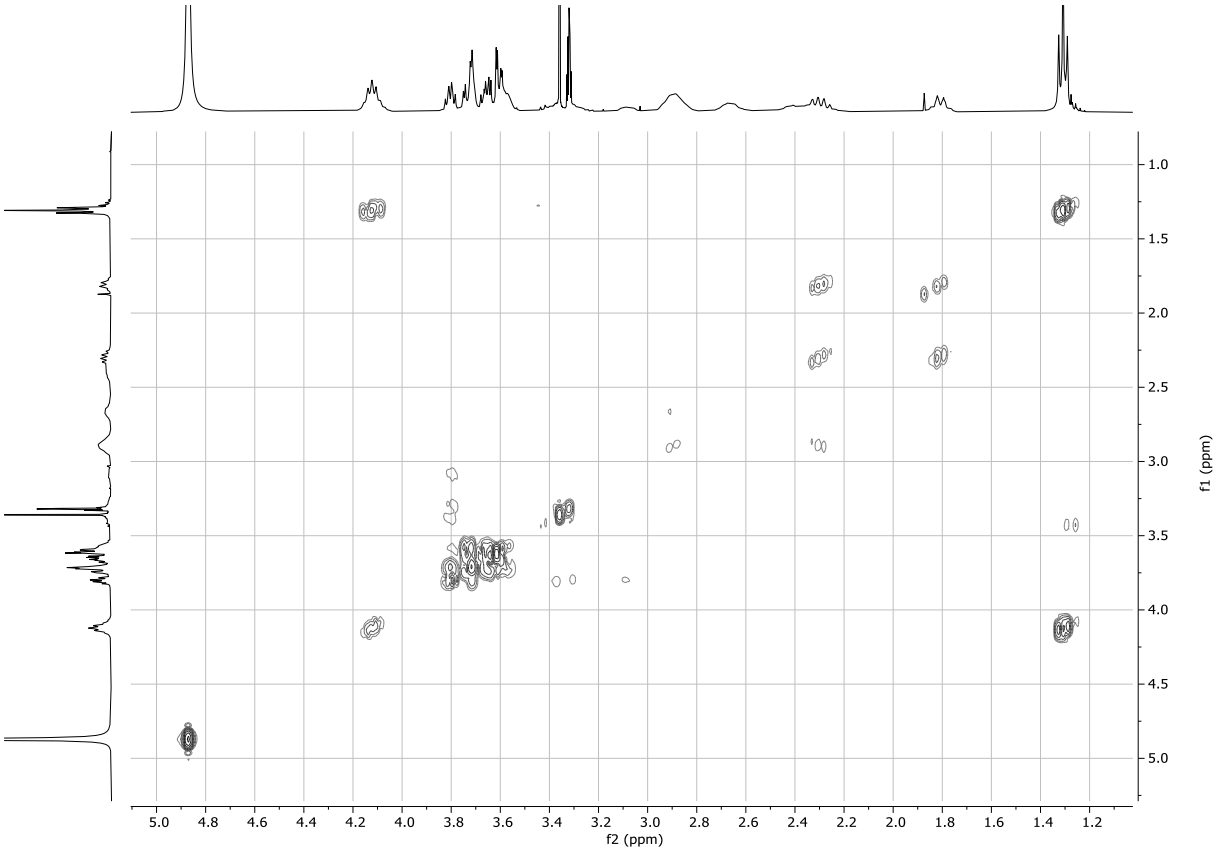
9

^{1}H NMR (400 MHz, MeOD [3.31 ppm], ppm)

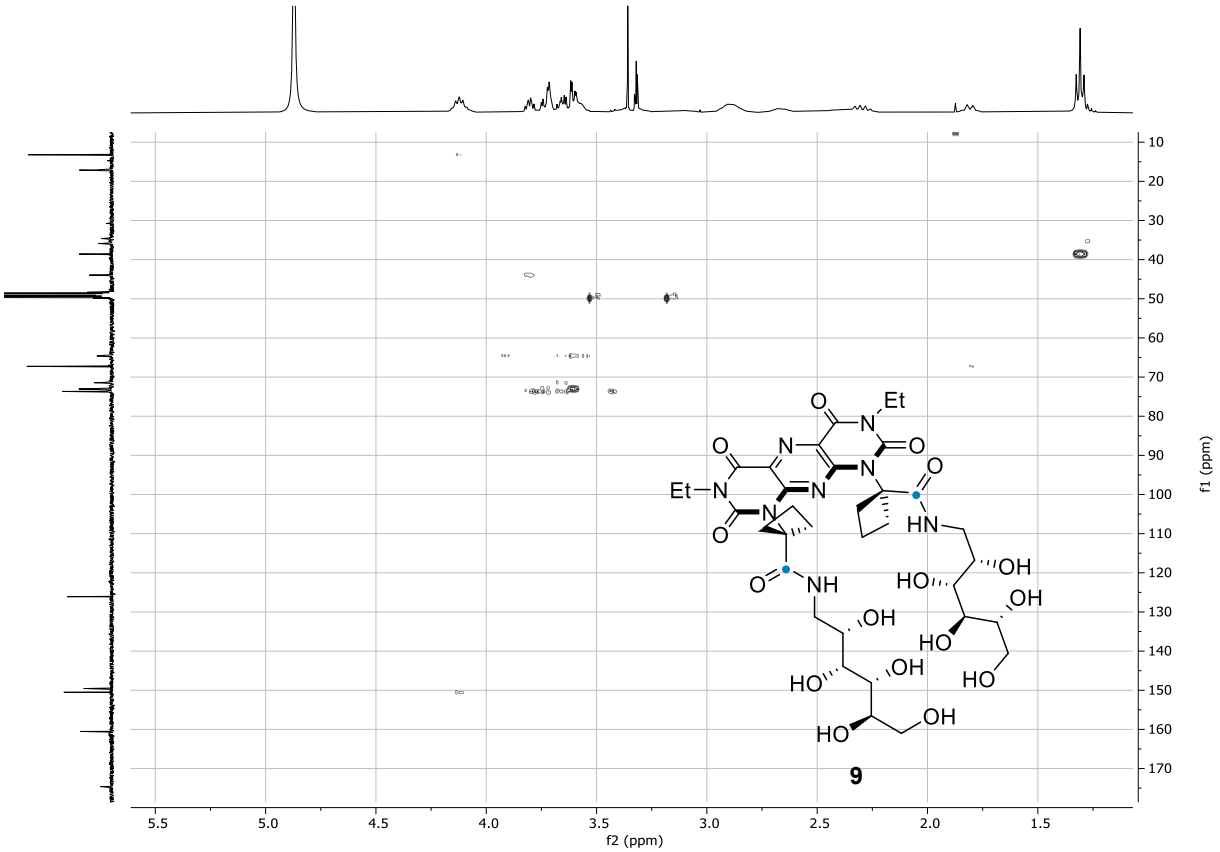


9

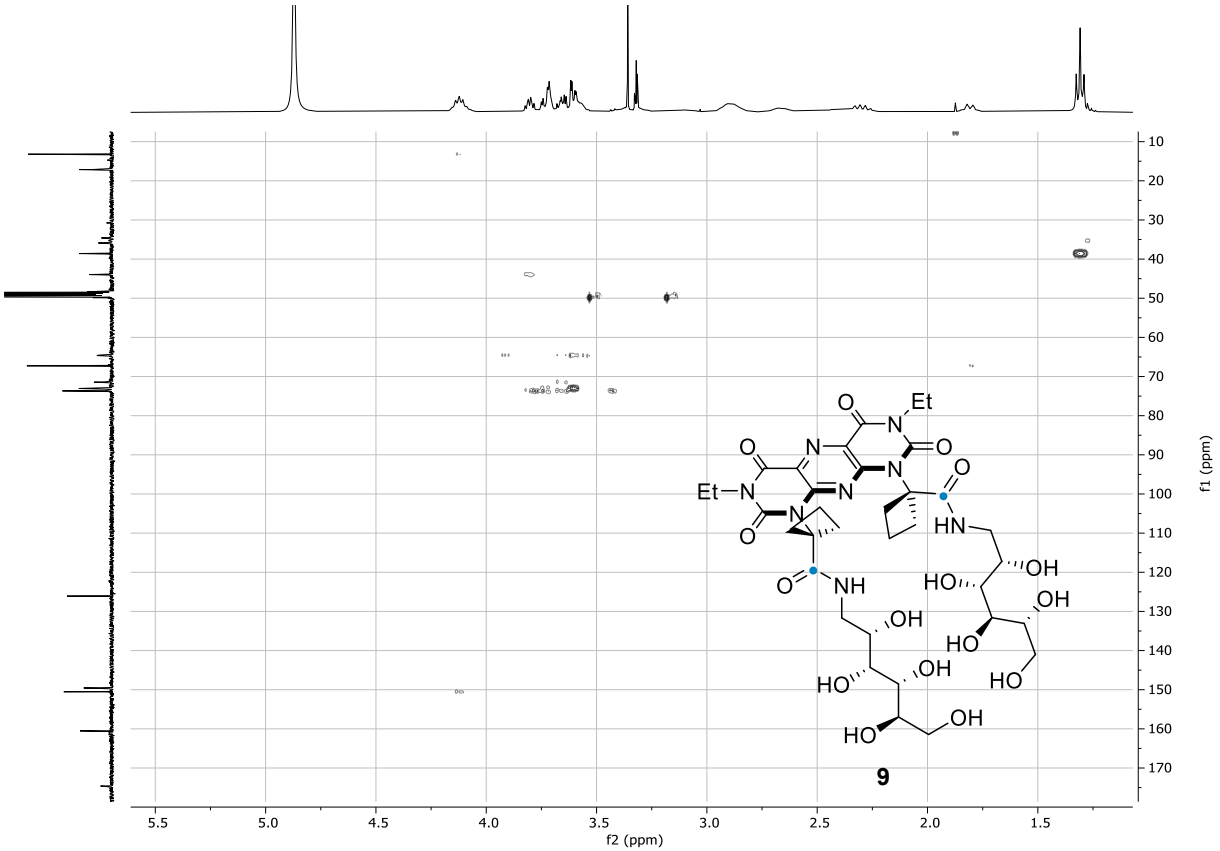
^{13}C NMR (101 MHz, MeOD [49.0 ppm], ppm)



^{1}H,^{1}H COSY-45



^{1}H,^{13}C HSQC



^{1}H,^{13}C HMBC

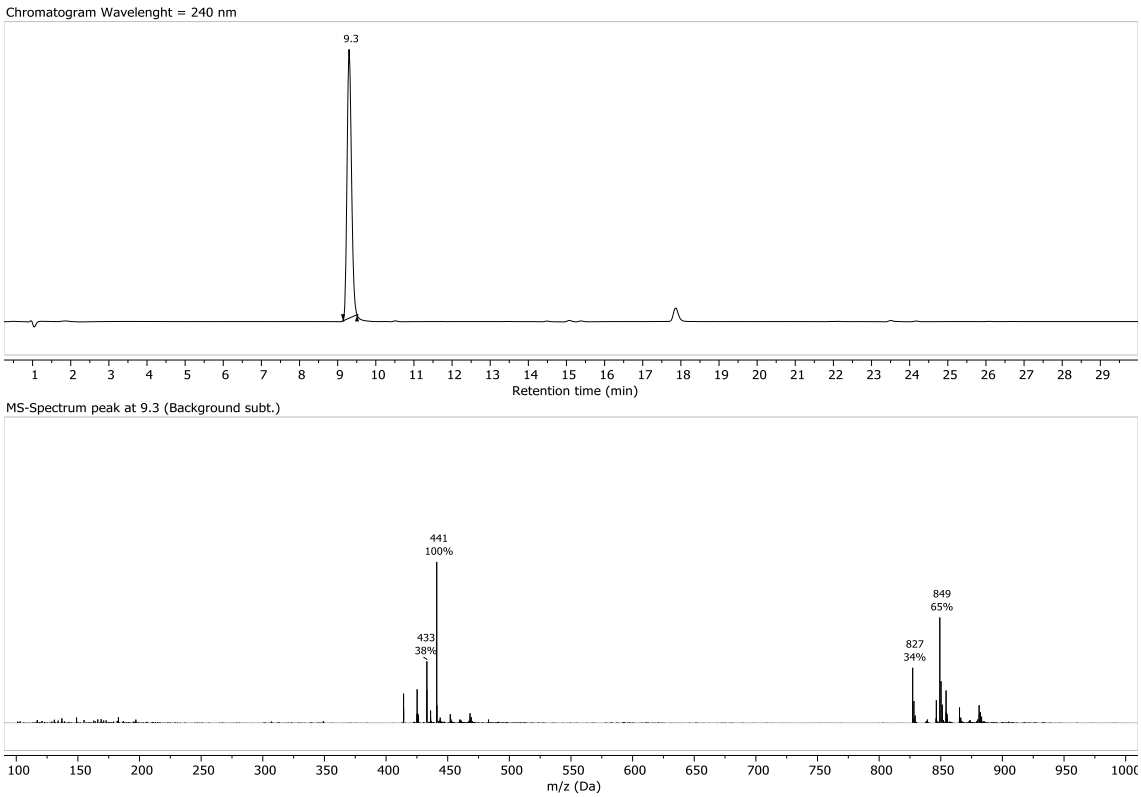
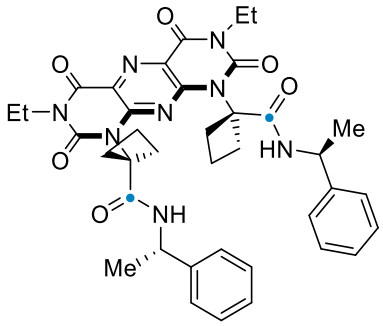


Figure S 6. Chromatogram and corresponding mass spectrum of isolated compound **9**; (Agilent, SB-C18, Water (0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI).

Synthesis of 1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(*N*-((*S*)-1-phenylethyl)cyclobutane-1-carboxamide) (10)



10

Carboxylic acid **7** (50.1 mg, 100 µmol, 1.00 equiv.), (1*S*)-1-phenylethanamine (36.4 mg, 300 µmol, 3.00 equiv.) HOBt (38.3 mg, 250 µmol, 2.50 equiv.) and EDC HCl (47.9 mg, 250 µmol, 2.50 equiv) were added in a flask. The flask was cooled to 0 °C using an ice bath and DMF (2.0 mL) was added. After stirring for 5 min, the reaction mixture was allowed to warm to ambient temperature and was stirred for 16 h. The crude mixture was then concentrated *in vacuo* and directly purified by reversed-phase column chromatography (H_2O:MeOH = 95:5 → 20:80). The collected fractions were concentrated *in vacuo* (around 10 ml of water left). The water phase was extracted with EtOAc (3 x 10 mL), dried over Na_2SO_4 and concentrated *in vacuo* yielding the title compound **10** (34.7 mg, 49.1 µmol, 49% yield) as an off-white solid.

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.45 – 6.99 (m, 10H), 6.94 – 6.79 (m, 2H), 5.11 (dq, *J* = 7.3, 7.1 Hz, 2H), 4.13 – 3.88 (m, 4H), 3.29 – 2.84 (m, 4H), 2.78 – 2.57 (m, 2H), 2.35 – 1.96 (m, 4H), 1.73 (d, *J* = 10.7 Hz, 2H), 1.59 – 1.35 (m, 6H), 1.29 – 1.21 (m, 6H).

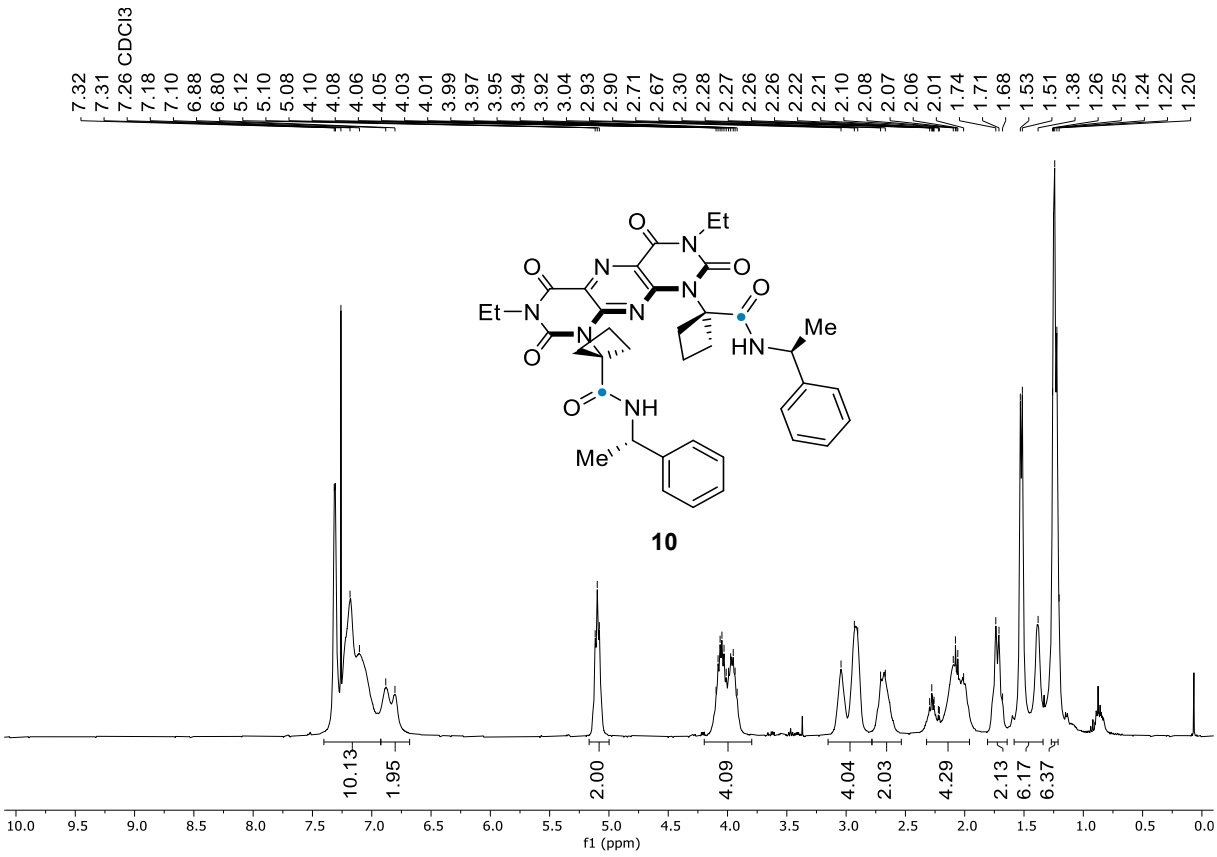
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 170.6, 170.4, 158.4, 149.4, 148.0, 128.8, 128.4, 127.2, 126.4, 126.1, 124.3, 66.2, 66.0, 49.6, 37.4, 35.1, 32.4, 22.2, 21.6, 16.6, 13.3.

HPLC (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI): tR = 24.5

MS (ESI): *m/z* (%) = 707 (100, $[M+H]^+$).

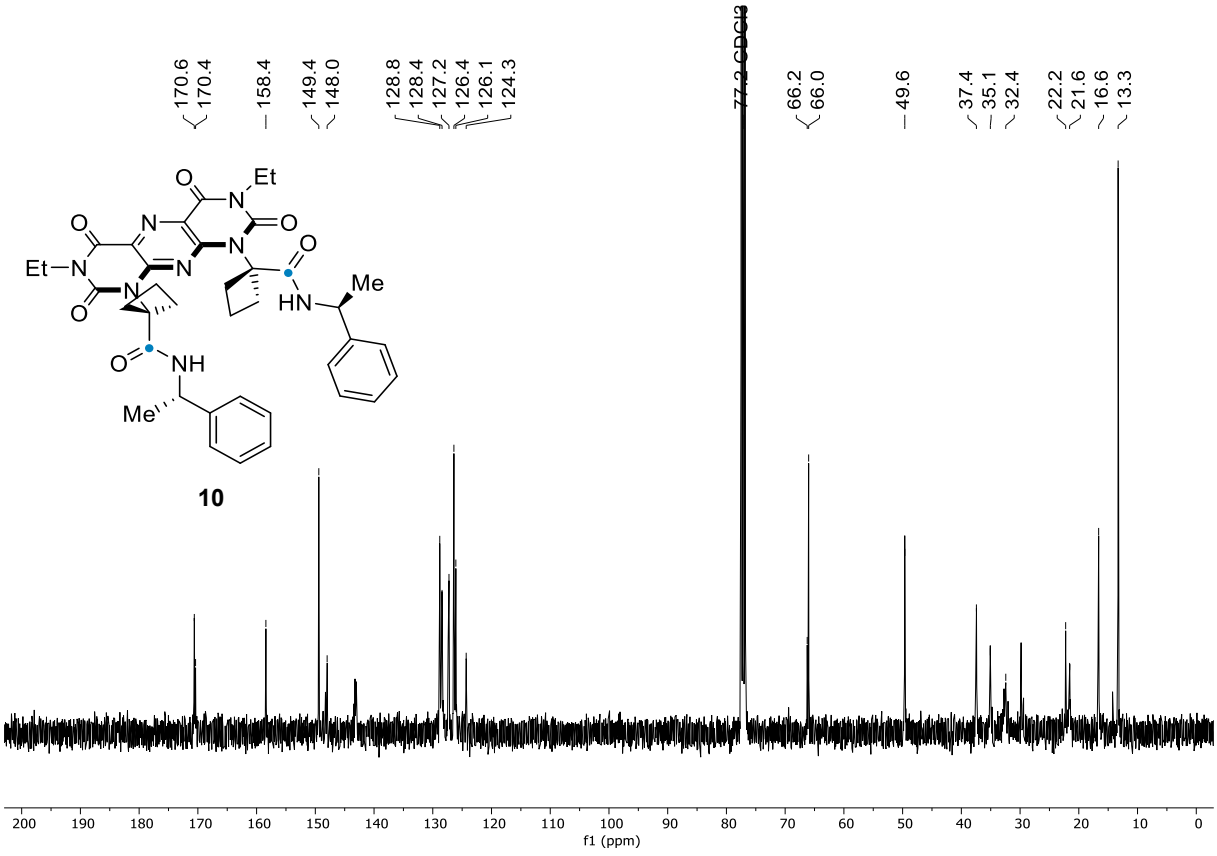
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{38}H_{42}N_8O_6Na]^+$: 729.3120; found: 729.3127 (Error PPM: 1.0).

IR (ATR, neat, $\tilde{\nu}$) = 3360 (vw), 2959 (w), 2932 (w), 1717 (s), 1662 (vs), 1554 (vs), 1509 (vs), 1433 (s), 1397 (vs), 1376 (vs), 1334 (vs), 1274 (vs), 1236 (vs), 1156 (m), 1126 (m), 1070 (s), 1050 (m), 1007 (m), 973 (m), 946 (m), 918 (w), 814 (s), 759 (vs), 718 (m), 697 (vs), 591 (m), 538 (s), 493 (vs) cm^{-1}.



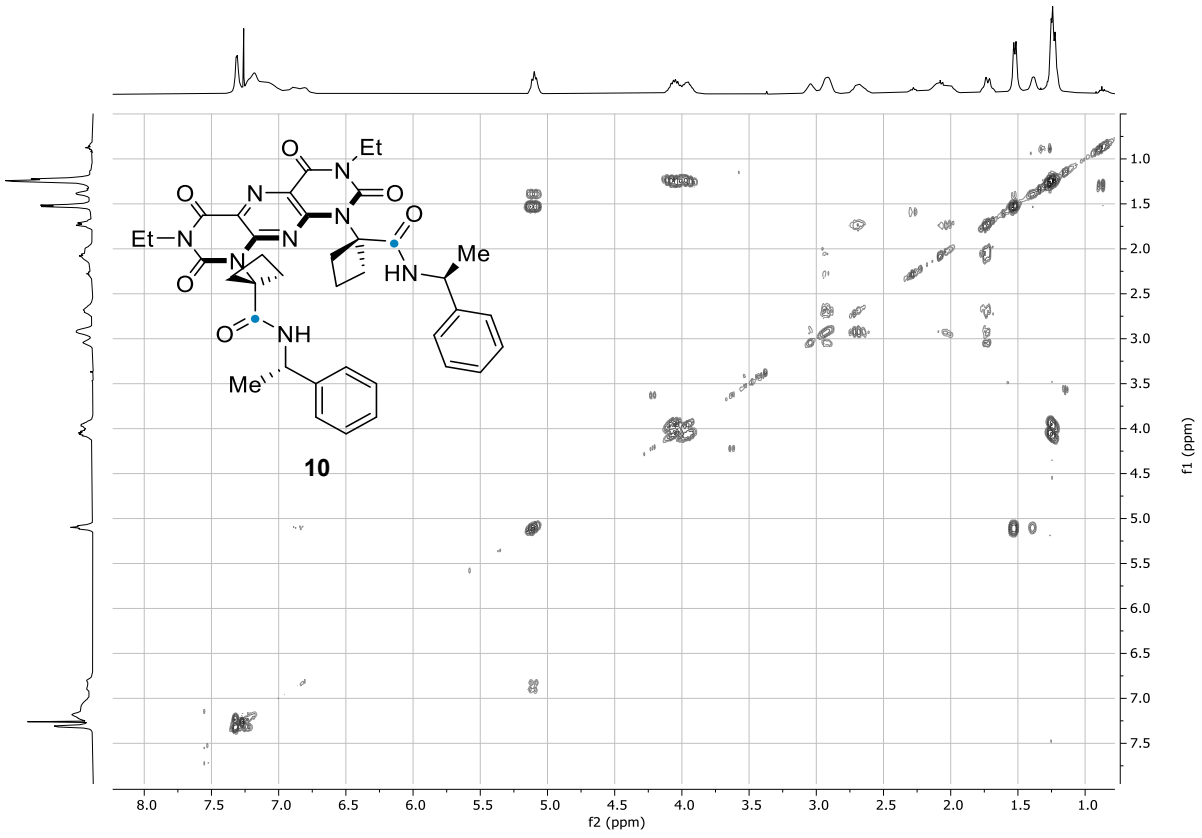
10

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)

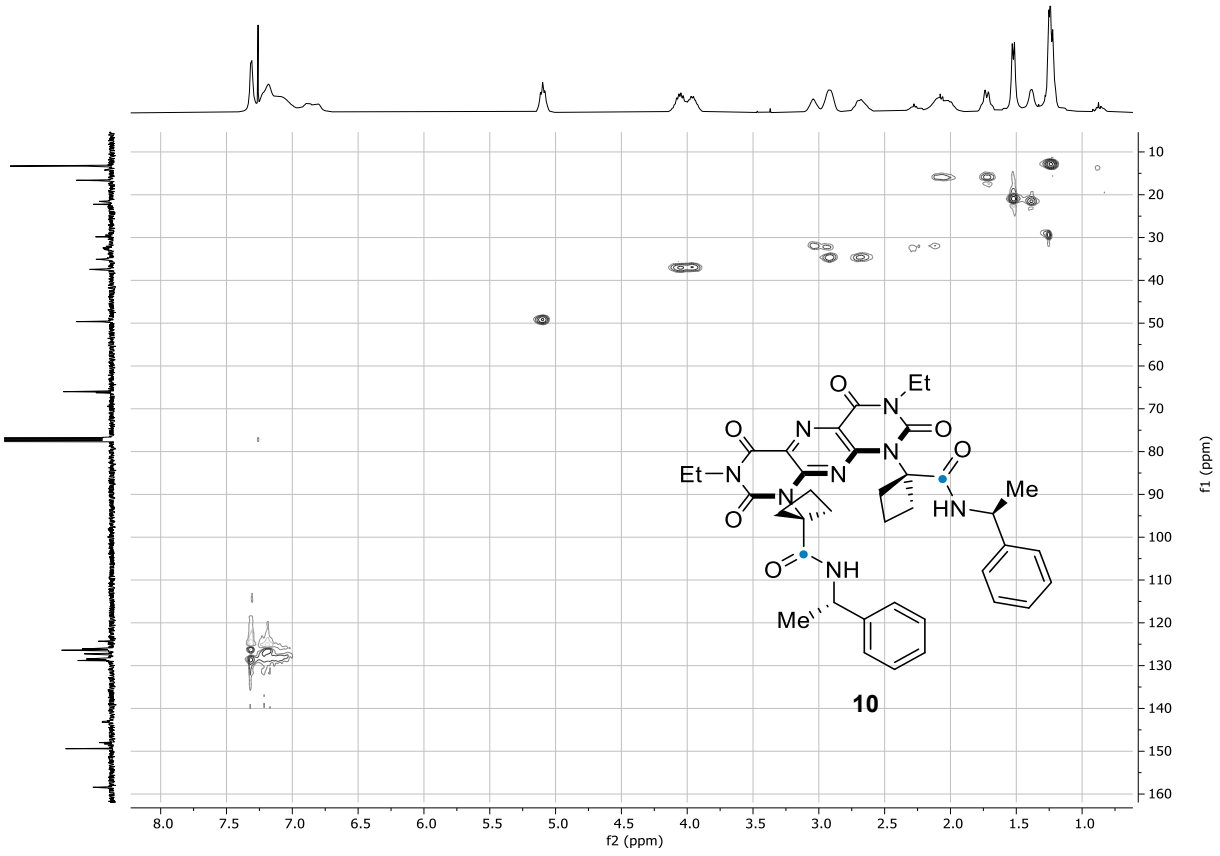


10

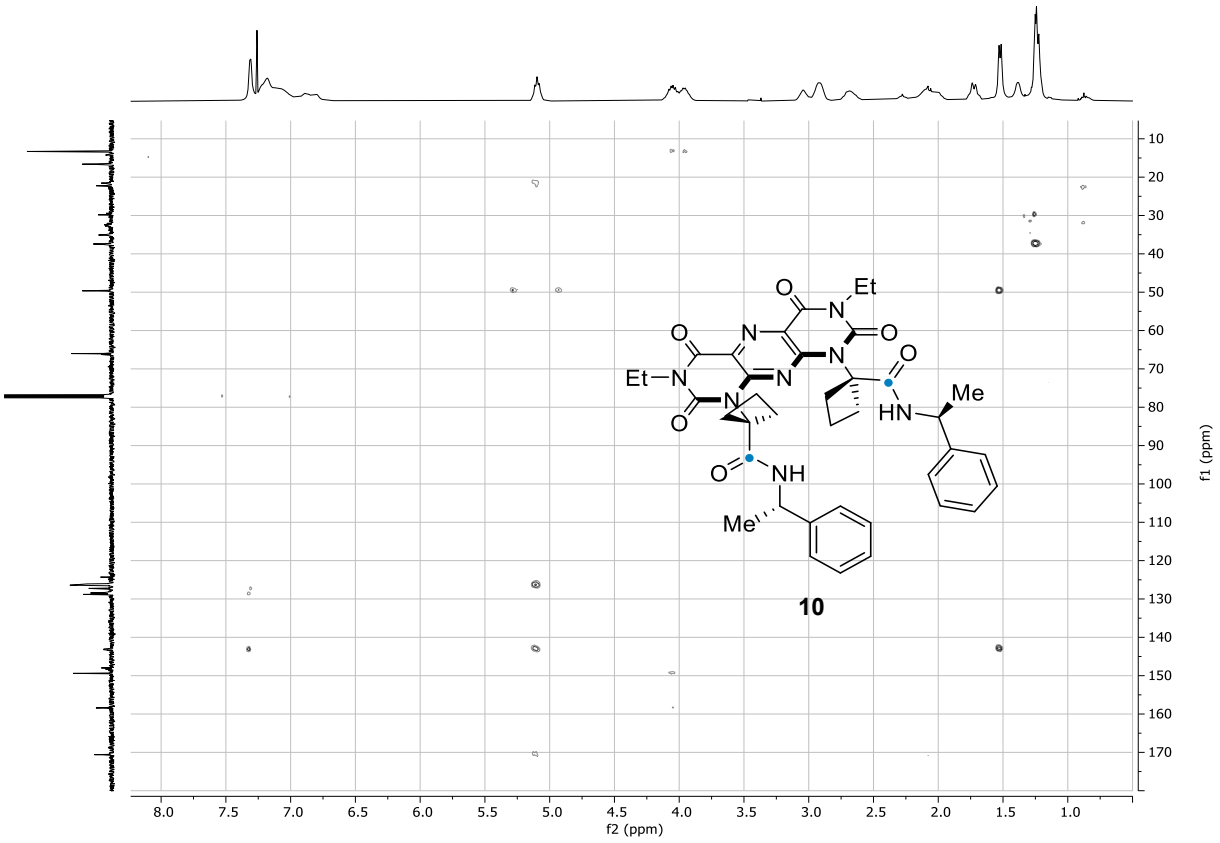
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)



^{1}H,^{1}H COSY-45 of compound **9**.



^{1}H,^{13}C HSQC of compound **9**.



^{1}H,^{13}C HMBC of compound **9**.

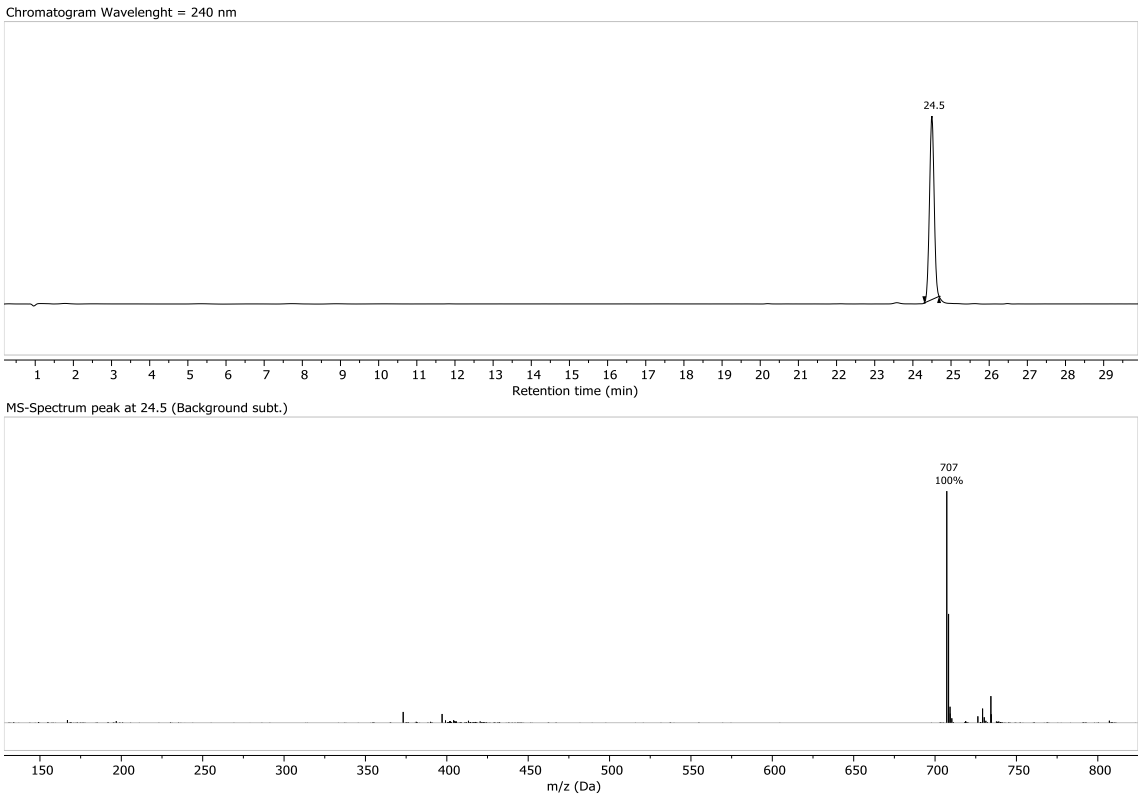
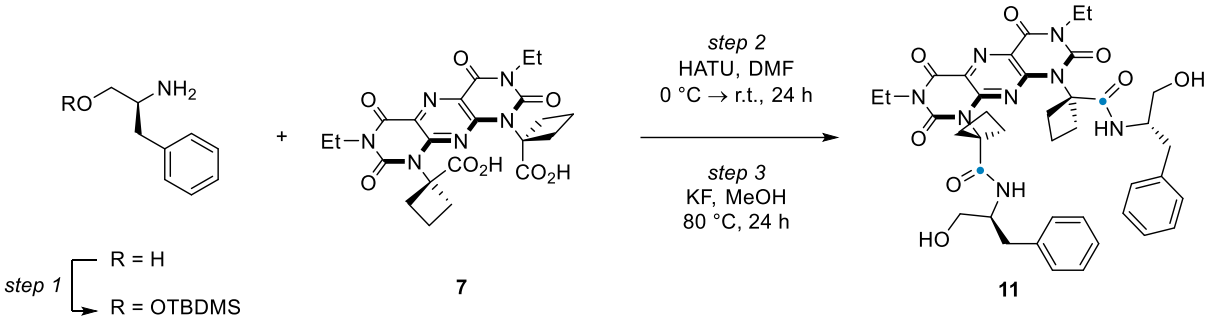


Figure S 7. Chromatogram and corresponding mass spectrum of isolated compound **10**; (Agilent, SB-C18, Water (0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI).

Synthesis of 1,1'-(3,7-diethyl-2,4,6,8-tetraoxo-3,4,7,8-tetrahydropyrimido[5,4-*g*]pteridine-1,9(2*H*,6*H*)-diyl)bis(*N*-((*S*)-1-hydroxy-3-phenylpropan-2-yl)cyclobutane-1-carboxamide) (11)



Step 1: *L*-Phenylalaninol was TBDMS-protected according to a literature known procedure.[11]

Step 2: Carboxylic acid **7** (105 mg, 200 µmol, 1.00 equiv.), (2*S*)-1-[tert-butyl(dimethyl)silyl]oxy-3-phenylpropan-2-amine (159 mg, 600 µmol, 3.00 equiv.) and HATU (167 mg, 440 µmol, 2.20 equiv.) were added in a flask. The flask was cooled down to 0 °C using an ice bath and DMF (4 mL) was added. After 5 min the reaction mixture was allowed to warm to ambient temperature and was stirred for 24 h.

Step 3: Following an adapted literature procedure, MeOH (1 mL) and KF (69.5 mg, 1.20 mmol, 6.00 equiv.) were added to the stirring solution.[12] Subsequently, the reaction was heated to 80 °C and stirred for another 24 h. The crude mixture was then concentrated *in vacuo* and directly purified by reversed-phase column chromatography (H_2O:MeOH= 95:5 → 20:80). The collected fractions were concentrated to dryness *in vacuo*, redissolved in CH_2Cl_2 and dried over Na_2SO_4. $Na_2SO_4$was removed by filtration and the solvent was removed *in vacuo* yielding the title compound **11** (112 mg, 147 µmol, 73% yield) as a colourless solid.

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.38 – 6.99 (m, 10H), 6.76 (dd, 7.6 Hz, 2H), 4.37 – 3.92 (m, 6H), 3.86 – 3.46 (m, 5H), 3.13 – 2.42 (m, 10H), 1.99 – 1.61 (m, 5H), 1.34 – 1.20 (m, 6H).

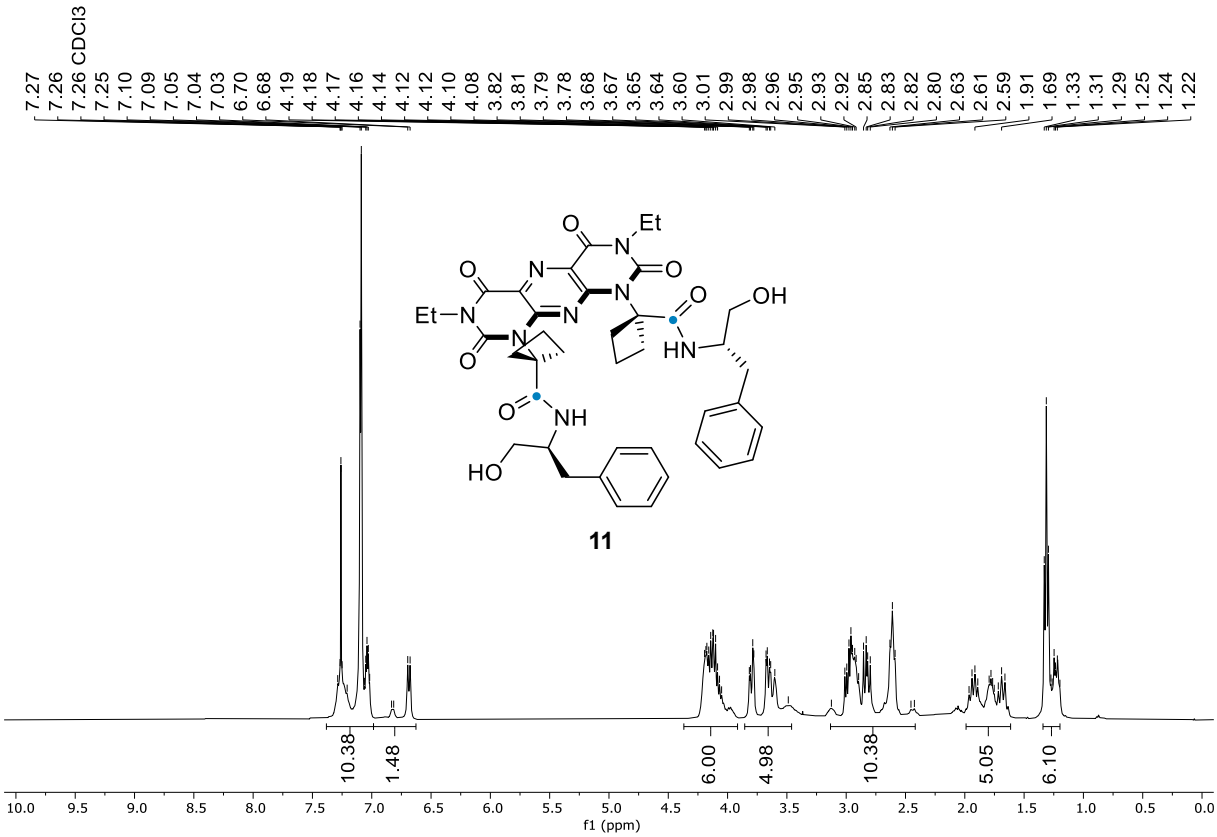
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 171.6, 157.8, 149.4, 147.4, 138.0, 129.6, 129.1, 128.6, 128.5, 126.7, 124.5, 65.6, 63.8, 53.3, 38.0, 37.3, 34.7, 31.5, 16.0, 13.2.

HPLC (Agilent, SB-C18, Water (0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI) tR = 22.8

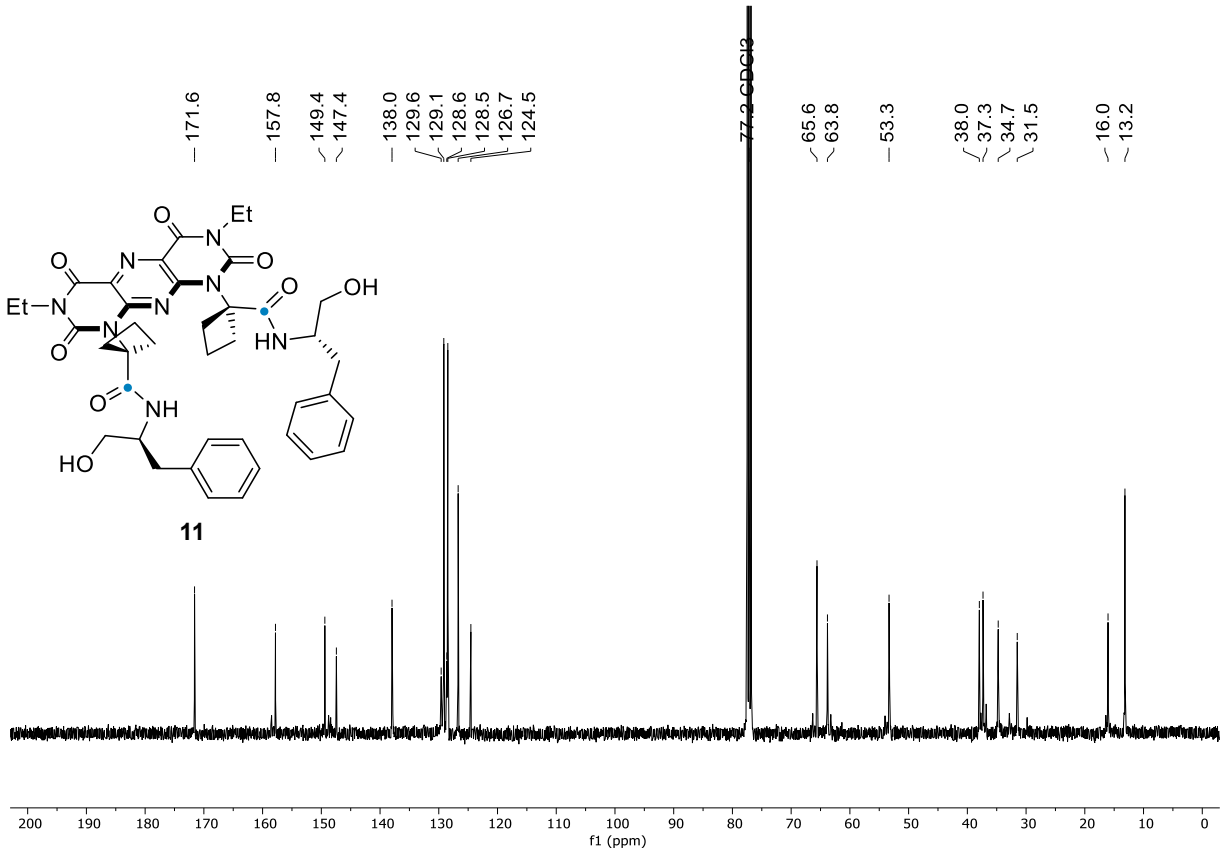
MS (ESI): *m/z* (%) = 795 (57), 767 (65, $[M+H]^+$), 434 (45), 411 (80), 403 (100).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{40}H_{46}N_8O_8Na]^+$: 789.3331; found: 789.3344 (Error PPM: 1.6).

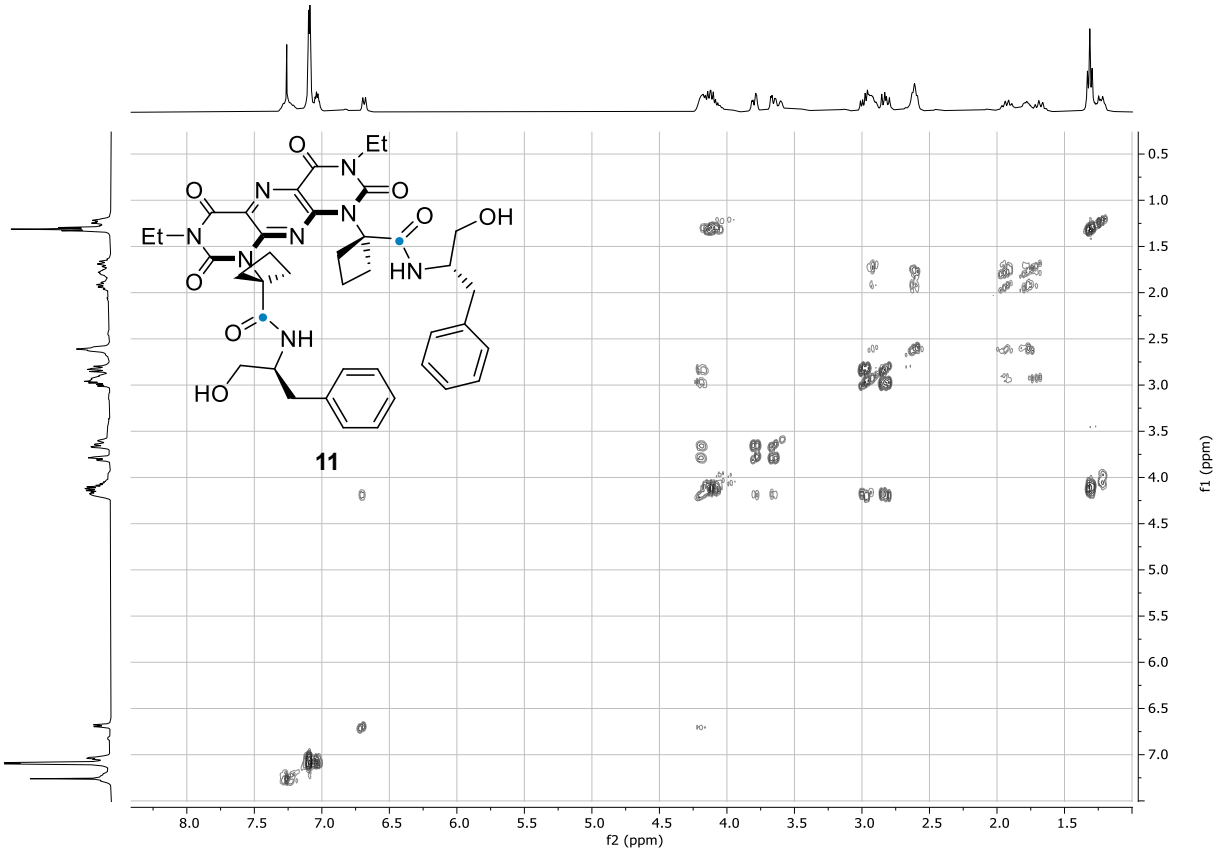
IR (ATR, neat, $\tilde{\nu}$) =3368 (w), 2938 (w), 2877 (w), 1719 (m), 1666 (vs), 1552 (vs), 1517 (vs), 1433 (s), 1395 (vs), 1376 (vs), 1331 (vs), 1264 (vs), 1236 (vs), 1158 (m), 1128 (w), 1068 (s), 1034 (s), 973 (w), 942 (m), 812 (m), 759 (s), 744 (s), 699 (vs), 555 (m), 495 (vs) cm^{-1}.



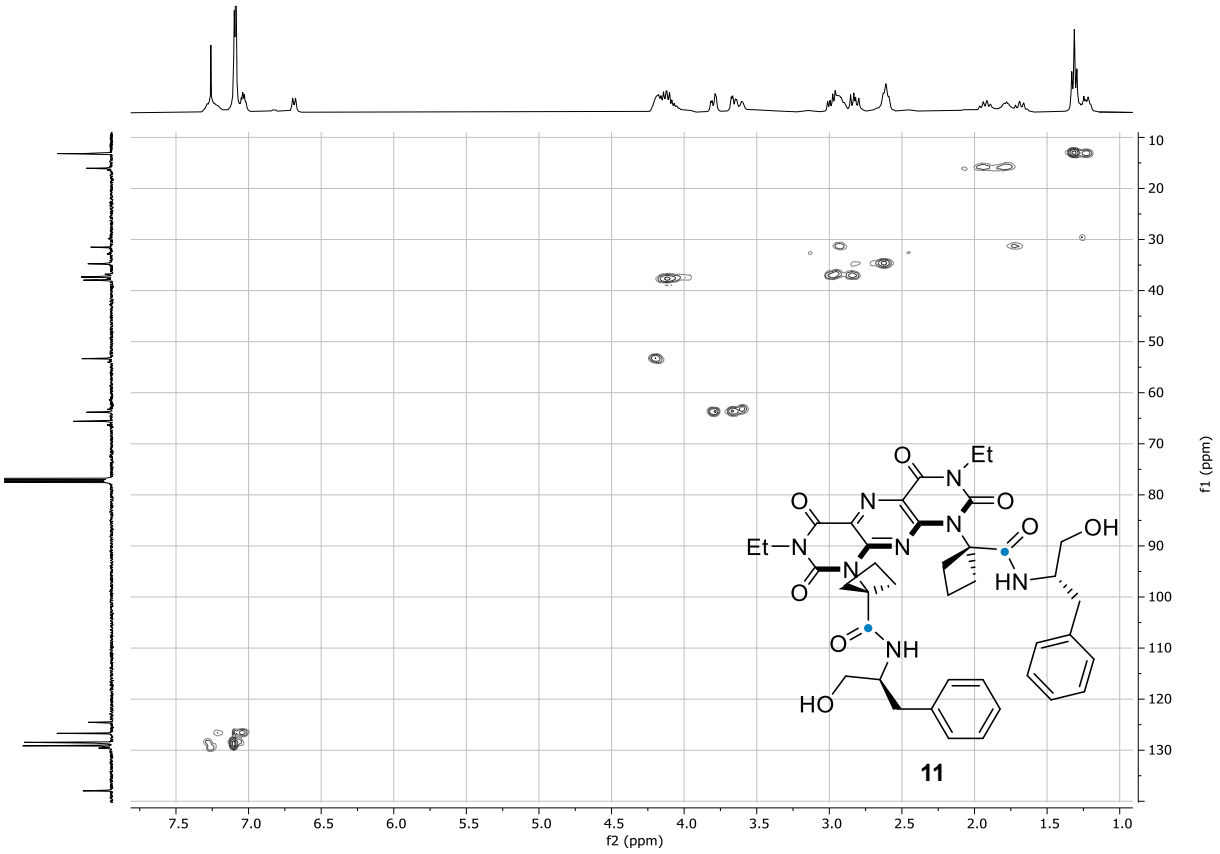
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



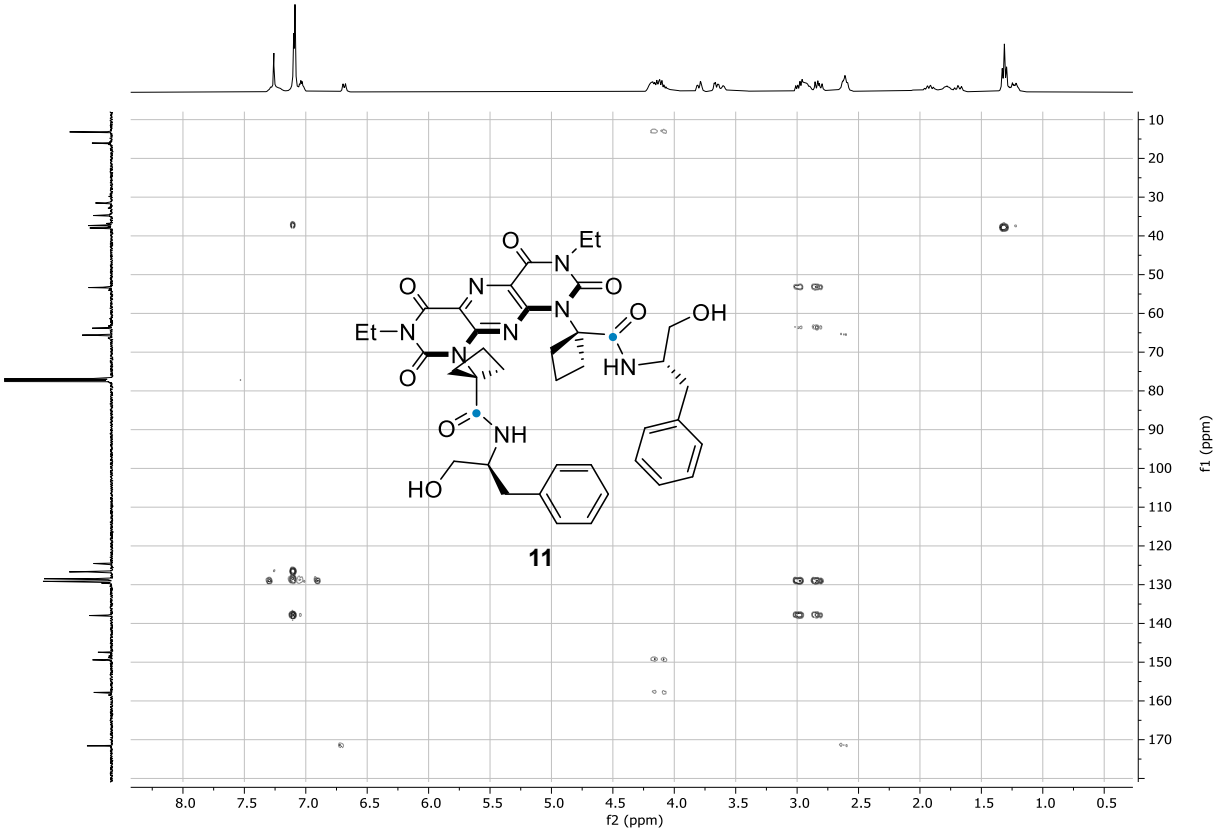
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)



^{1}H,^{1}H COSY-45 of compound **11**.



^{1}H,^{13}C HSQC of compound **11**.



^{1}H,^{13}C HMBC of compound **11**.

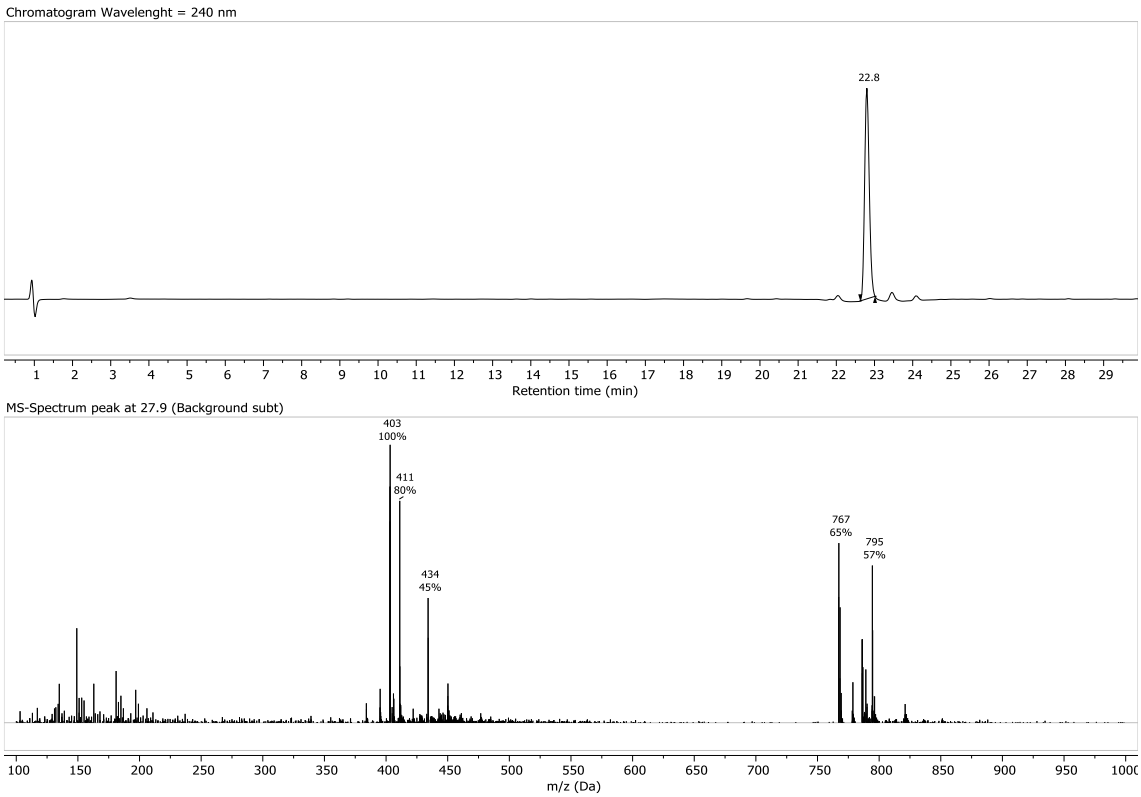
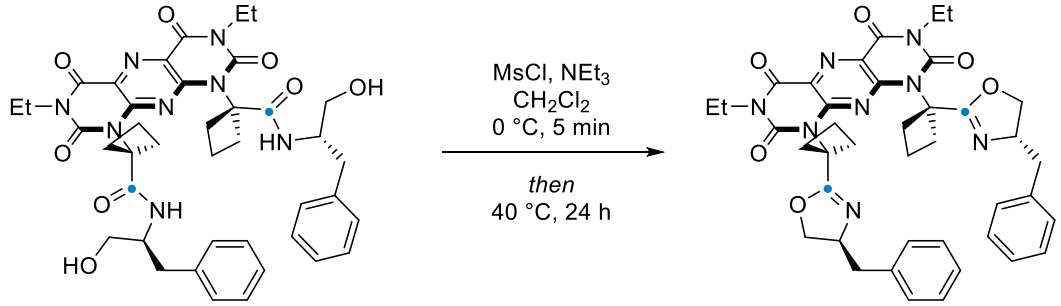


Figure S 8. Chromatogram and corresponding mass spectrum of isolated compound **11**; (Agilent, SB-C18, Water (0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI)

Synthesis of 1-(1-(4-benzyl-2,5-dihydrooxazol-2-yl)cyclobutyl)-9-(1-((*S*)-4-benzyl-4,5-dihydrooxazol-2-yl)cyclobutyl)-3,7-diethylpyrimido[5,4-*g*]pteridine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetraone (12)



11 **12**

The title compound was synthesized using an modified literature procedure.[13] Alcohol **11** (76.7 mg, 100 µmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (1.0 mL) and triethylamine (60.7 mg, 600 µmol, 6.00 equiv.) was added. The reaction mixture was subsequently cooled to 0 °C using an ice bath and methanesulfonyl chlorid (34.4 mg, 300 µmol, 3.0 equiv.) was added. Subsequently, the reaction mixture is heated to reflux and stirred for 24 h. Afterwards, the reaction mixture is transferred to a separatory funnel containing 10 mL *aq*. $NaHCO_3$ solution and extracted CH_2Cl_2 (3 x 10 mL). The combined organic layer were combined, dried *in vacuo* and purified by column chromatography (CH_2Cl_2:EtOAc = 2:3) to afford the title compound **12** (52.9 mg, 72.4 µmol, 72% yield) as a colorless solid.

R_f = 0.25 (CH_2Cl_2: EtOAc = 2:3, UV)

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 7.46 – 6.97 (m, 10H), 4.58 – 3.85 (m, 10H), 3.24 – 2.82 (m, 5H), 2.81 – 2.23 (m, 9H), 1.94 – 1.70 (m, 2H), 1.30 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 166.5, 157.9, 148.7, 148.2, 137.5, 129.6, 128.6, 126.7, 124.4, 73.1, 67.2, 61.2, 41.6, 37.7, 35.3, 33.6, 16.0, 13.1.

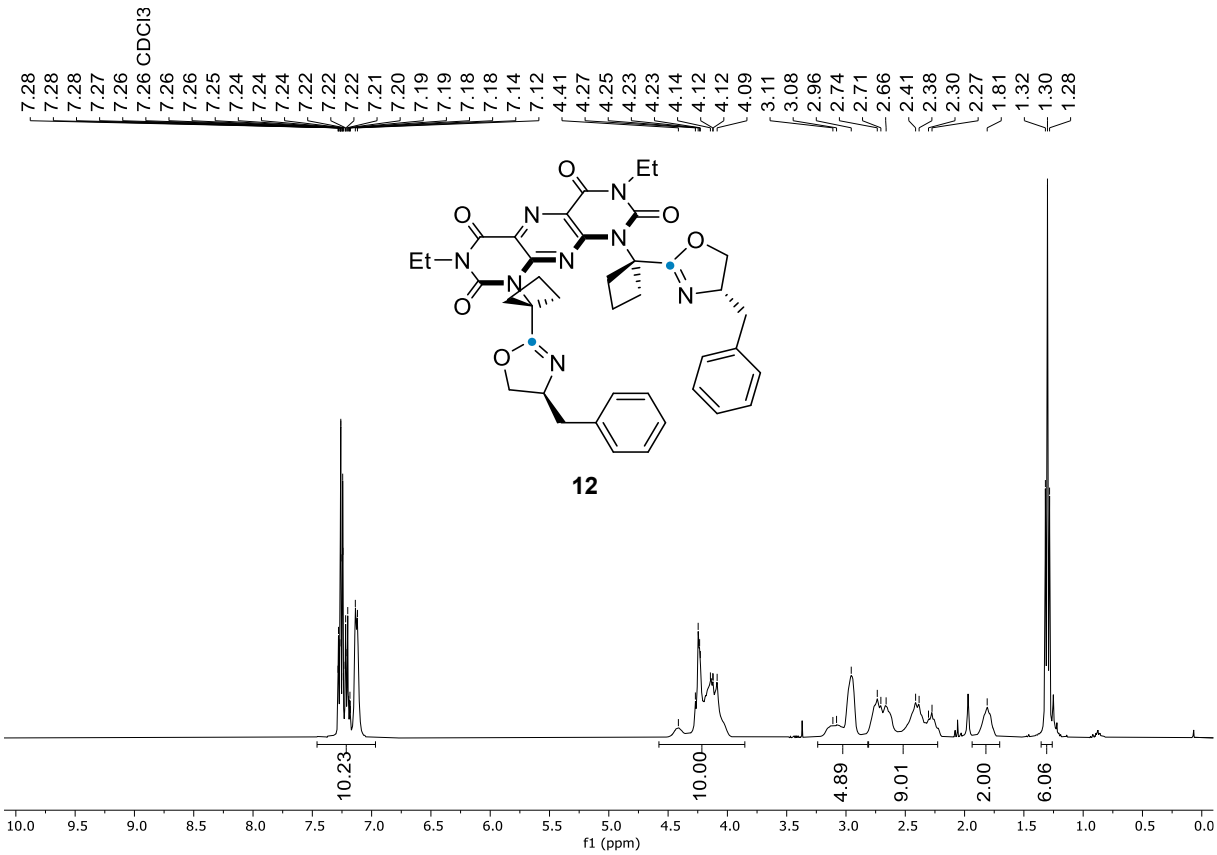
HPLC (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI):

t_R = 27.9

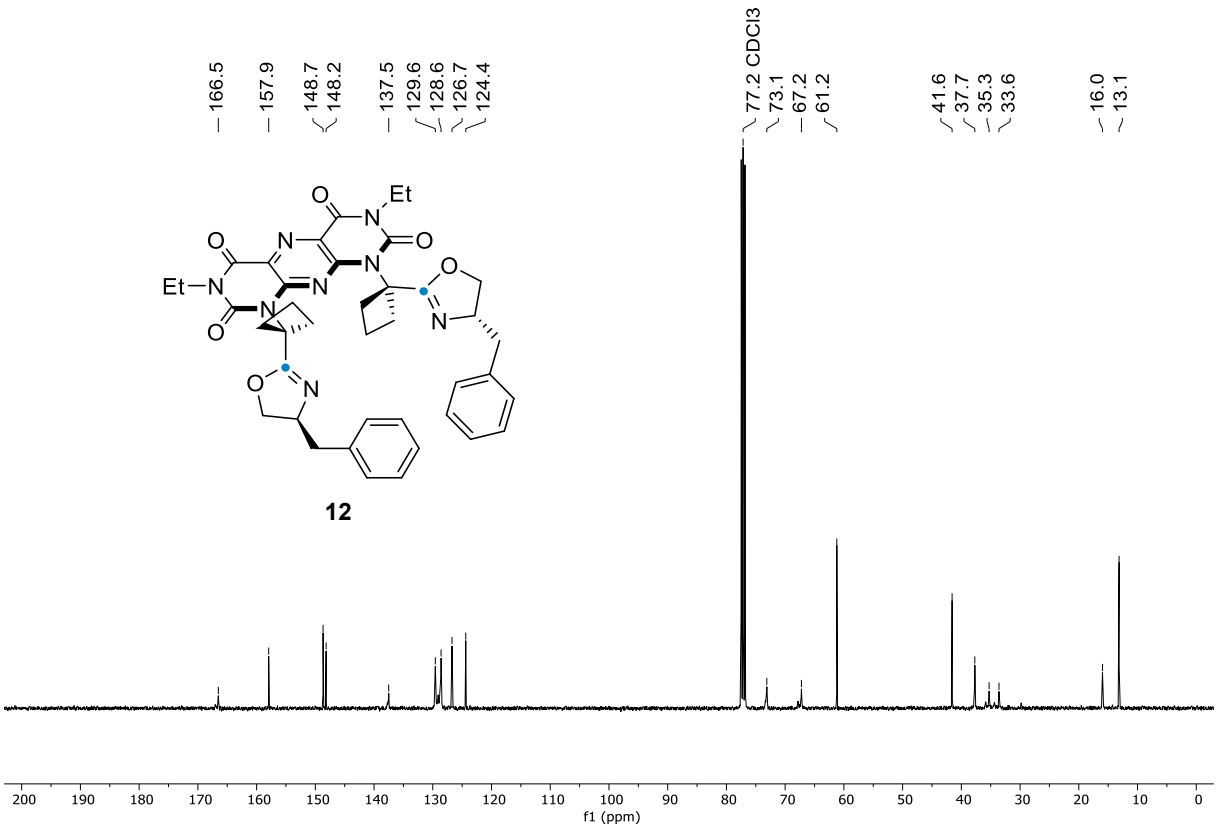
MS (ESI): *m/z* (%) = 731 (100, $[M+H]^+$), 366 (60).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{40}H_{43}N_8O_6]^+$: 731.3301; found: 731.3306 (Error PPM: 0.7).

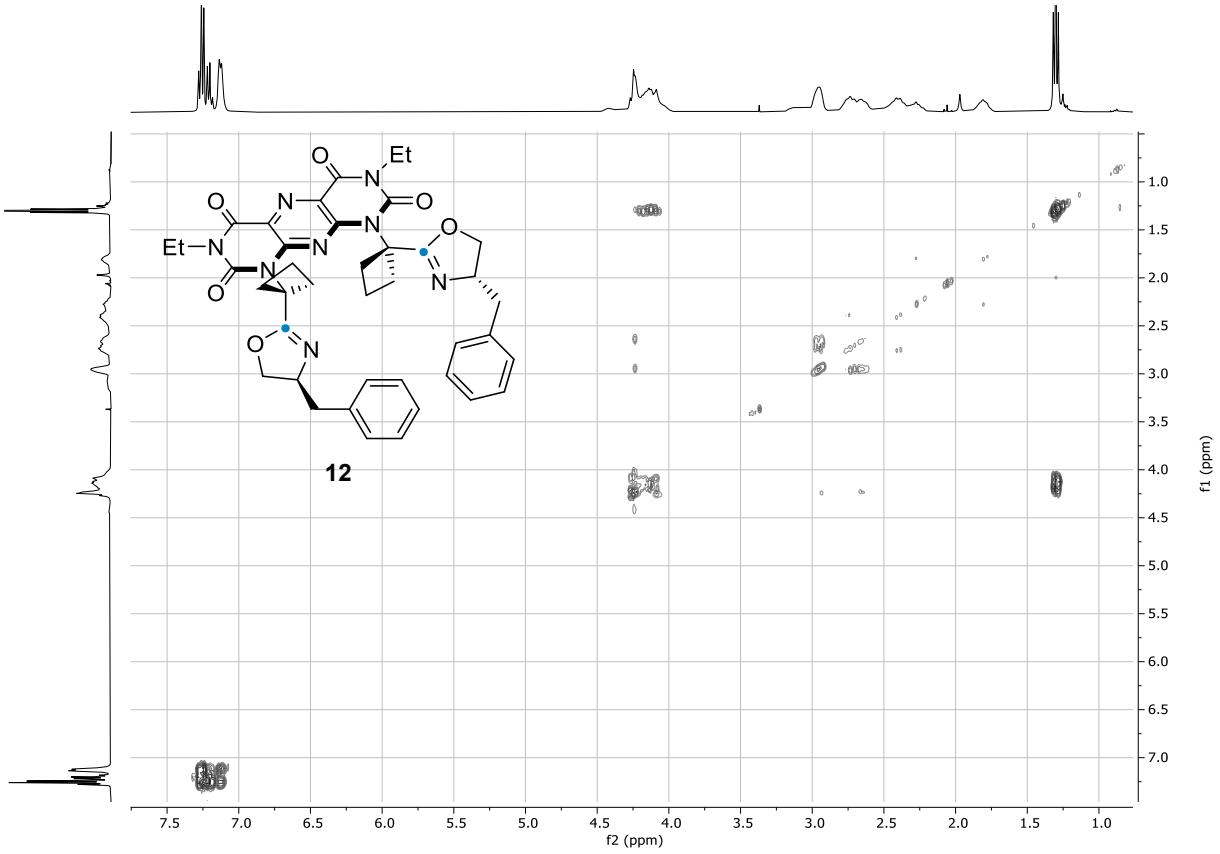
IR (ATR, neat, ṽ) = 2957 (w), 1723 (m), 1680 (vs), 1658 (vs), 1550 (vs), 1495 (w), 1431 (m), 1393 (vs), 1374 (vs), 1331 (vs), 1264 (vs), 1236 (vs), 1203 (s), 1156 (m), 1107 (m), 1070 (m), 962 (m), 812 (m), 756 (m), 718 (m), 701 (vs), 644 (w), 500 (vs) cm^{-1}.



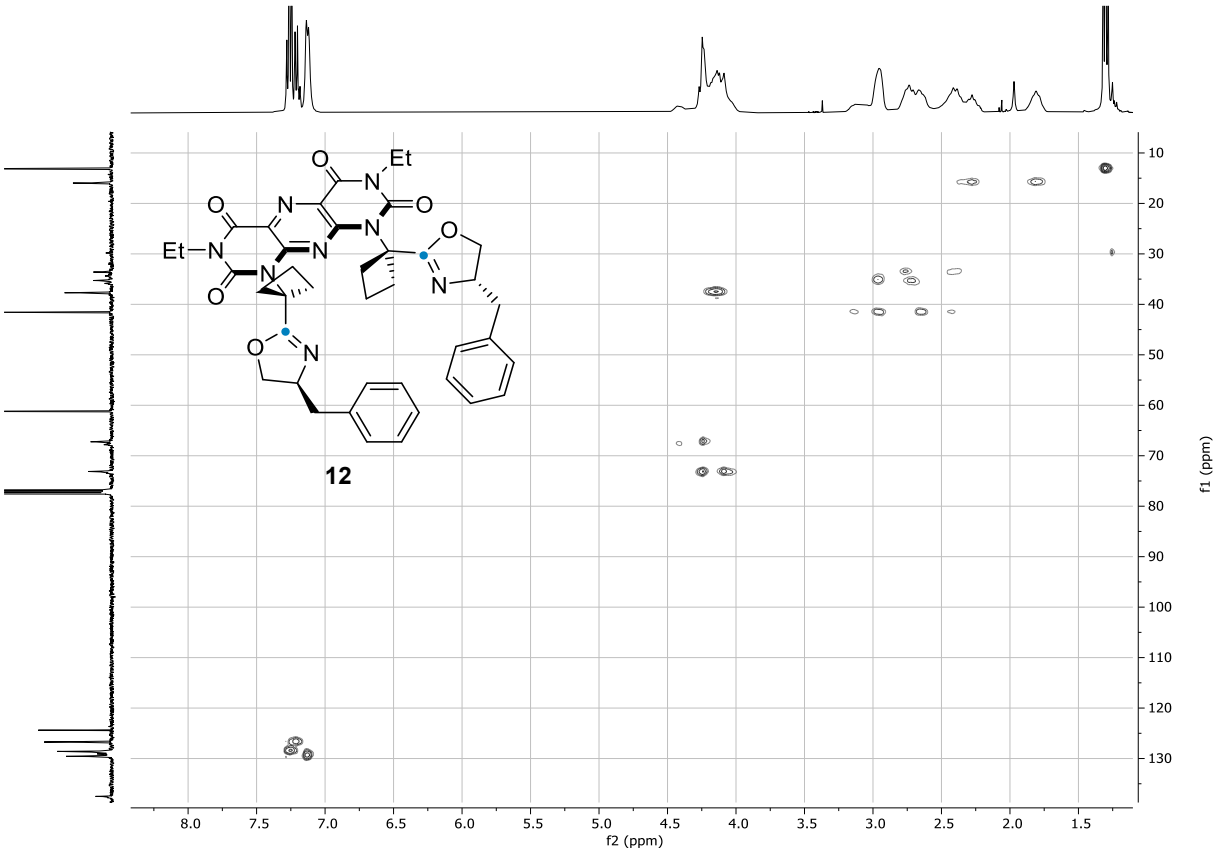
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



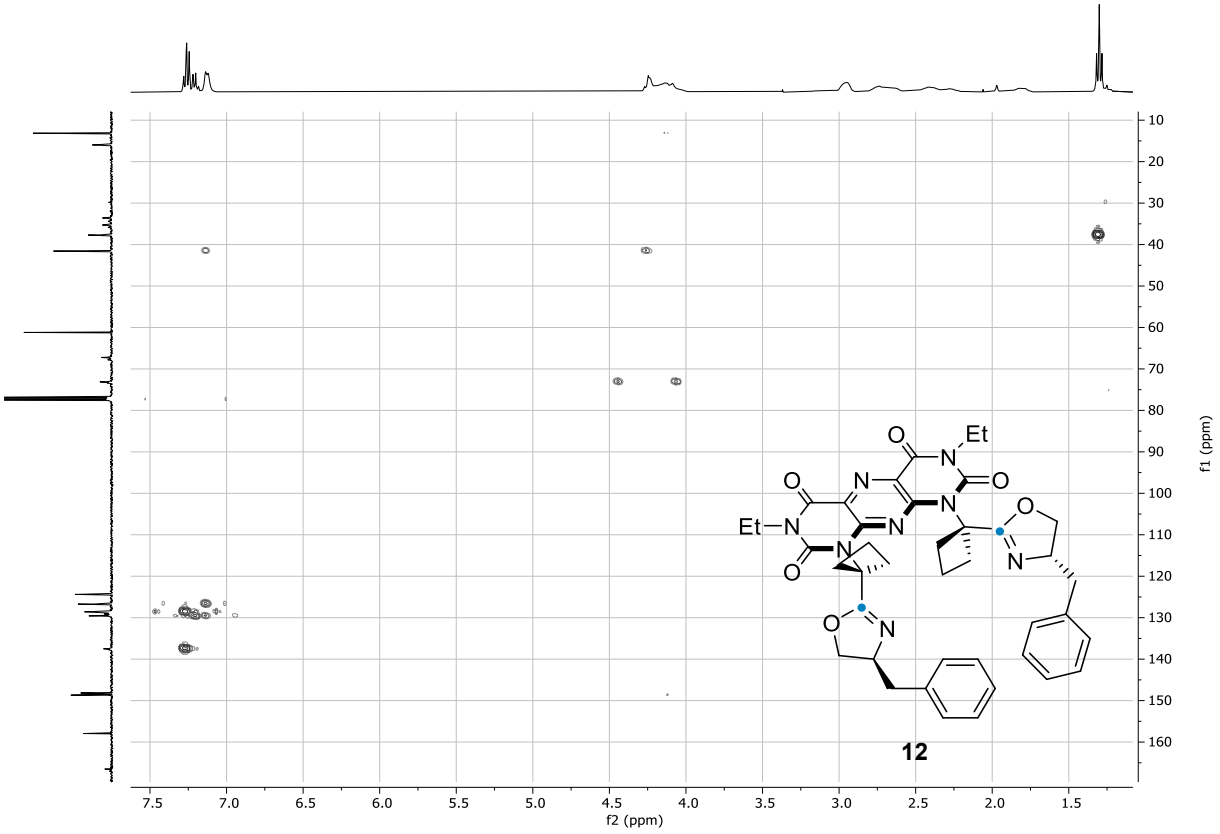
^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)



^{1}H,^{1}H COSY-45



^{1}H,^{13}C HSQC



^{1}H,^{13}C HMBC

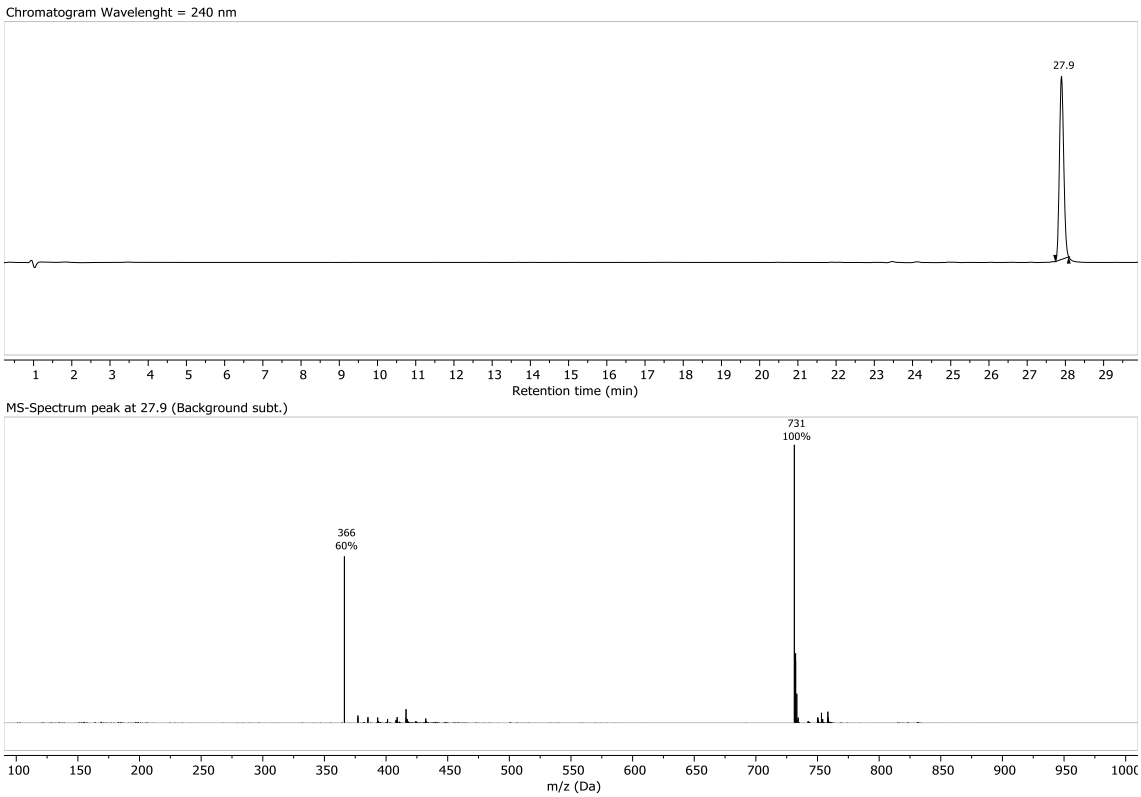


Figure S 9. Chromatogram and corresponding mass spectrum of isolated compound **12**; (Agilent, SB-C18, Water (0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI)

5 Single crystal X-ray diffraction data

X-ray quality crystals were selected in Fomblin YR-1800 perfluoroether (ALFA AESAR) at low temperature. Diffraction data were collected at 150(2) K on a STOE IPDS II using Mo-Kα radiation for **ks1736** and **ks1749** or on a BRUKER Kappa APEX II Duo diffractometer using Mo-K_α radiation for **AX2767**, **AX2854** and **AX2840** or Cu-K_α radiation for **AX2850**, **AX2866**, **AX2882** and **AX2917**. The structures were solved by iterative (SHELXT) or direct methods (SHELXS-97) and refined by full matrix least square techniques against F^2 (SHELXL-2014). Semi-empirical absorption corrections were applied (SADABS/ BRUKER). The non-hydrogen atoms were refined anisotropically.

The hydrogen atoms, except for the hydrogens in water molecules of **AX2767** and for O*H* in **ks1749**(determination and refinement from electron density), were placed in the theoretical positions and were refined by using the riding model. Contributions of solvent molecules was removed in **ks1749** from the diffraction data with PLATON/SQUEEZE.

DIAMOND (Crystal Impact GbR) was used for structure representations.

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited at the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge, CB21EZ, UK (fax: int. code + (1223) 336-033; e-mail: deposit@ccdc.cam.ac.uk.

Table S 5. Crystallographic data.

Compound	**2c**	(*S*)-**2k**	**2d**
Chem. Formula	$C_{24}H_{28}N_6O_8$	$C_{26}H_{36}N_6O_8$	$C_{26}H_{32}N_6O_8$
Formula weight [g/mol]	528.52	560.61	556.57
Colour	colorless	colorless	colorless
Crystal system	triclinic	monoclinic	monoclinic
Space group	$P\bar{1}$	$P2_1$	$P2_1/c$
a [Å]	8.4394(4)	12.0690(15)	9.8461(7)
b [Å]	13.0868(7)	7.6497(9)	24.1926(16)
c [Å]	13.2386(7)	16.1743(19)	11.2997(7)
α [°]	119.294(3)	90	90
β [°]	103.450(3)	110.812(2)	97.556(2)
γ [°]	91.119(4)	90	90
V [Å^3]	1224.76(11)	1395.8(3)	2668.2(3)
Z	2	2	4
$\rho_{calcd.}$ [g/cm^3]	1.433	1.334	1.385
μ [mm^{-1}]	0.922	0.100	0.104
Measured reflections	19043	39943	65193

Independent reflections	3821	5495	5236
Reflections with $I > 2\sigma(I)$	3177	4923	3972
R_{int}	0.0464	0.0548	0.0616
$F(000)$	556	596	1176
$R_1(R[F^2>2\sigma(F^2)])$	0.0730	0.0417	0.0396
w$R_2(F^2)$	0.2392	0.1058	0.1071
GooF	1.097	1.044	1.013
No. of Parameters	347	380	383
Flack parameter		0.1(3)	
CCDC #	2466913	2466914	2466916

Table S 1Table S 5, continued.

Compound	(*S*)-**2j**	**2a**	**2x**
Chem. Formula	$C_{28}H_{50}N_6O_8$	2 x $C_{24}H_{32}N_6O_8 \cdot Et_2O \cdot H_2O$	$C_{36}H_{36}N_6O_8$
Formula weight [g/mol]	588.66	1175.26	680.71
Colour	colorless	colorless	colorless
Crystal system	orthorhombic	monoclinic	triclinic
Space group	$P2_12_12_1$	$P2_1/c$	$P\bar{1}$
a [Å]	8.7897(5)	27.446(5)	10.4253(4)
b [Å]	15.3948(8)	11.970(2)	12.0534(4)
c [Å]	21.7326(12)	18.952(3)	13.6573(5)
α [°]	90	90	85.7950(10)
β [°]	90	107.682(4)	72.1880(10)
γ [°]	90	90	78.1290(10)
V [Å^3]	2940.8(3)	5932.3(18)	1598.87(10)
Z	4	4	2
$\rho_{calcd.}$ [g/cm^3]	1.330	1.316	1.414
μ [mm^{-1}]	0.818	0.101	0.842
Measured reflections	24765	156426	20982
Independent reflections	5182	11659	5598
Reflections with $I > 2\sigma(I)$	5138	8522	4932
R_{int}	0.0254	0.0605	0.0345
$F(000)$	1256	2504	716
$R_1(R[F^2>2\sigma(F^2)])$	0.0318	0.0451	0.0428

w$R_2(F^2)$	0.0839	0.1226	0.1241
GooF	1.049	1.054	1.036
No. of Parameters	387	782	453
Flack parameter	0.04(3)		
CCDC #	2466915	2469766	2477199

Table S 1Table S 5, continued.

Compound	**2e**	**S11**	(*S*,*S*)-**6**
Chem. Formula	$C_{26}H_{32}N_6O_8$	$C_{40}H_{46}N_8O_8$	$C_{34}H_{40}N_8O_{10}$· DCM
Formula weight [g/mol]	556.57	766.85	762.06
Colour	colorless	colorless	colorless
Crystal system	monoclinic	triclinic	tetragonal
Space group	$P2_1$	$P1$	$P4_32_12$
a [Å]	12.5101(4)	9.5279(6)	14.5984(3)
b [Å]	7.7677(2)	12.7827(9)	14.5984(3)
c [Å]	14.8027(5)	17.7588(13)	17.1526(4)
α [°]	90	94.807(6)	90
β [°]	113.431(3)	96.335(6)	90
γ [°]	90	108.423(5)	90
V [Å^3]	1319.83(8)	2023.3(2)	3655.45(17)
Z	2	2	4
$\rho_{calcd.}$ [g/cm^3]	1.401	1.259	1.385
μ [mm^{-1}]	0.106	0.090	1.490
Measured reflections	14108	35278	29433
Independent reflections	5149	14183	3238
Reflections with $I > 2\sigma(I)$	4661	10464	2974
R_{int}	0.0169	0.0391	0.0409
F(000)	588	812	1602
$R_1(R[F^2>2\sigma(F^2)])$	0.0387	0.0563	0.0702
w$R_2(F^2)$	0.1042	0.1451	0.2099
GooF	1.009	1.023	1.105
No. of Parameters	365	1033	289
Flack parameter	0.0(3)	-0.6(7)	0.035(17)
CCDC #	2558190	2558192	2558191

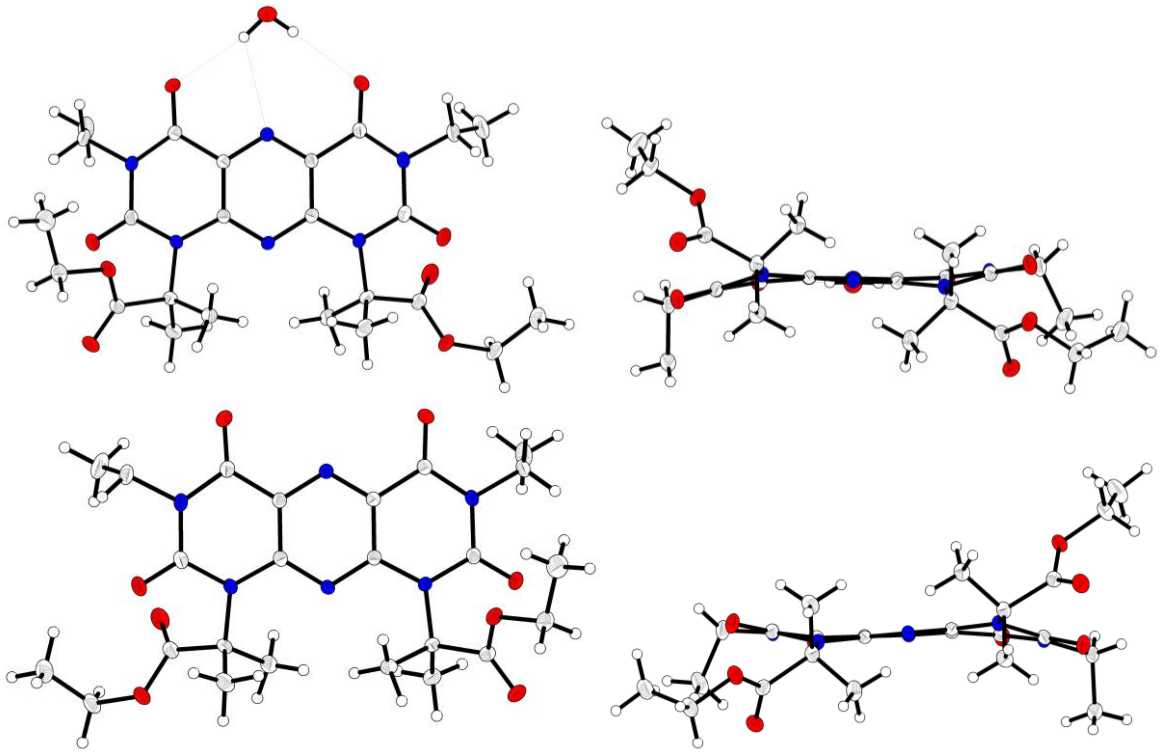


Figure S 10. ORTEP representations of both enantiomers of **2a** shown in two orientations. Thermal ellipsoids are drawn at the 50% probability level.

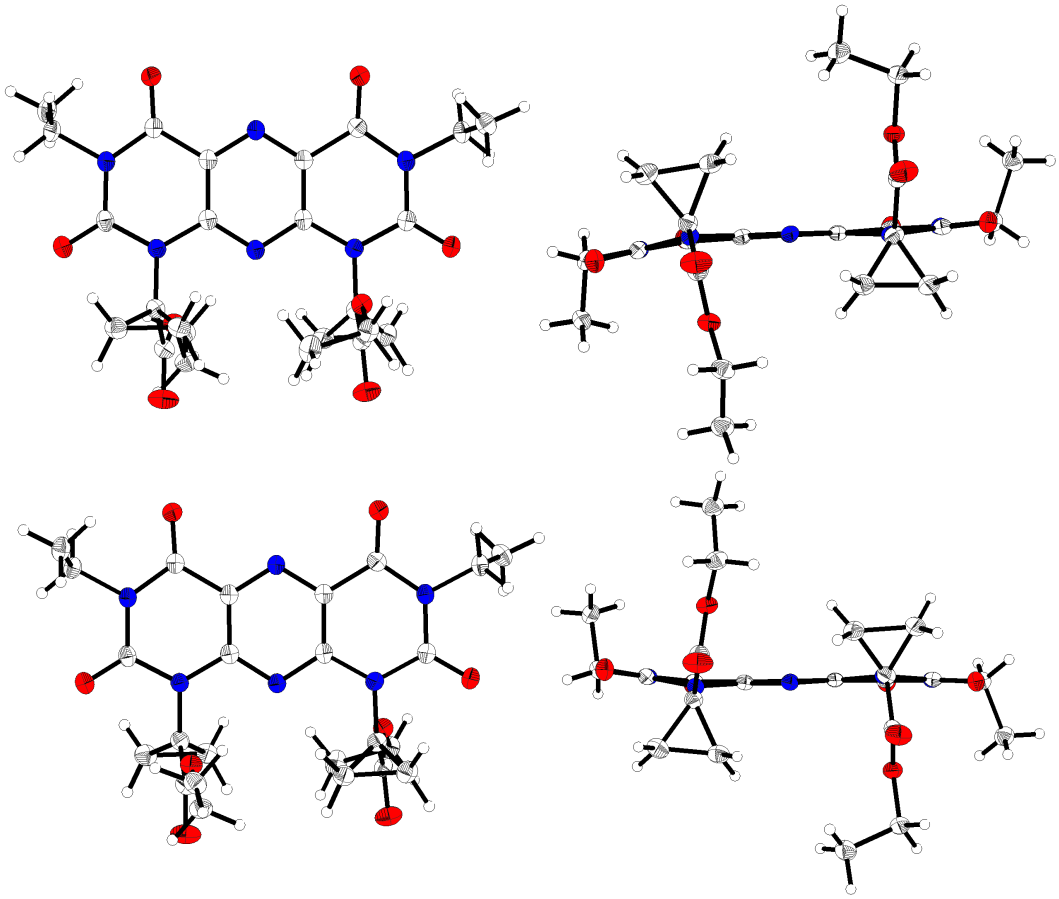


Figure S 11. ORTEP representations of both enantiomers of **2c** shown in two orientations. Thermal ellipsoids are drawn at the 40% probability level.

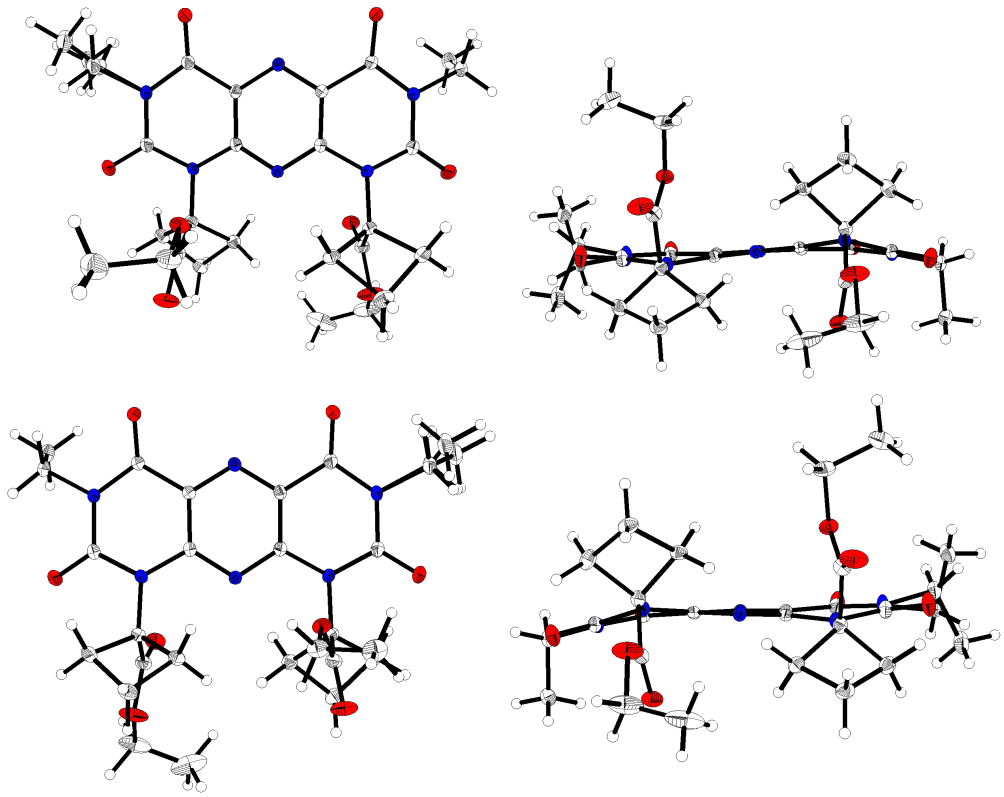


Figure S 12. ORTEP representations of both enantiomers of **2d** shown in two orientations. Thermal ellipsoids are drawn at the 50% probability level.

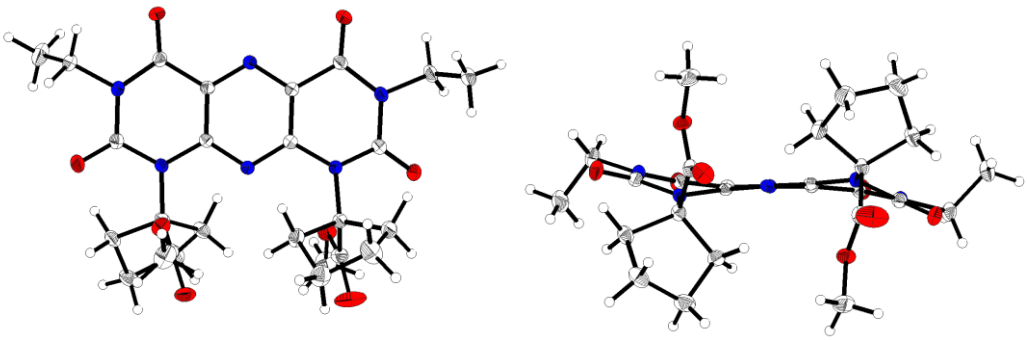


Figure S 13. ORTEP representations of both enantiomers of **2e** shown in two orientations. Thermal ellipsoids are drawn at the 40% probability level.

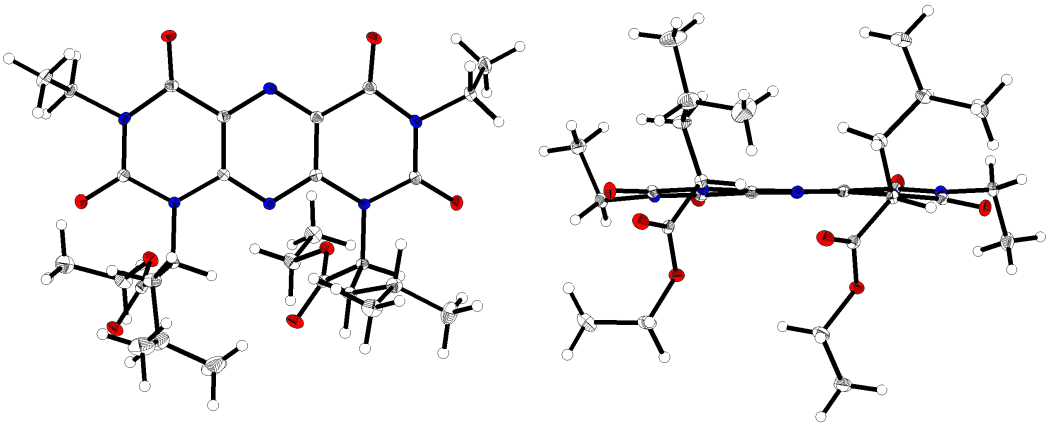


Figure S 14. ORTEP representations of (*S*)-**2j** shown in two orientations. Thermal ellipsoids are drawn at the 50% probability level.

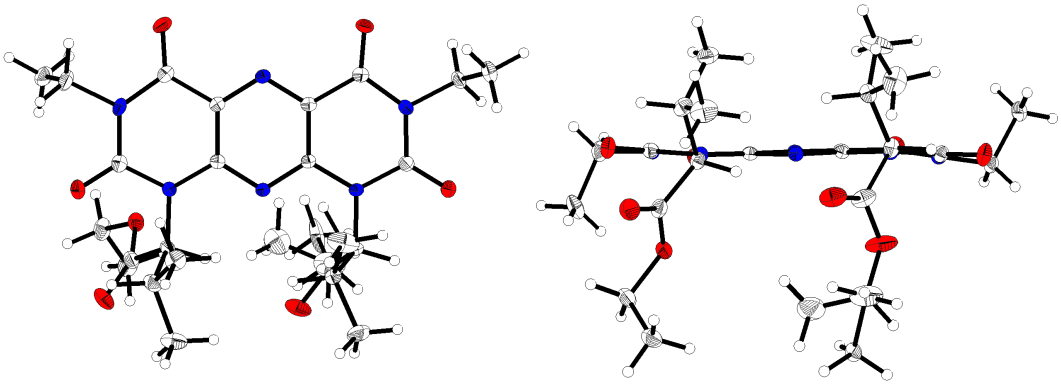


Figure S 15. ORTEP representations of (*S*)-**2k** shown in two orientations. Thermal ellipsoids are drawn at the 50% probability level. Alkyl ester misplaced.

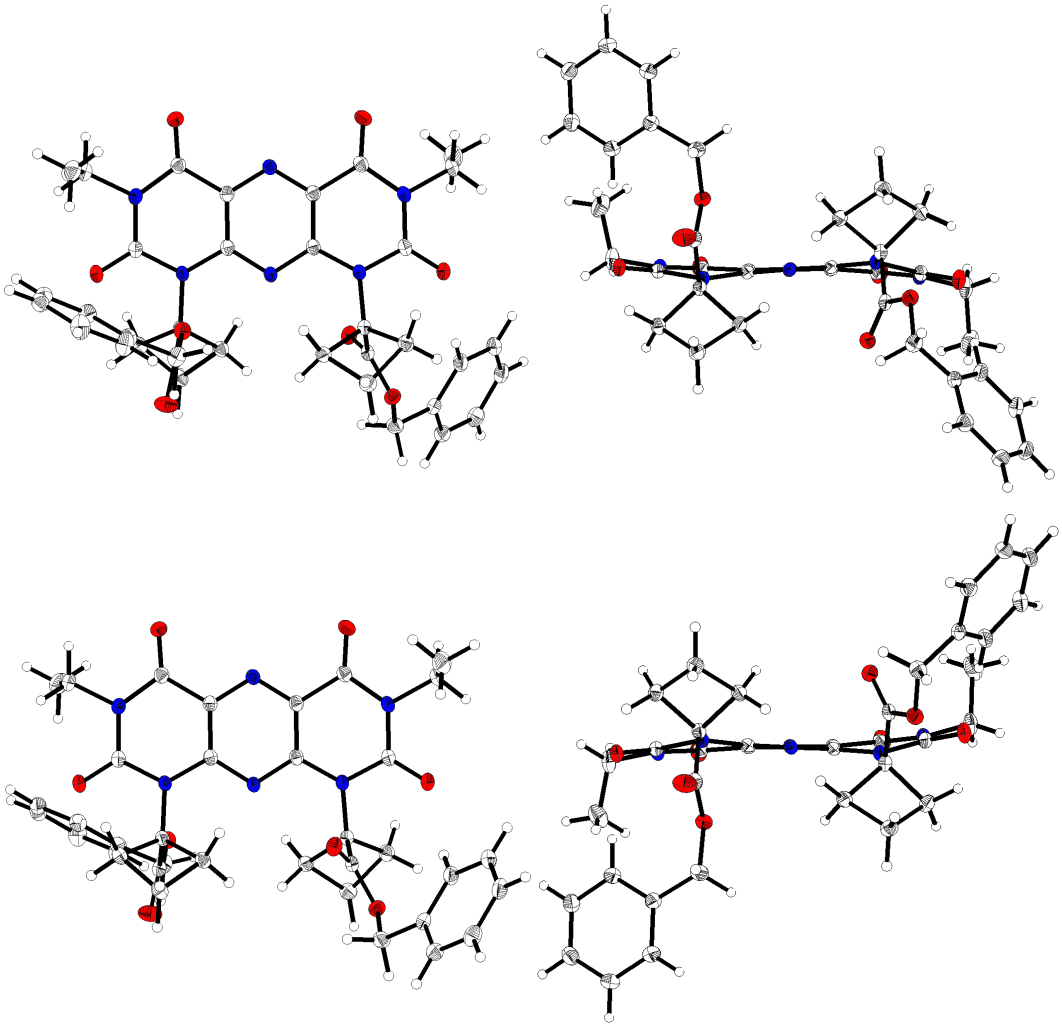


Figure S 16. ORTEP representations of both enantiomers of **2x** shown in two orientations. Thermal ellipsoids are drawn at the 30% probability level.

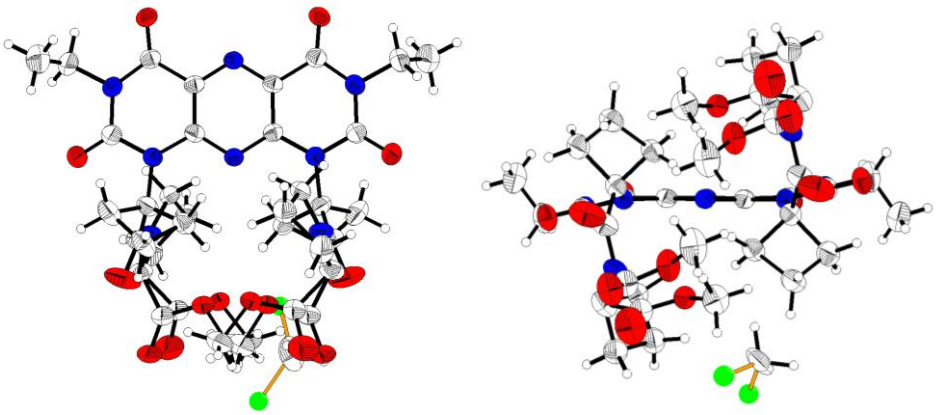


Figure S 17. ORTEP representations of (*S*,*S*)-**6** shown in two orientations. Thermal ellipsoids are drawn at the 50% probability level.

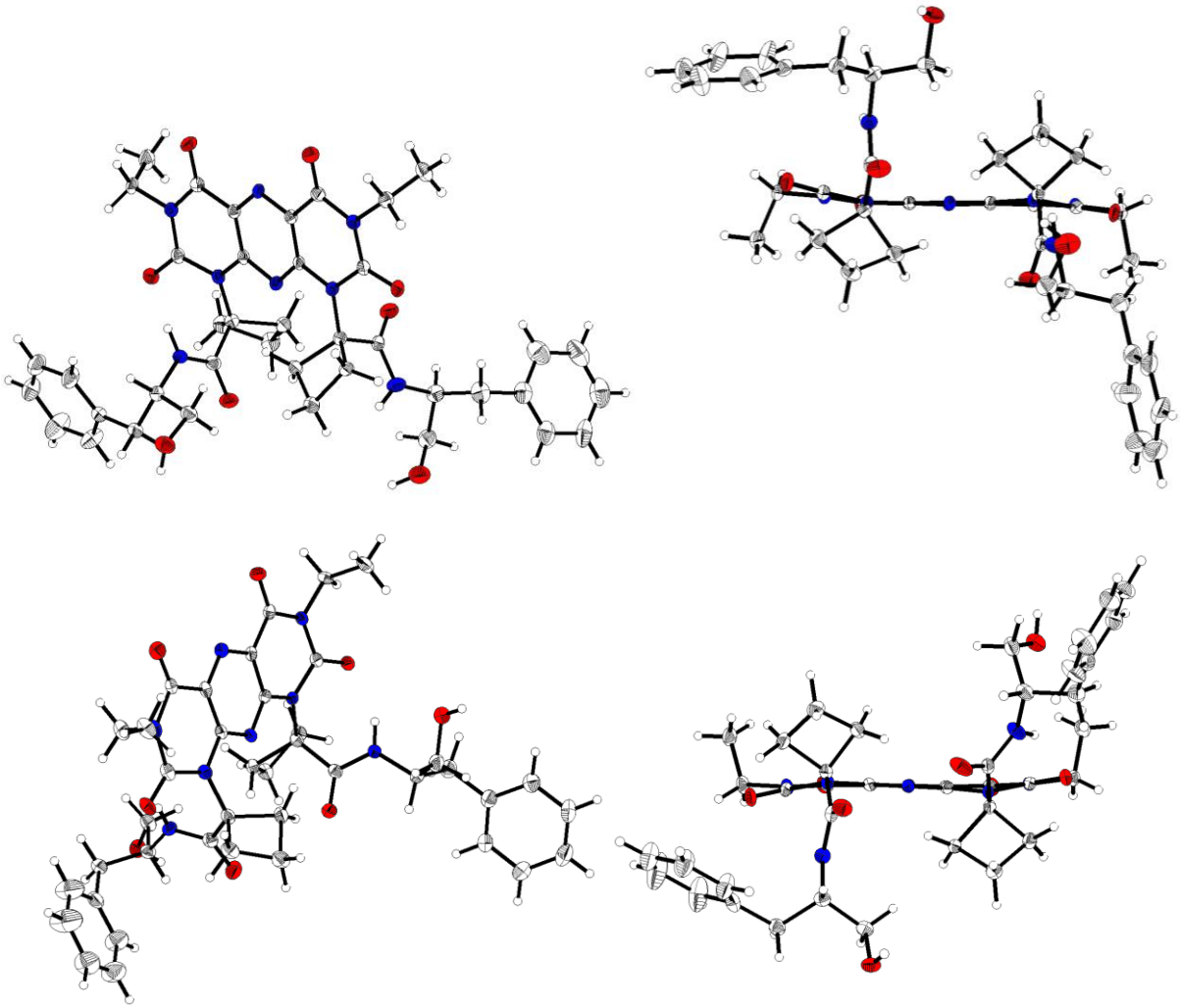
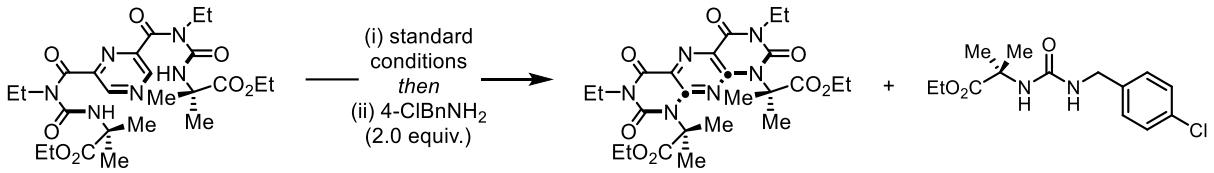


Figure S 18. ORTEP representations of both diastereomers of **S11** shown in two orientations. Thermal ellipsoids are drawn at the 50% probability level.

6 Mechanistic studies

6.2 Isocyanate trapping

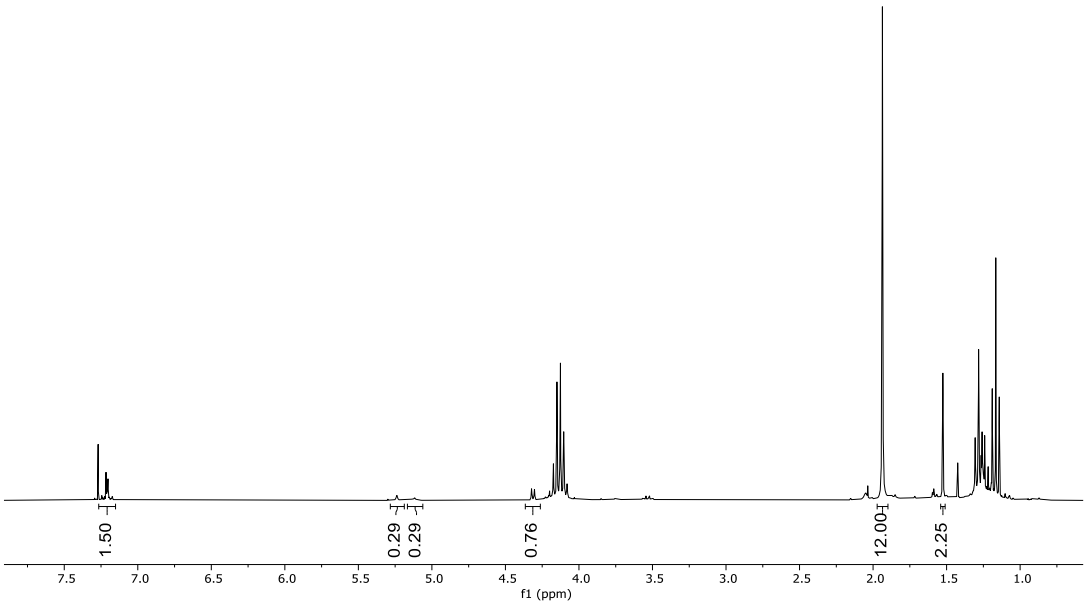


1a **2a**, 59% **13**, 22%

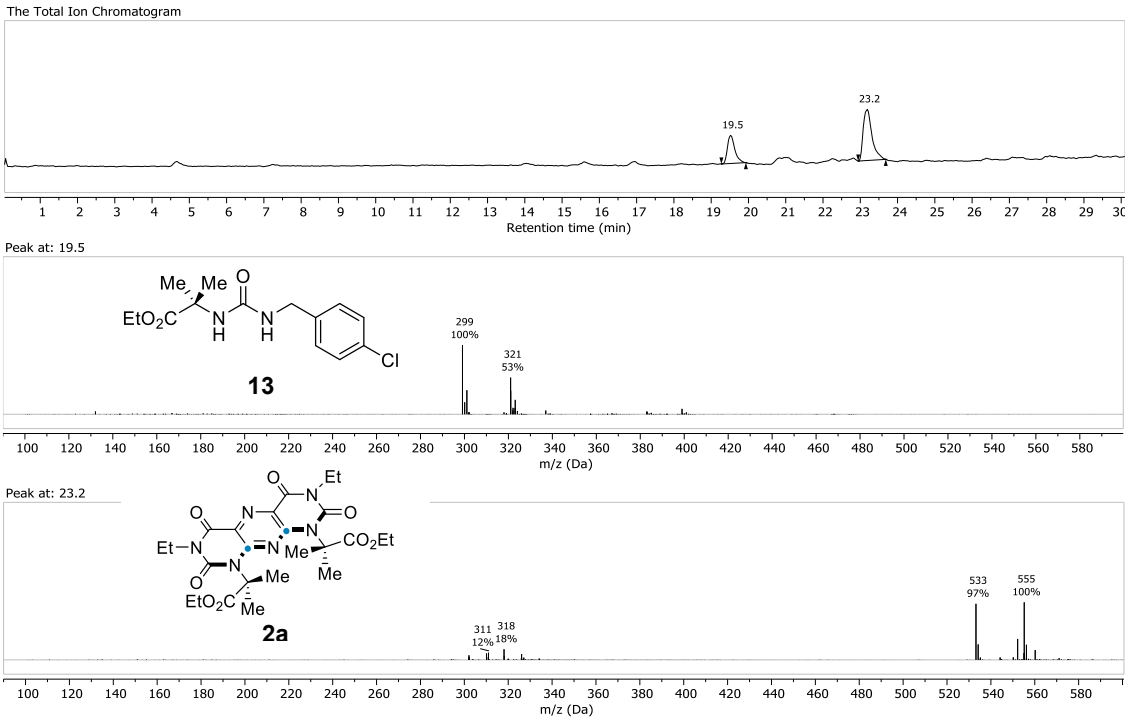
To investigate the mass balance, efforts were made to identify possible side reactions. One side reaction that was considered is the cleavage of the urea moiety of **1a**, resulting in the corresponding amide and isocyanate. To verify this hypothesis, 4-chlorobenzylamine was added to trap the isocyanate as the corresponding urea.

(*i*) The cyclization was performed with **1a** according to the general procedure (0.2 mmol scale).

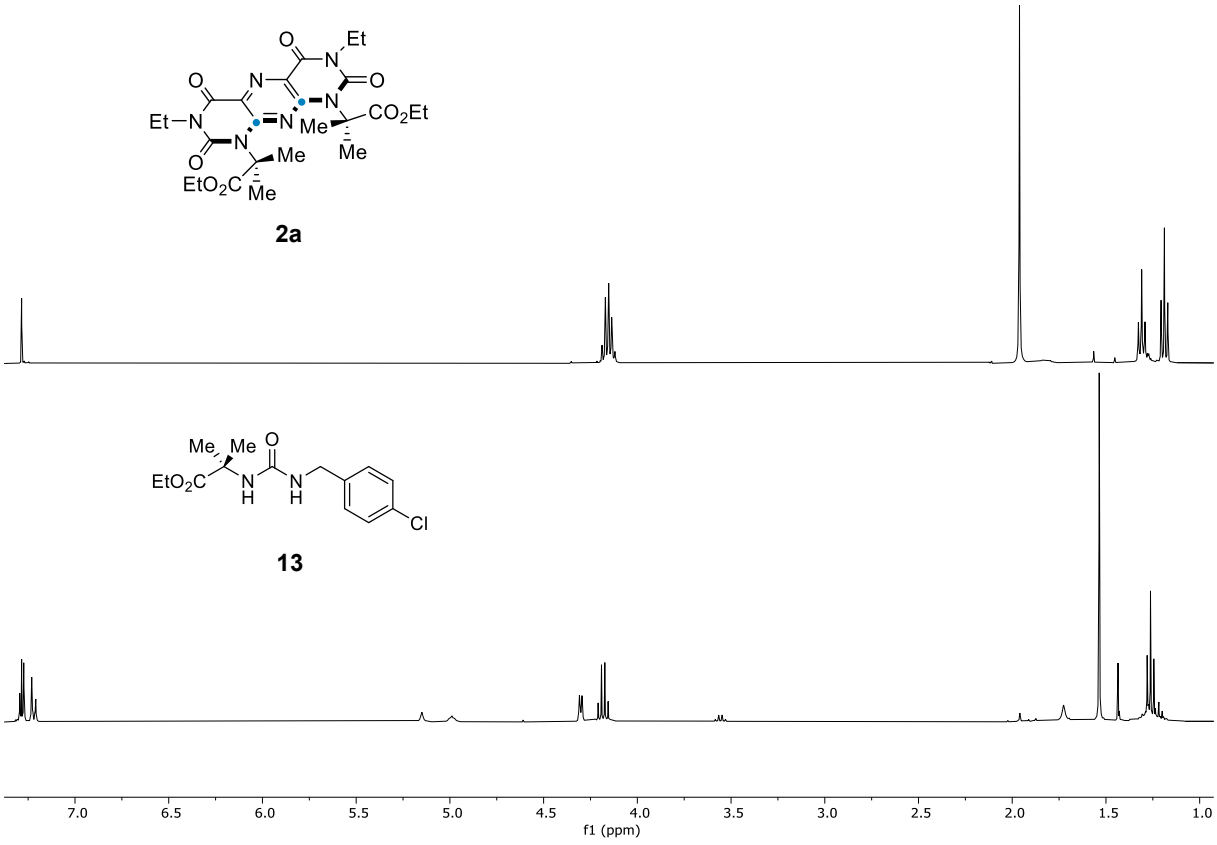
(*ii*) After the reaction time of 30 minutes, 4-chlorobenzylamine (57.1 mg, 403 µmol, 2.01 equiv) was added and the reaction mixture was stirred at room temperature for an additional 30 minutes. The crude reaction mixture was directly purified by column chromatography (*n*-pentane:ethyl acetate = 1:1) and PPT **2a** and the chlorobenzyl urea **13** were isolated together (76.6 mg). The ratio and individual yield was determined by 1H-NMR (13.3 mg, 22%, **13**) (63.3 mg, 59%,) comparing the integral from the *gem*-dimethyl groups. The spectrum from which the yield is calculated is displayed and the the total ion chromatogram and the individual mass spectra are displayed *vide infra*.



Spectrum of the mixed fraction **2a** and **13**; ^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm).



Total ion chromatogram and the individual mass spectra of the mixed fraction PPT **2a** and urea **13**; HPLC (Agilent, SB-C18, Water(0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI)

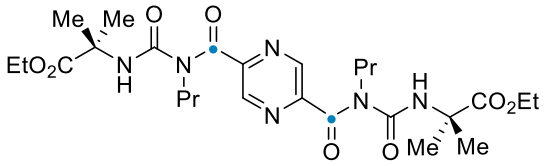


Spectra of the isolated materials **2a** and **13**; ^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm).

6.1 Structure-reactivity relationship of radical cyclization

To elucidate the structure-reactivity relationship of the radical amidation for heteroarene synthesis, a subset of different derivatives was synthesized.

Synthesis of diethyl 2,2'-((((pyrazine-2,5-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis(azanediyl))bis(2-methylpropanoate) (1z)



1z

Following the general procedure GP2, using 2,5-pyrazinedicarboxylic acid (168 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea **S1a** (505 mg, 2.50 mmol, 2.50 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1), afforded the title compound **1z** (458 mg, 854 µmol, 85% yield) as a yellow solid.

R_f = 0.19 (*n*-pentane:ethyl acetate = 2:1, UV)

m.p. = 139-140 °C

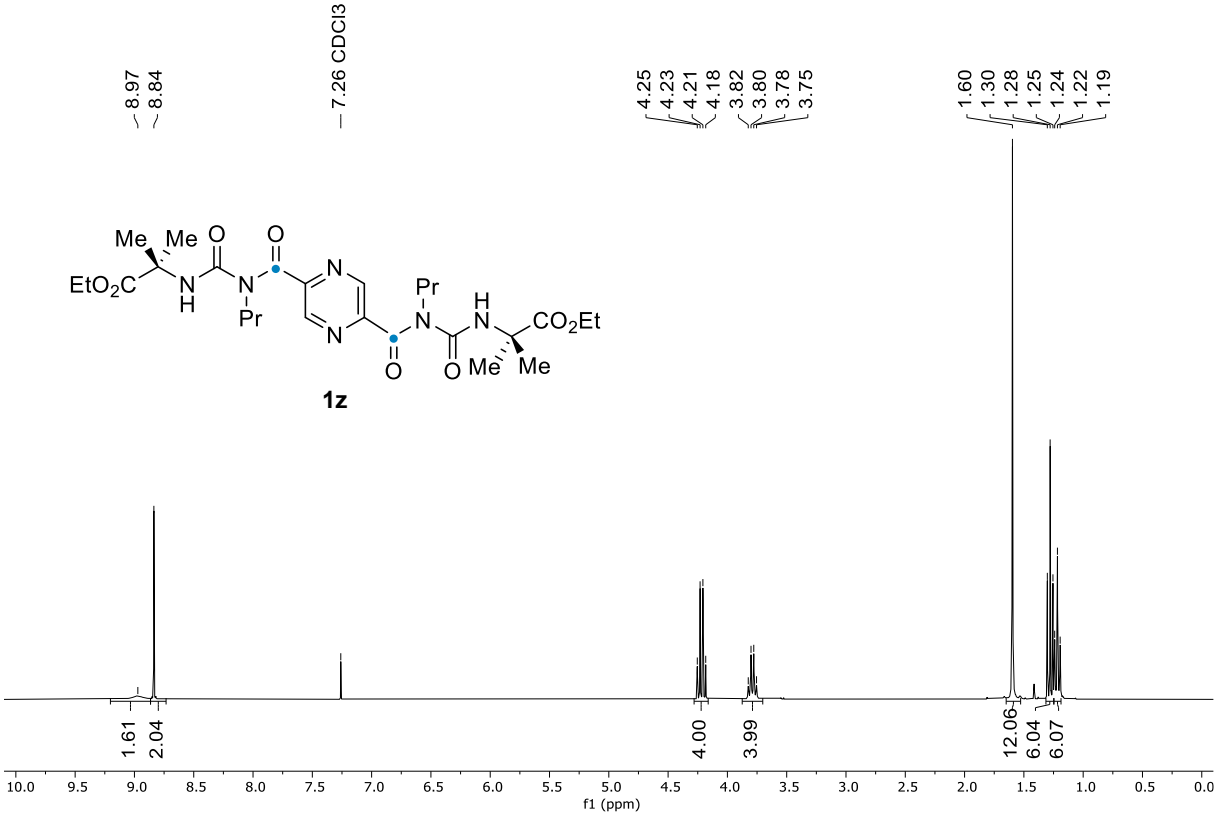
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.97 (bs, 2H), 8.84 (s, 2H), 4.22 (q, *J* = 7.1 Hz, 4H), 3.79 (q, *J* = 7.0 Hz, 4H), 1.60 (s, 12H), 1.28 (t, *J* = 7.1 Hz, 6H), 1.22 (t, *J* = 6.9 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.2, 169.0, 152.6, 150.2, 142.2, 61.7, 57.1, 41.5, 25.0, 15.0, 14.3.

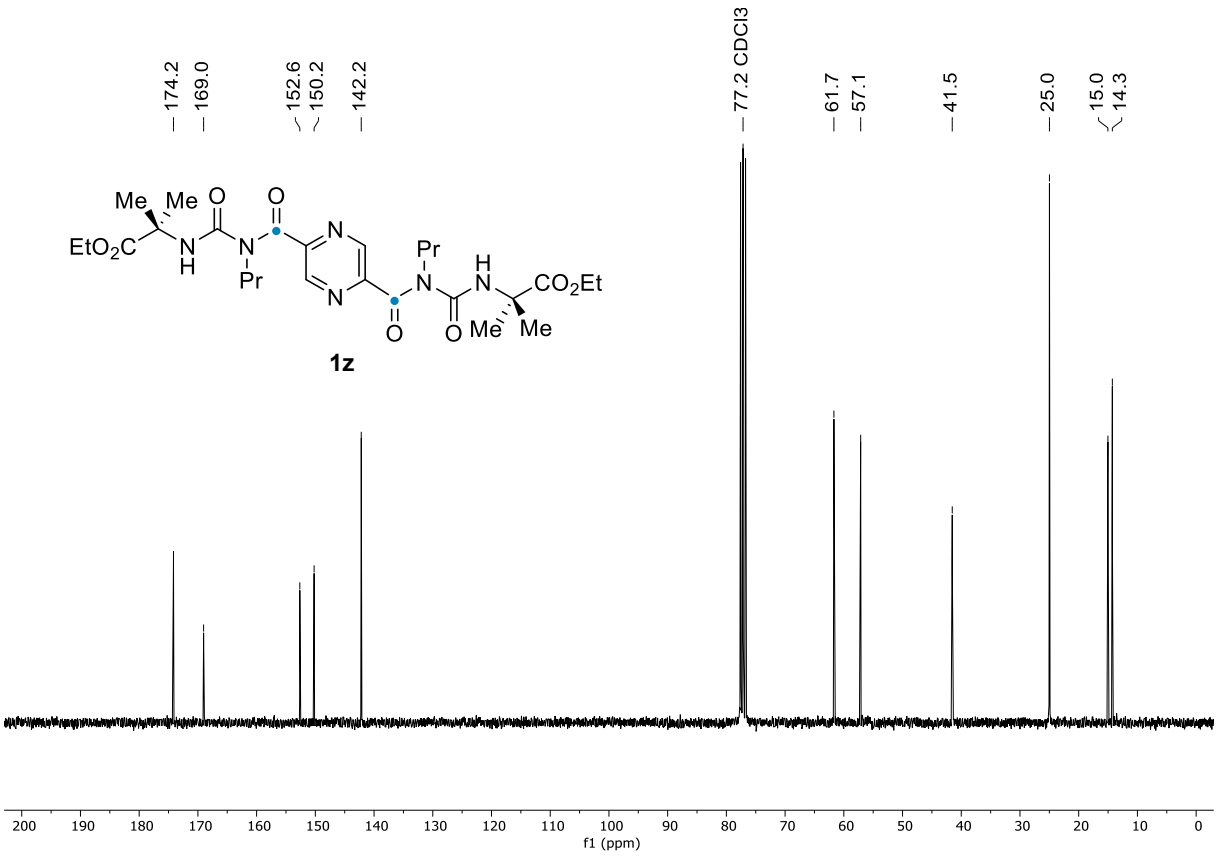
MS (ESI): *m/z* (%) = 559 (100, $[M+Na]^+$), 380 (21), 223 (29).

HRMS (ESI-TOF) : *m/z* $[M+Na]^+$ calcd. for $[C_{24}H_{36}N_6O_8Na]^+$: 559.2487; found: 559.2488 (Error PPM: 0.2).

IR (ATR, neat, ṽ) = 3305 (w), 2985 (w), 2940 (w), 1739 (s), 1660 (vs), 1527 (vs), 1466 (s), 1456 (s), 1380 (vs), 1364 (s), 1340 (w), 1293 (s), 1276 (vs), 1264 (vs), 1221 (s), 1201 (m), 1172 (vs), 1148 (vs), 1091 (s), 1070 (vs), 1026 (vs), 966 (w), 926 (m), 889 (w), 854 (m), 818 (s), 765 (m), 744 (s), 622 (m), 559 (s), 526 (w), 432 (vs) cm^{-1}.

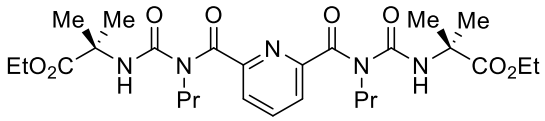


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



13C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-((((pyridine-2,6-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis(azanediyl))bis(2-methylpropanoate) (S16)



S16

Following the general procedure GP2, using 2,6-pyridinedicarboxylic acid (251 mg, 1.50 mmol, 1.00 equiv.) and the corresponding urea **S1a** (759 mg, 3.75 mmol, 2.50 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1 to 1:1), afforded the title compound S**16** (671 mg, 1.25 mmol, 84% yield) as an off white solid.

R_f = 0.34 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. = 92-96 °C

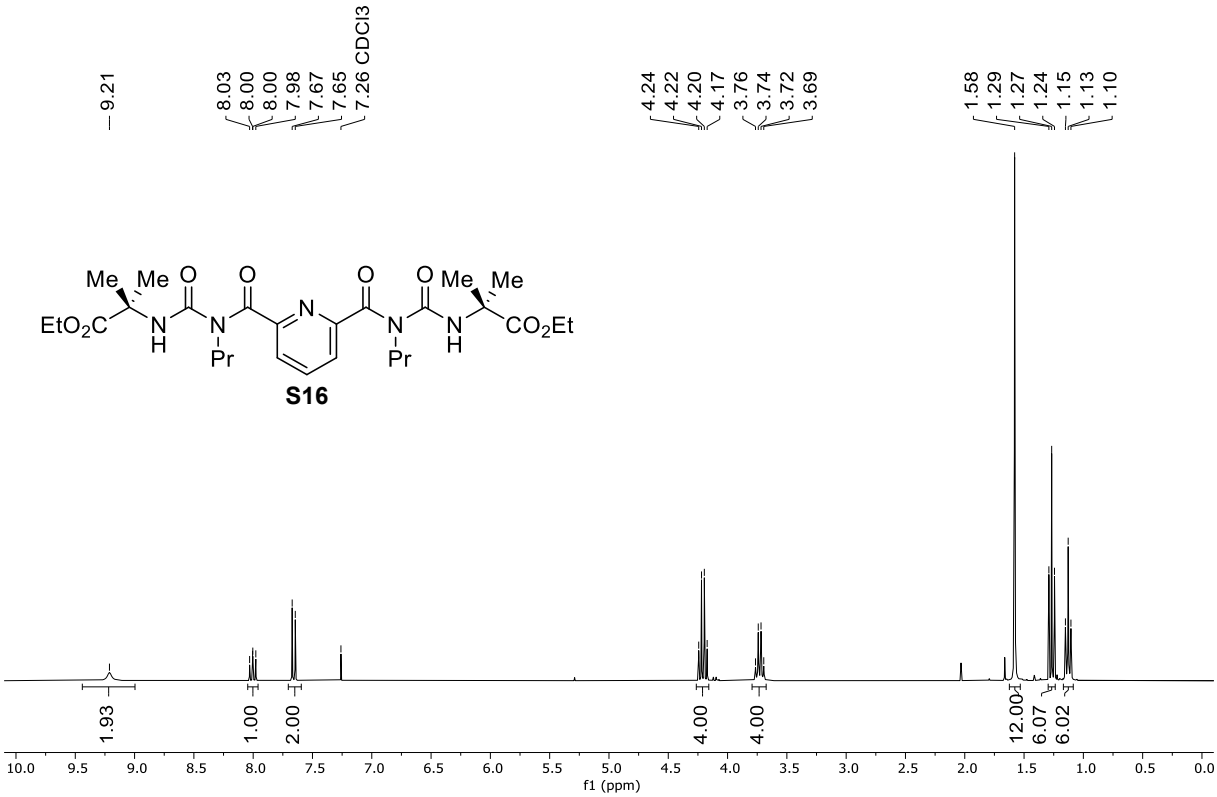
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.21 (bs, 2H), 8.06 – 7.95 (m, 1H), 7.66 (d, *J* = 7.8 Hz, 2H), 4.21 (q, *J* = 7.1 Hz, 4H), 3.73 (q, *J* = 6.9 Hz, 4H), 1.58 (s, 12H), 1.27 (t, *J* = 7.1 Hz, 6H), 1.13 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.2, 171.0, 153.2, 152.8, 138.9, 123.7, 61.6, 57.0, 41.4, 25.1, 15.0, 14.3.

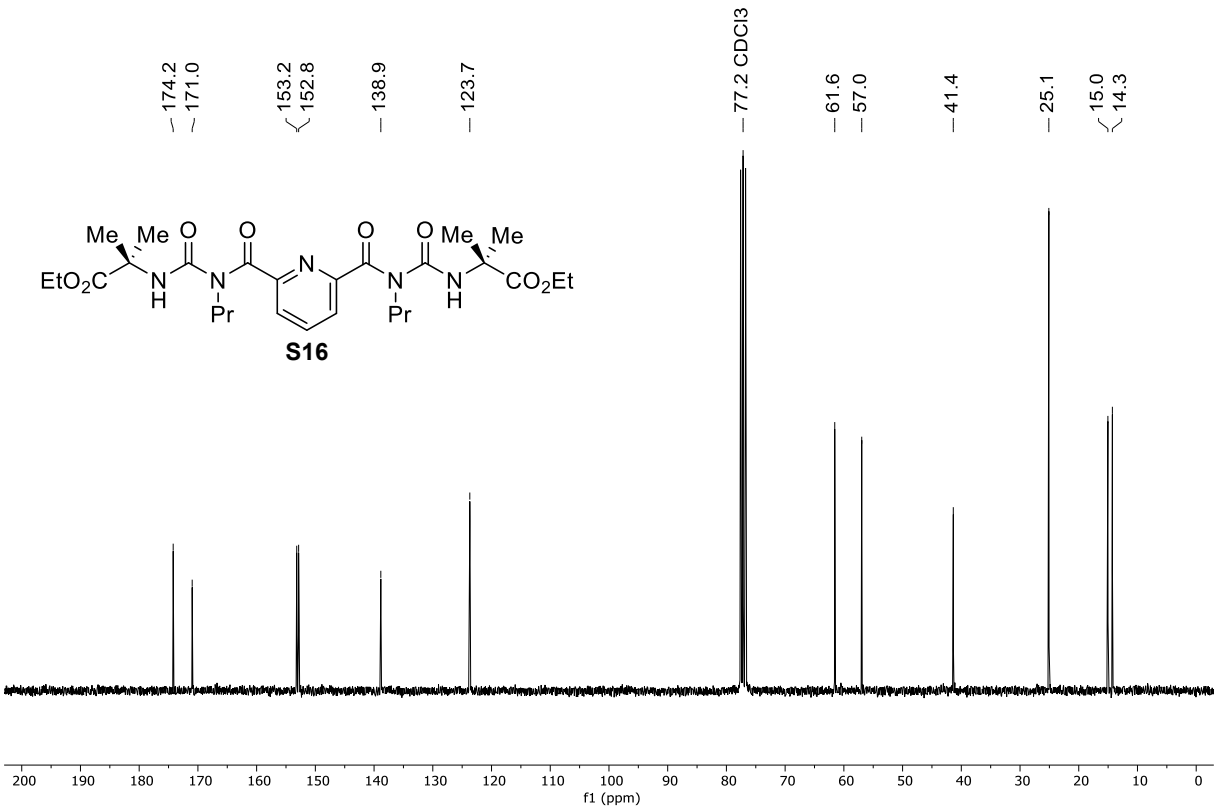
MS (ESI): *m/z* (%) = 558 (100, $[M+Na]^+$), 405 (76), 379, (19) 296 (12), 288 (22), 222 (11).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{25}H_{37}N_5O_8Na]^+$: 558.2534; found: 558.2541 (Error PPM: 1.3).

IR (ATR, neat, $\tilde{\nu}$) = 3364 (w), 2981 (w), 2936 (w), 1696 (vs), 1670 (vs), 1656 (vs), 1590 (w), 1515 (vs), 1468 (m), 1450 (s), 1415 (w), 1384 (s), 1370 (s), 1358 (vs), 1315 (s), 1283 (s), 1270 (vs), 1250 (vs), 1215 (m), 1179 (vs), 1162 (vs), 1091 (s), 1068 (vs), 1028 (s), 995 (m), 942 (w), 928 (w), 881 (w), 869 (m), 838 (s), 820 (m), 797 (w), 765 (m), 752 (vs), 697 (s), 646 (w), 626 (m), 597 (w), 567 (s), 524 (m), 502 (m), 463 (m), 436 (w), 416 (s) cm^{-1}.

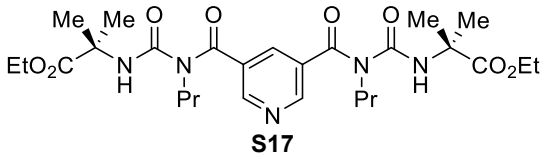


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 2,2'-((((pyridine-3,5-dicarbonyl)bis(ethylazanediyl))bis(carbonyl))bis(azanediyl))bis(2-methylpropanoate) (S17)



S17

Following the general procedure GP2, using 3,5-pyridinedicarboxylic acid (167 mg, 1.00 mmol, 1.00 equiv.) and the corresponding urea **S1a** (506 mg, 2.50 mmol, 2.50 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = 2:1 to 1:1), afforded the title compound S**17** (73.0 mg, 136 µmol, 14% yield) as a colourless resin.

R_f = 0.18 (*n*-pentane:ethyl acetate = 1:1, UV)

m.p. =N/A

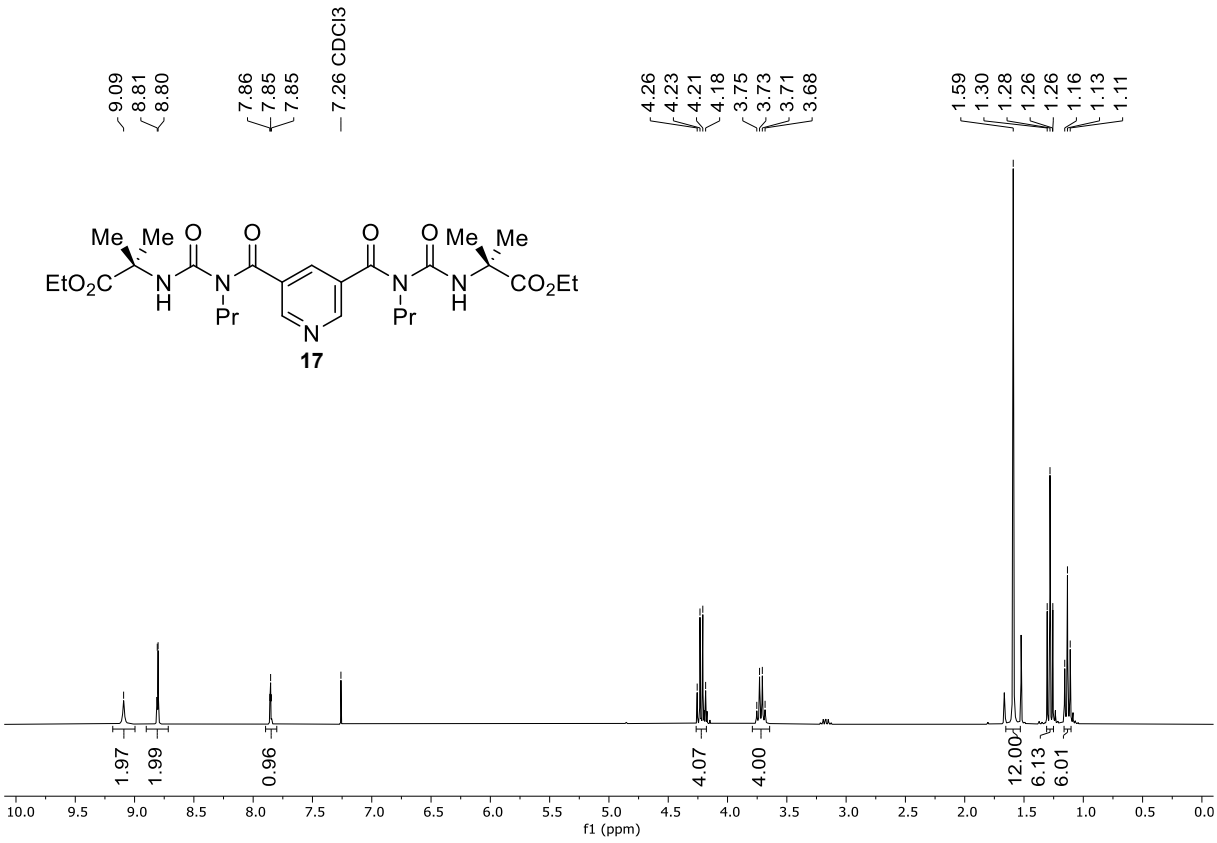
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.09 (bs, 2H), 8.81 (d, *J* = 2.1 Hz, 2H), 7.85 (t, *J* = 2.1 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 4H), 3.72 (q, *J* = 7.0 Hz, 4H), 1.59 (s, 12H), 1.28 (t, *J* = 7.1 Hz, 6H), 1.13 (t, *J* = 6.9 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.1, 170.9, 152.5, 148.2, 132.4, 131.7, 61.7, 57.1, 42.1, 25.1, 15.1, 14.3.

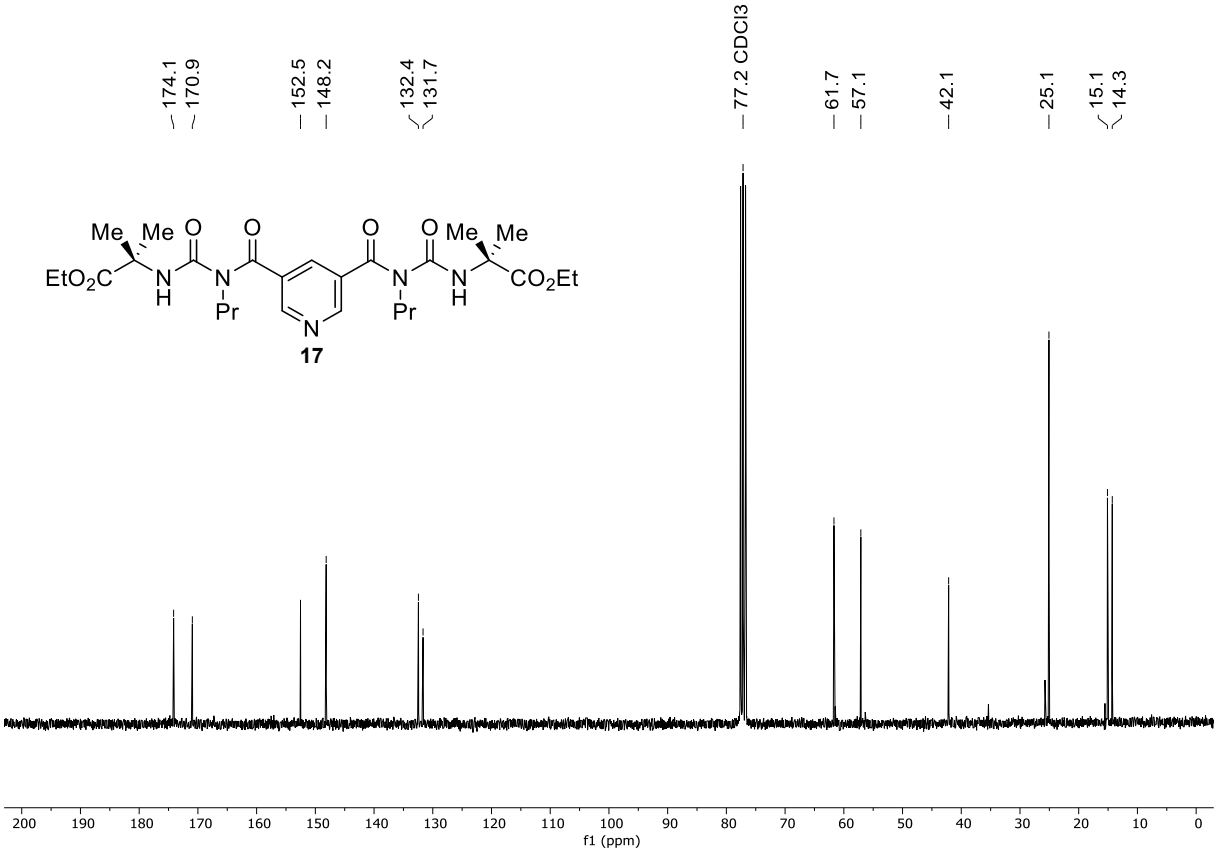
MS (ESI): *m/z* (%) = 558 (18, $[M+Na]^+$) 536 (100, $[M+H]^+$).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{25}H_{38}N_5O_8]^+$: 536.2715; found: 536.2726 (Error PPM: 2.1).

IR (ATR, neat, $\tilde{\nu}$) = 3307 (w), 2983 (w), 2938 (w), 2910 (vw), 2875 (vw), 1737 (s), 1703 (vs), 1650 (vs), 1595 (w), 1515 (vs), 1454 (m), 1380 (vs), 1366 (s), 1276 (vs), 1254 (vs), 1221 (s), 1172 (vs), 1146 (vs), 1091 (s), 1064 (vs), 1026 (s), 940 (w), 916 (w), 860 (w), 818 (w), 789 (w), 771 (m), 744 (m), 722 (w), 691 (w), 626 (m), 604 (m), 544 (m), 461 (w) cm^{-1}.

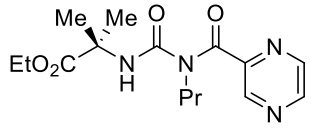


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm.

Ethyl 2-(3-ethyl-3-(pyrazine-2-carbonyl)ureido)-2-methylpropanoate (18)



18

Following general procedure GP2', using pyrazine-2-carboxylic acid (62.0 mg, 0.50 mmol, 1.00 equiv.), the corresponding urea **S1a** (126 mg, 0.62 mmol, 1.25 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 3:1), afforded the title compound **18** (135 mg, 0.44 mmol, 87% yield) as a colorless crystalline solid.

$\mathbf{R}_f$ = 0.20 (*n*-pentane:ethyl acetate = 3:1, UV)

m.p. = 66-70 °C

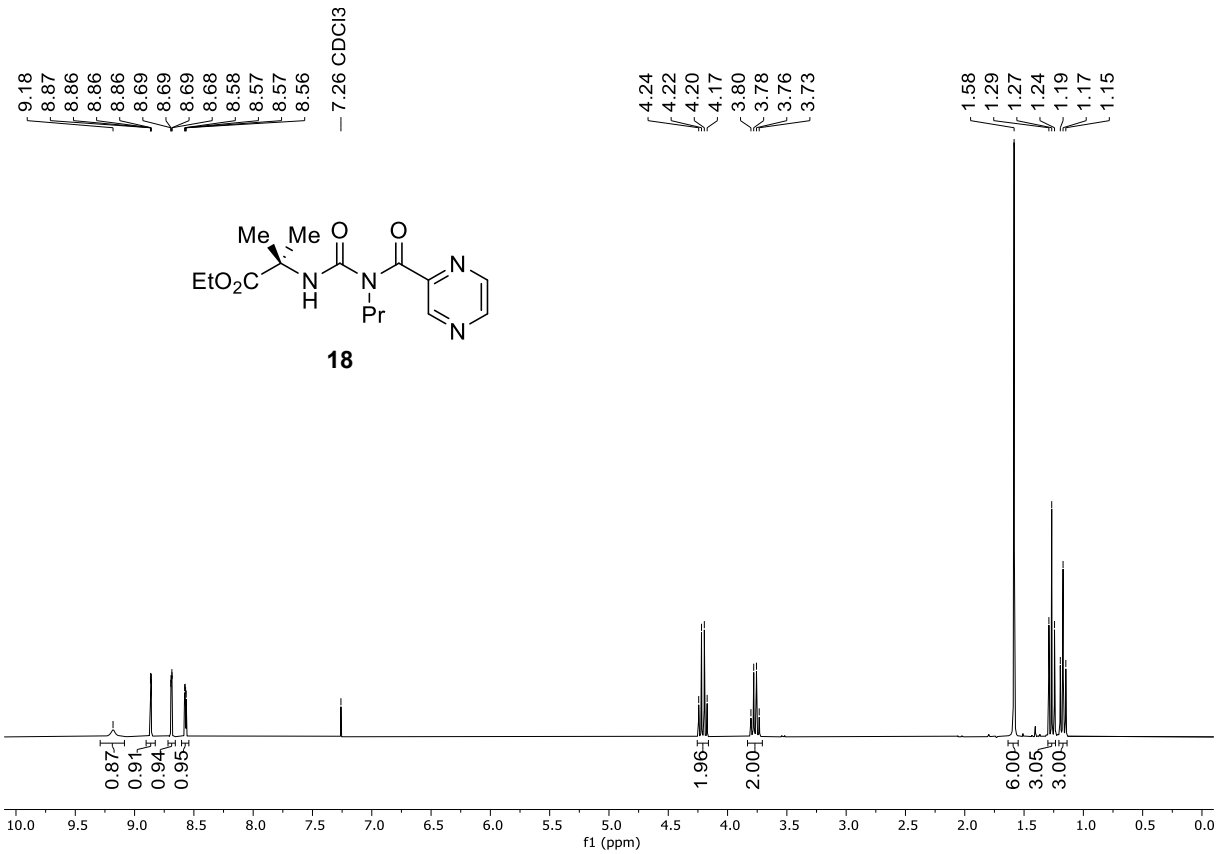
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.18 (s, 1H), 8.90 – 8.83 (m, 1H), 8.72 – 8.66 (m, 1H), 8.57 (dd, *J* = 2.5, 1.5 Hz, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.77 (q, *J* = 7.0 Hz, 2H), 1.58 (s, 6H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.17 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, Chloroform-d [77.2 ppm], ppm) δ = 174.1, 169.9, 152.8, 149.8, 146.1, 144.2, 142.9, 61.6, 57.0, 41.4, 25.1, 15.0, 14.3.

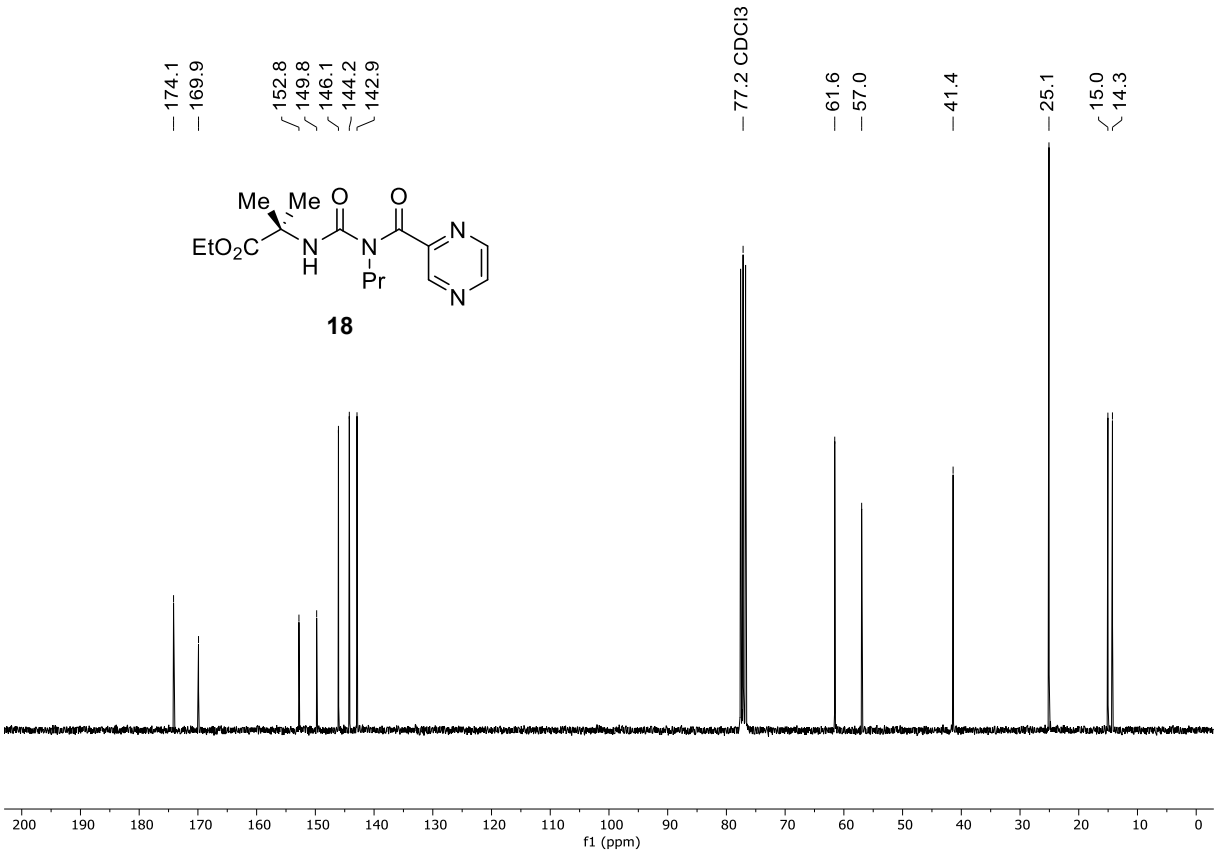
MS (ESI): *m/z* (%) = 331 (100, $[M+Na]^+$), 309 (81, $[M+H]^+$), 152 (35).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{14}H_{20}N_4O_4Na]^+$: 331.1377; found: 331.1384 (Error PPM: 2.1).

IR (ATR, neat, $\tilde{\nu}$) = 3315 (w), 3052 (w), 2987 (w), 2942 (w), 1739 (s), 1698 (s), 1692 (s), 1650 (vs), 1578 (w), 1529 (vs), 1468 (w), 1456 (m), 1417 (m), 1387 (m), 1368 (vs), 1356 (s), 1315 (s), 1295 (m), 1262 (vs), 1234 (s), 1213 (w), 1185 (m), 1154 (vs), 1113 (m), 1091 (s), 1073 (s), 1044 (m), 1026 (vs), 981 (w), 964 (w), 936 (w), 895 (s), 858 (vs), 824 (w), 781 (w), 767 (vs), 732 (w), 716 (m), 634 (s), 618 (m), 573 (m), 551 (s), 510 (w), 461 (m), 414 (vs) cm^{-1}.

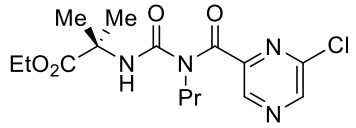


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (100 MHz, Chloroform-d [77.2 ppm], ppm)

Synthesis of ethyl 2-(3-(6-chloropyrazine-2-carbonyl)-3-ethylureido)-2-methylpropanoate (19)



19

Following general procedure GP2', using 6-chloropyrazine-2-carboxylic acid (159 mg, 1.00 mmol, 1.00 equiv), the corresponding urea **S1a** (253 mg, 1.25 mmol, 1.25 equiv), and purification by column chromatography (*n*-pentane:ethyl acetate = = 3:1 to 4:1), afforded the title compound **19** as an off-white solid.

R_f = 0.30 (*n*-pentane:ethyl acetate = 4:1, UV)

m.p. = 98-102 °C

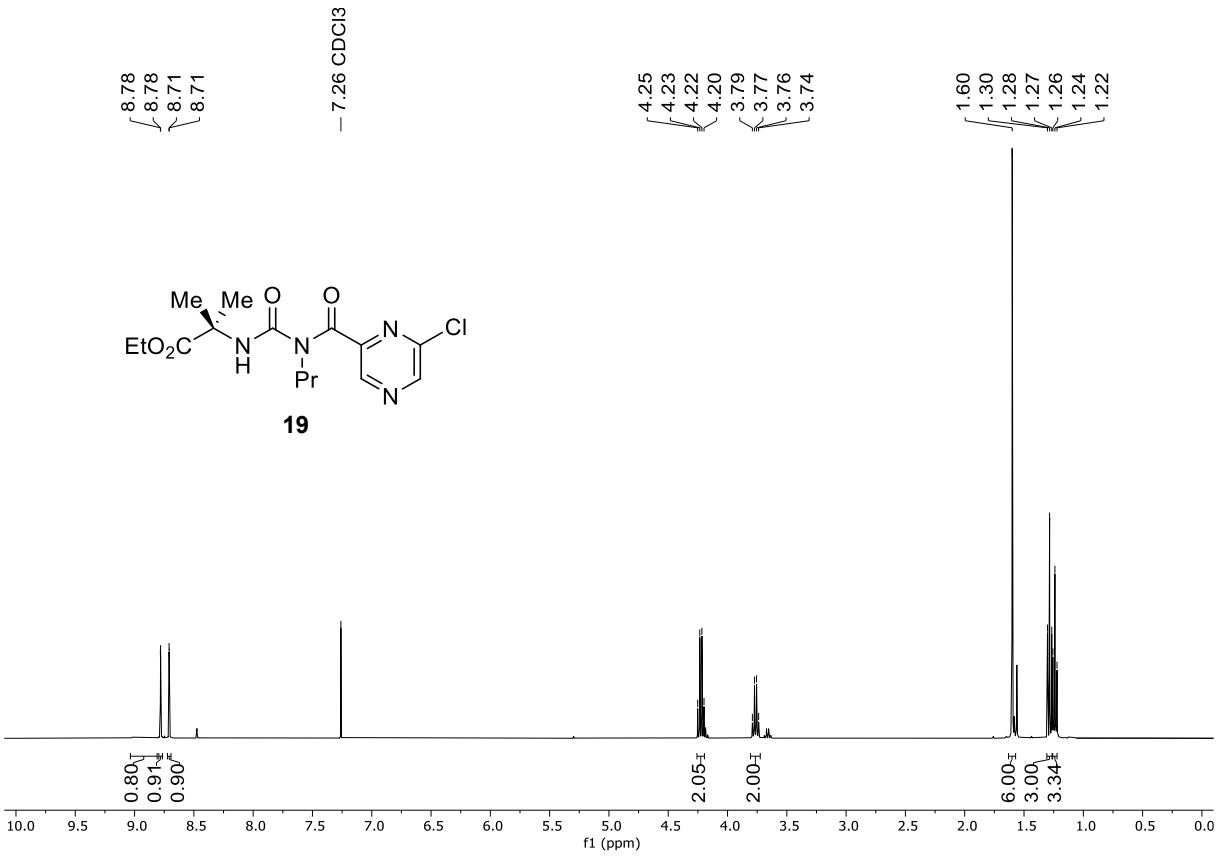
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.04 – 8.81 (bs, 1H), 8.78 (d, *J* = 0.6 Hz, 1H), 8.71 (d, *J* = 0.5 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 3.76 (q, *J* = 7.0 Hz, 2H), 1.60 (s, 6H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.2, 168.2, 164.9, 152.6, 148.6, 147.8, 146.2, 141.7, 61.7, 57.1, 41.6, 25.0, 14.9, 14.3.

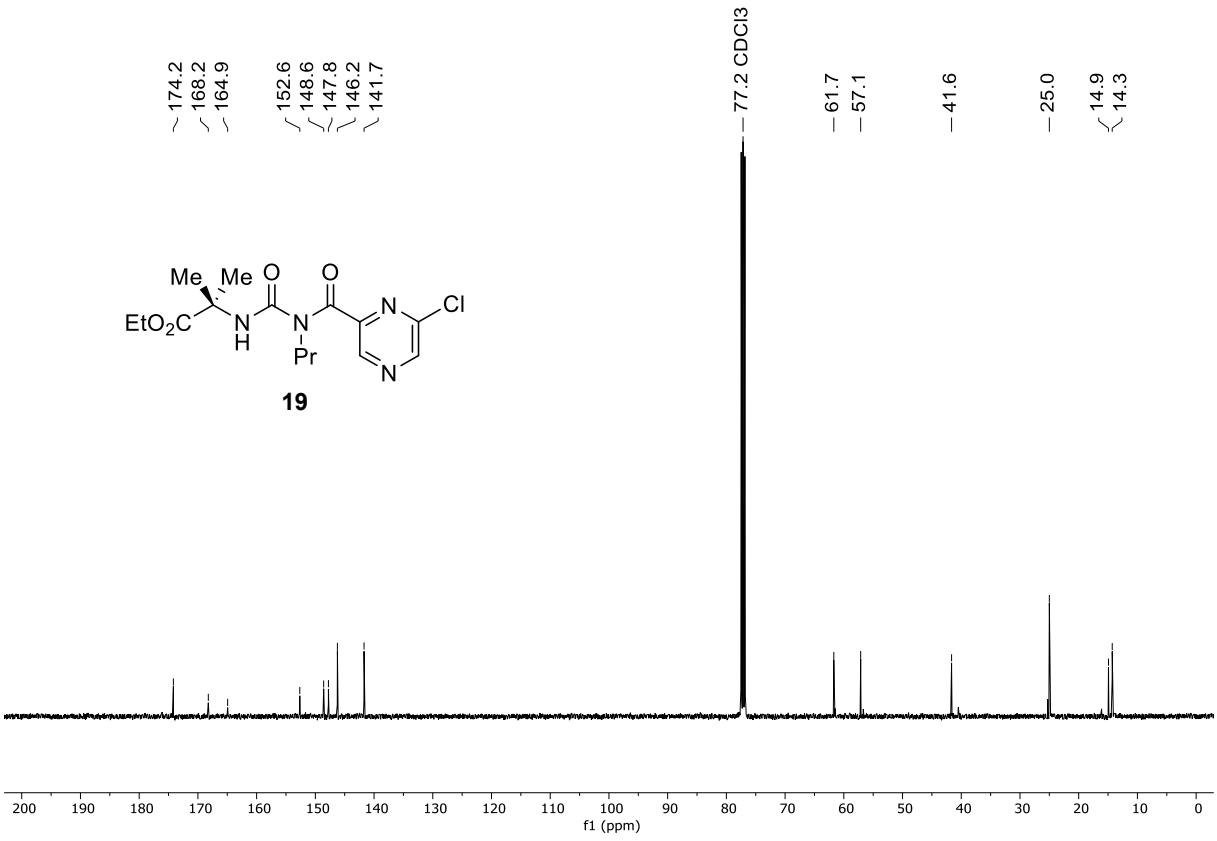
MS (ESI): *m/z* (%) = 365 (63, $[M+Na]^+$) 343 (100, $[M+H]^+$) 186 (7).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{14}H_{29}ClN_4O_4Na]^+$: 365.0987; found: 365.0996 (Error PPM: 2.5).

IR (ATR, $\tilde{\nu}$) = 3315 (w), 2983 (w), 2938 (w), 1737 (s), 1684 (vs), 1650 (vs), 1531 (vs), 1470 (m), 1456 (m), 1403 (m), 1384 (m), 1366 (vs), 1311 (s), 1289 (m), 1264 (vs), 1219 (vs), 1156 (vs), 1132 (vs), 1089 (vs), 1070 (vs), 1028 (s), 1013 (vs), 964 (w), 930 (w), 911 (m), 901 (s), 887 (w), 860 (m), 828 (m), 805 (s), 763 (vs), 748 (m), 724 (w), 616 (s), 589 (m), 559 (m), 477 (m), 438 (vs) cm^{-1}.

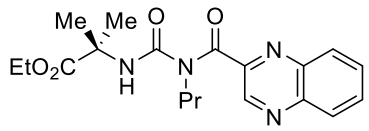


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl 2-(3-ethyl-3-(quinoxaline-2-carbonyl)ureido)-2-methylpropanoate (20)



20

Following general procedure GP2', using quinoxaline-2-carboxylic acid (435 mg, 2.50 mmol, 1.00 equiv.), the corresponding urea **S1a** (632 mg, 3.12 mmol, 1.25 equiv.), and purification by column chromatography (*n*-pentane:ethyl acetate = 3:1), afforded the title compound **20** (826 mg, 2.30 mmol, 92% yield) as a yellow solid.

R_f = 0.27 (*n*-pentane:ethyl acetate = 3:1, UV)

m.p. = 106-108 °C

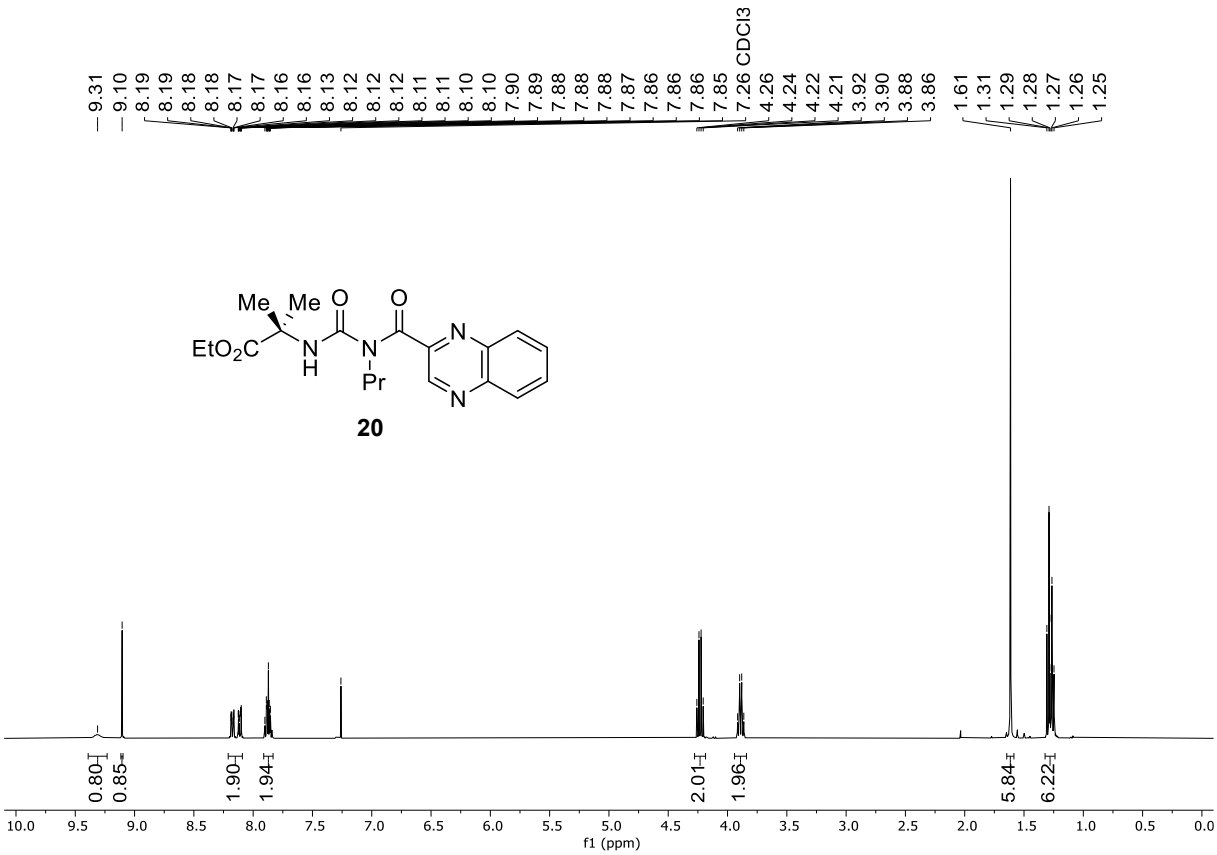
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.31 (bs, 1H), 9.11 (s, 1H), 8.19–8.10 (m, 2H), 7.89–7.85 (m, 2H), 4.23 (q, *J* = 7.1 Hz, 2H), 3.89 (q, *J* = 7.0 Hz, 2H), 1.61 (s, 6H), 1.31–1.25 (m, 6H).

^{13}C NMR (100 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.2, 170.4, 152.8, 148.3, 143.8, 142.8, 140.3, 131.7, 131.3, 129.9, 129.6, 61.6, 57.0, 41.6, 25.2 (2C), 15.3, 14.3.

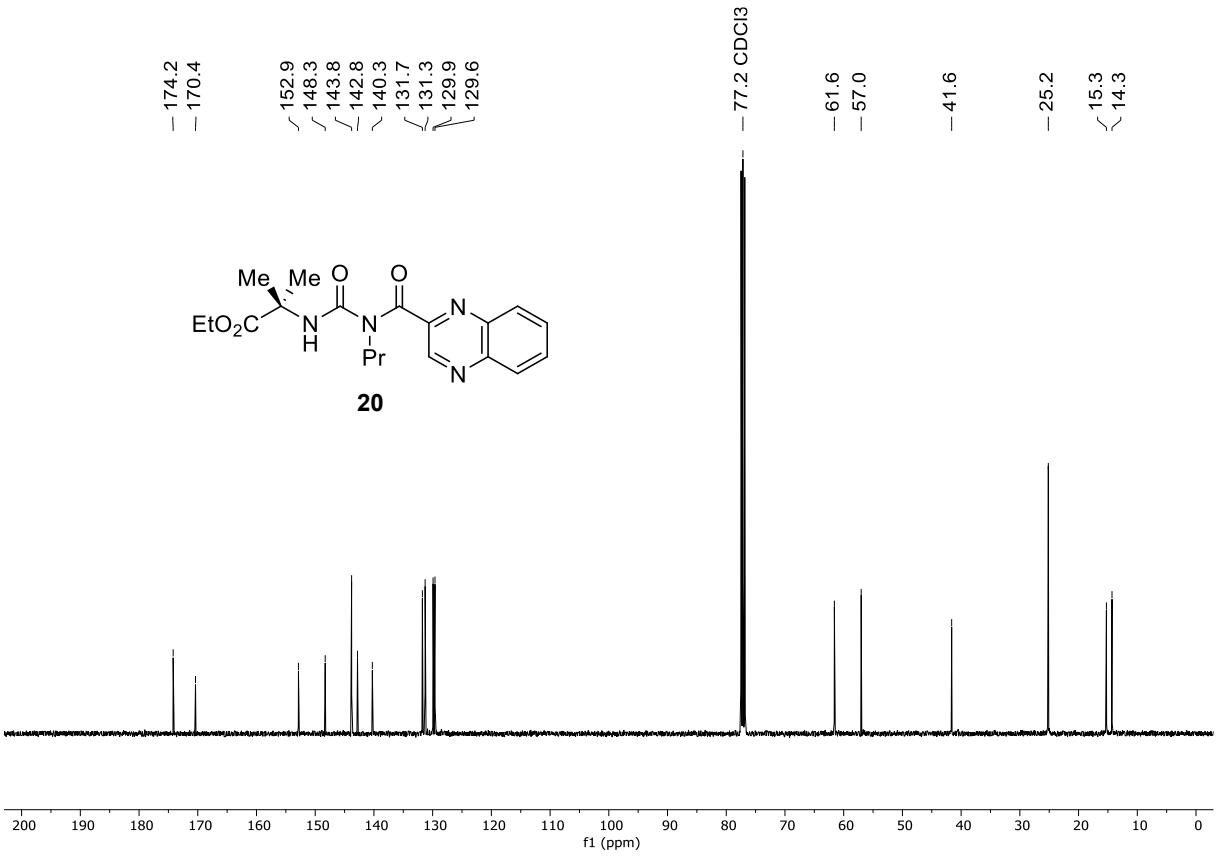
MS (ESI): *m/z* (%) = 381 (71, $[M+Na]^+$), 359 (100, $[M+H]^+$) 202 (26).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{18}H_{23}N_4O_4]^+$: 359.1714; found: 359.1713 (Error PPM: -0.3).

IR (ATR, neat, $\tilde{\nu}$) = 3317 (w), 2985 (w), 2975 (w), 1731 (s), 1678 (vs), 1662 (vs), 1570 (w), 1531 (vs), 1491 (m), 1466 (m), 1450 (m), 1415 (w), 1382 (m), 1372 (s), 1362 (vs), 1325 (s), 1295 (vs), 1266 (s), 1244 (s), 1225 (s), 1160 (vs), 1119 (s), 1087 (m), 1066 (vs), 1030 (s), 1013 (m), 971 (s), 952 (w), 938 (m), 903 (m), 883 (w), 865 (w), 848 (m), 812 (s), 775 (vs), 765 (vs), 665 (w), 628 (m), 618 (m), 591 (m), 563 (s), 548 (m), 502 (w), 493 (w), 467 (m), 455 (m), 434 (w), 408 (vs) cm^{-1}.

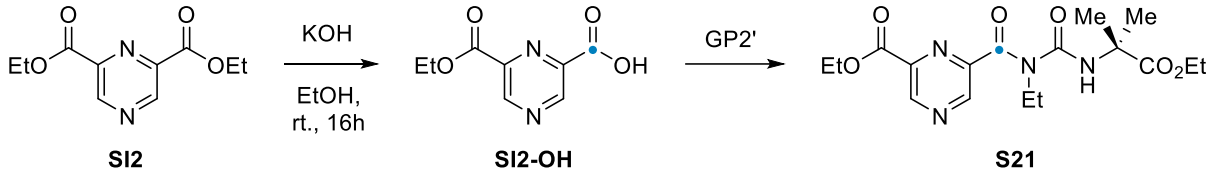


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (100 MHz, Chloroform-d [77.16 ppm], ppm).

Synthesis of ethyl 6-(((1-ethoxy-2-methyl-1-oxopropan-2-yl)carbamoyl)(ethyl)carbamoyl)-pyrazine-2-carboxylate (S21)



(*i*) Adapting a literature known procedure, diethyl pyrazine-2,6-dicarboxylate (**SI2**) (243 mg, 1.08 mmol, 1.00 equiv.) was added to a flask and dissolved in ethanol (10.7 ml, 0.1 M).[14] Subsequently KOH (72.8 mg, 1.30 mmol, 1.20 equiv.) was added and the reaction mixture was stirred at ambient temperature for 16 h. The crude reaction mixture was then evaporated *in vacuo* resulting in the crude 6-(ethoxycarbonyl)pyrazine-2-carboxylic acid (**SI2-OH)**.

(*ii*) Afterwards GP2' was followed, using the corresponding urea **S1a** (271 mg, 1.34 mmol, 1.25 equiv.). Column chromatography (*n*-pentane:ethyl acetate = 2:1) afforded the title compound **S21** (306 mg, 803 µmol, 75% yield) as an off white solid.

R_f = 0.30 (*n*-pentane:ethyl acetate = 2:1, Detection)

m.p. = 92-94 °C

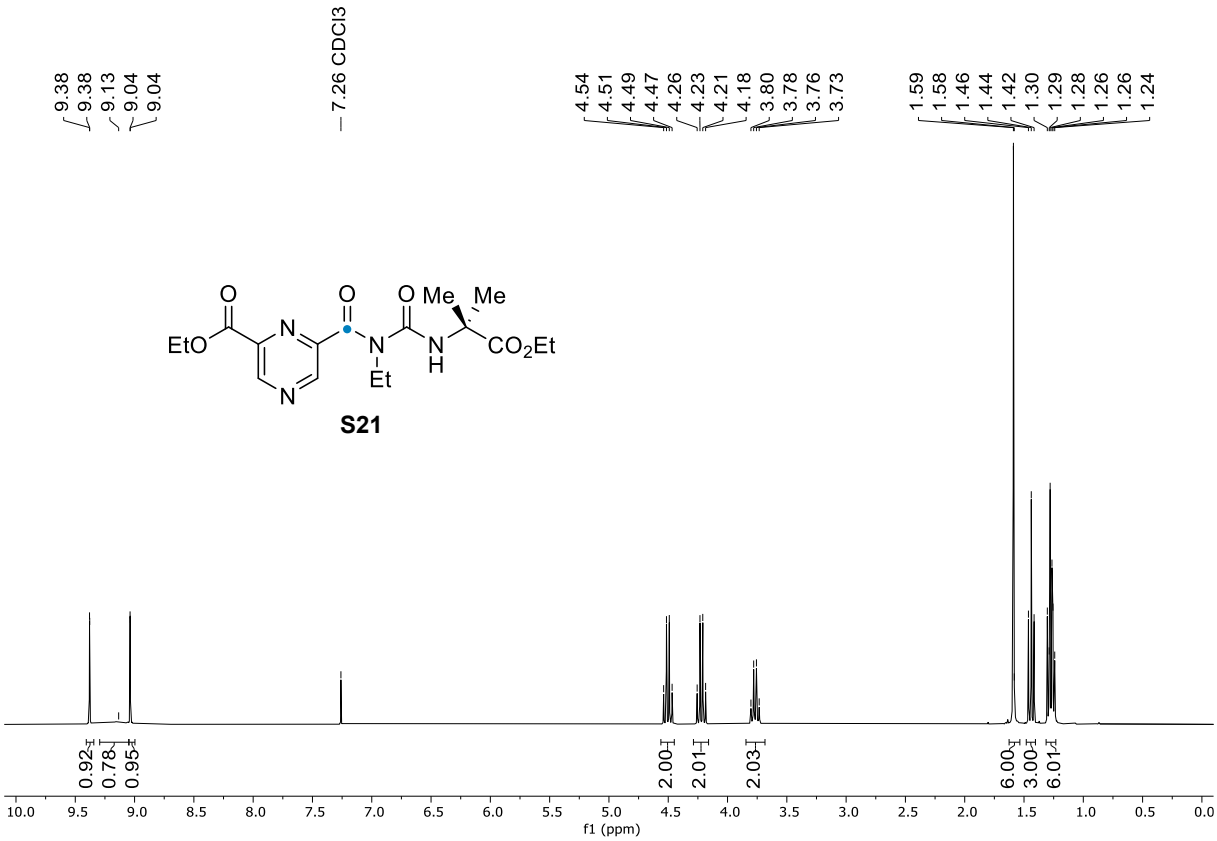
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.38 (d, *J* = 0.5 Hz, 1H), 9.13 (bs, 1H), 9.04 (d, *J* = 0.5 Hz, 1H), 4.50 (q, *J* = 7.1 Hz, 2H), 4.22 (q, *J* = 7.1 Hz, 2H), 3.77 (q, *J* = 7.0 Hz, 2H), 1.59 (s, 6H), 1.44 (t, *J* = 7.1 Hz, 3H), 1.32 – 1.21 (m, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 174.1, 169.1, 163.3, 152.6, 148.9, 147.0, 146.9, 141.4, 62.8, 61.6, 57.0, 41.8, 25.1, 15.0, 14.3, 14.3.

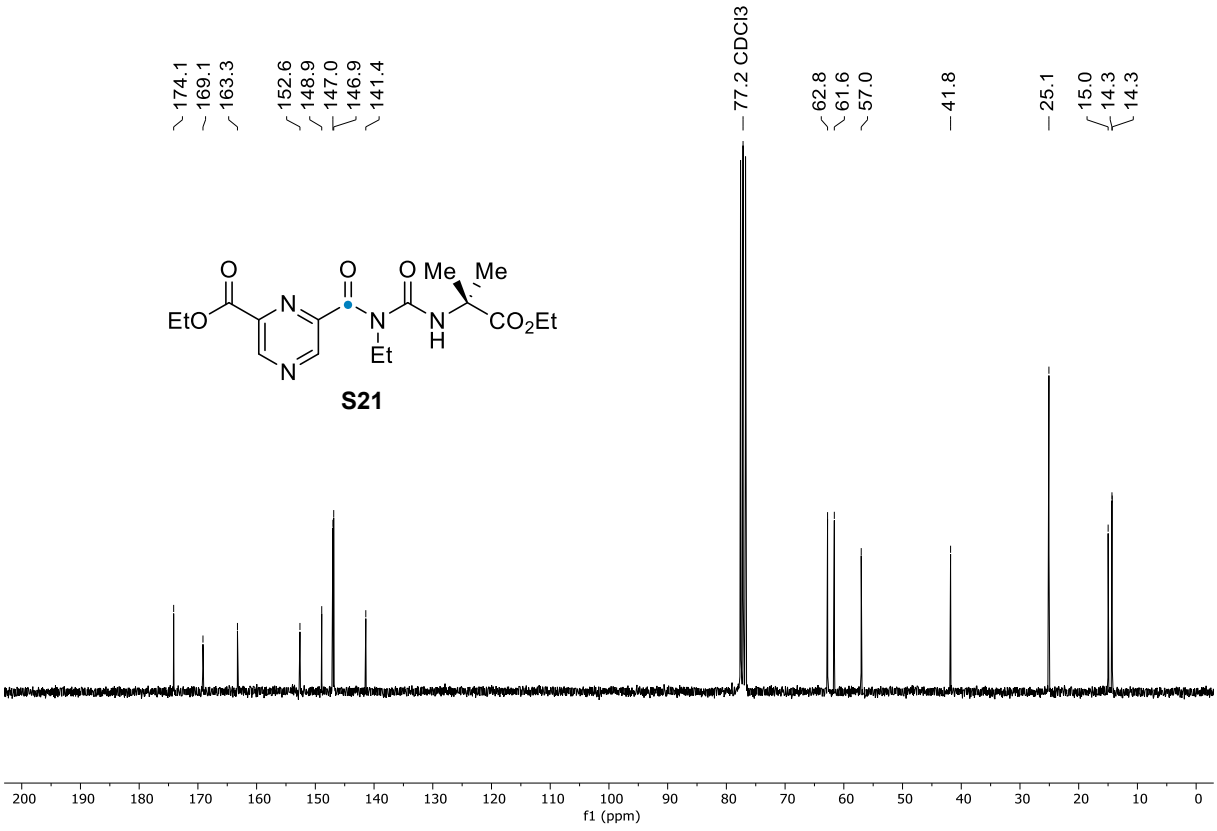
MS (ESI): *m/z* (%) = 403 (100, $[M+Na]^+$), 381 (9, $[M+H]^+$), 324 (14), 224 (28).

HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{17}H_{24}N_4O_6+H]^+$: 381.1769; found: 381.1770 (Error PPM: 0.3).

IR (ATR, neat, $\tilde{\nu}$) = 3346 (w), 2983 (w), 2944 (vw), 1725 (vs), 1668 (vs), 1525 (vs), 1468 (m), 1452 (m), 1433 (w), 1389 (m), 1374 (s), 1362 (vs), 1321 (s), 1303 (vs), 1258 (s), 1225 (w), 1205 (m), 1162 (vs), 1128 (vs), 1091 (s), 1070 (vs), 1034 (s), 1017 (vs), 964 (w), 944 (m), 913 (w), 883 (w), 867 (w), 856 (m), 820 (w), 803 (w), 787 (w), 769 (s), 736 (w), 712 (w), 632 (m), 587 (m), 557 (m), 451 (m), 408 (s) cm^{-1}.

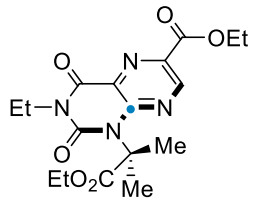


^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of ethyl 1-(1-ethoxy-2-methyl-1-oxopropan-2-yl)-3-ethyl-2,4-dioxo-1,2,3,4-tetrahydro-pteridine-6-carboxylate (21)



21

Following the general procedure GP3, but using 2.5 equiv. of AgF_2 and heating the reaction mixture to 40 °C using the corresponding 6-(carbamoyl)pyrazine-2-carboxylate **S21** (95.0 mg, 250 µmol, 1.00 equiv.) purification by column chromatography (*n*-pentane:ethyl acetate = 2:1) afforded the title compound **21** (63.3 mg, 167 µmol, 67%) as a colorless oil.

R_f = 0.25 (*n*-pentane:ethyl acetate = 2:1, UV)

m.p. = N/A

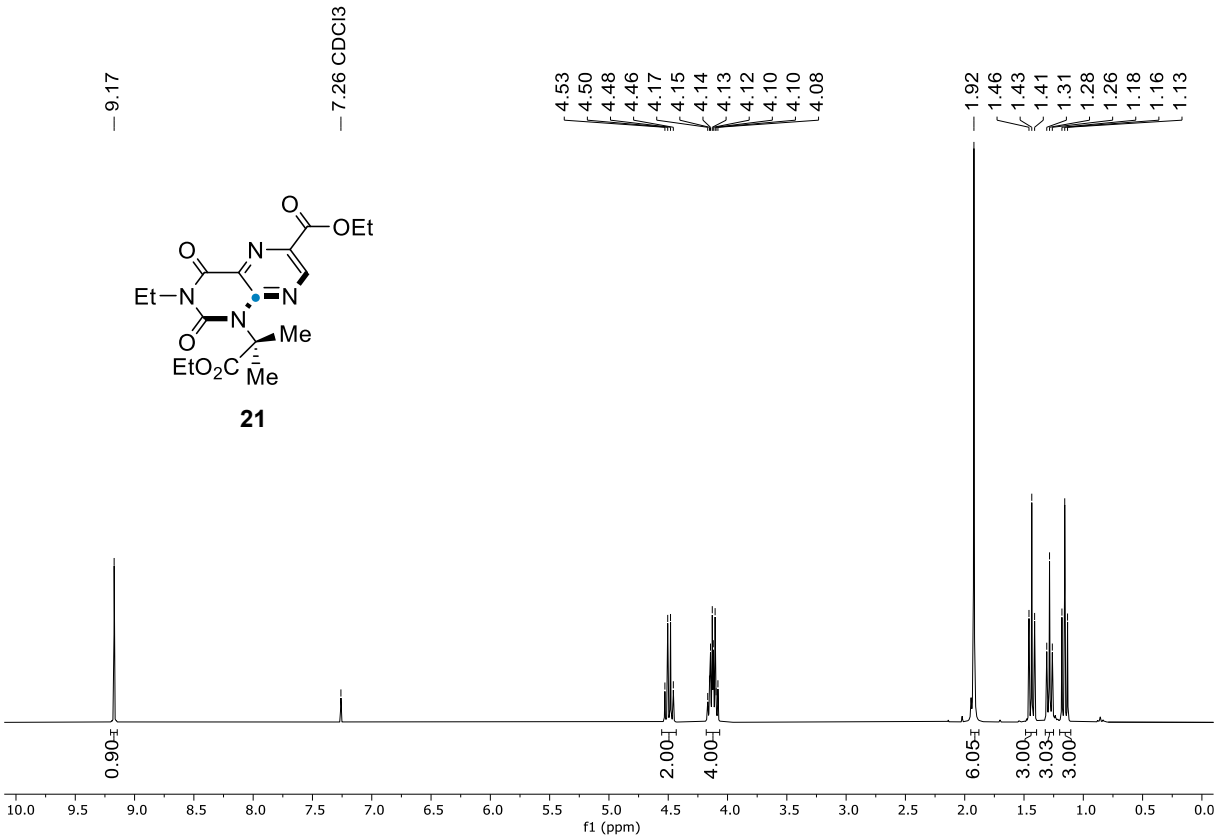
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.17 (s, 1H), 4.49 (q, *J* = 7.1 Hz, 2H), 4.21 – 3.98 (m, 4H), 1.92 (s, 6H), 1.43 (t, *J* = 7.1 Hz, 3H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.16 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 172.8, 163.2, 158.6, 149.8, 149.4, 146.9, 139.1, 127.7, 66.0, 62.7, 61.7, 38.2, 24.9, 14.4, 14.1, 13.0.

MS (GC-EI): *m/z* (%) = 378 (14, $[M]^+$), 305 (80), 234 (100), 192 (26).

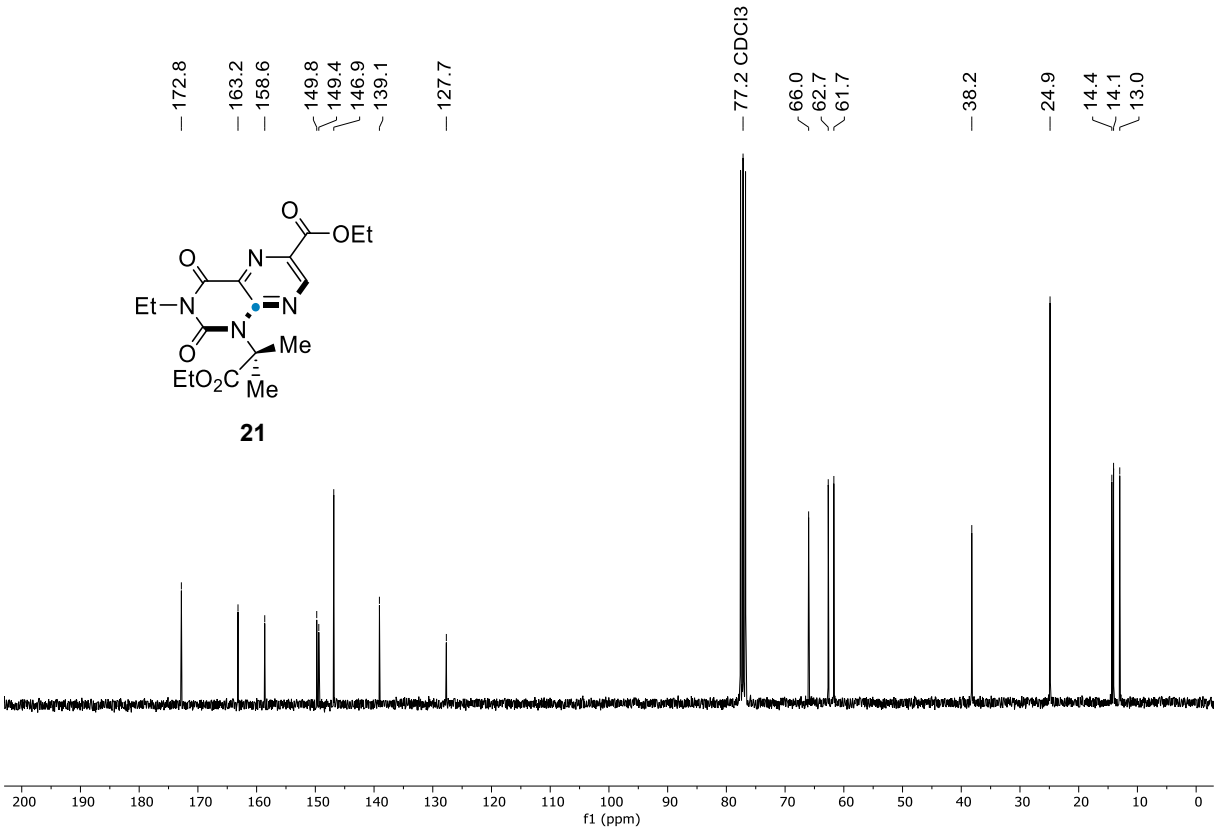
HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{17}H_{22}N_4O_6Na]^+$: 401.1431; found: 401.1427 (Error PPM: -1.0).

IR (ATR, neat, $\tilde{\nu}$) = 2983 (w), 2938 (w), 2908 (vw), 1719 (vs), 1678 (vs), 1574 (w), 1546 (s), 1484 (m), 1458 (m), 1442 (s), 1423 (w), 1370 (vs), 1331 (s), 1317 (m), 1285 (vs), 1240 (vs), 1215 (m), 1172 (vs), 1140 (vs), 1093 (s), 1079 (s), 1026 (s), 958 (m), 860 (m), 834 (w), 816 (m), 801 (w), 783 (w), 756 (vs), 738 (w), 714 (m), 677 (w), 616 (vw), 581 (w), 557 (w), 528 (w), 495 (m), 442 (s) cm^{-1}.



21

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



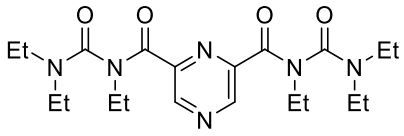
21

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

6.2 Structure-reactivity relationship on C(sp²)–H bond fluorination

Within our study, substrates containing protic N–H bonds exhibited a complete absence of fluorinated products, in contrast to the AgF_2-mediated heteroaromatic fluorination reactions reported by Hartwig and co-workers.[15] To further probe the viability of a direct C(sp²)–H fluorination pathway under our reaction conditions, the reported fluorination reactivity was reproduced using substrates **SI1** and **SI2** which do not contain protic N–H functionalities. In these cases, C(sp²)–H fluorination was successfully observed.

Synthesis of *N*²,*N*⁶-bis(diethylcarbamoyl)-*N*²,*N*⁶-diethylpyrazine-2,6-dicarboxamide (SI1)



SI1

Following the general procedure GP2, using 2,6-pyrazinedicarboxylic acid (1.00 mmol) with purification by column chromatography (*n*-pentane:ethyl acetate = 1:2) afforded the title compound **SI1** (282 mg, 0.67 mmol, 67% yield) as a colorless crystalline solid.

R_f = 0.31 (*n*-pentane:ethyl acetate = 1:2, UV)

m.p. = 72-73 °C

^{1}H NMR (400 MHz, 333 K, Chloroform-d [7.26 ppm], ppm) δ = 9.01 (s, 2H), 3.74 (q, *J* = 7.1 Hz, 4H), 3.23 (q, *J* = 7.1 Hz, 8H), 1.29 (t, *J* = 7.1 Hz, 6H), 1.04 (t, *J* = 7.2 Hz, 12H).

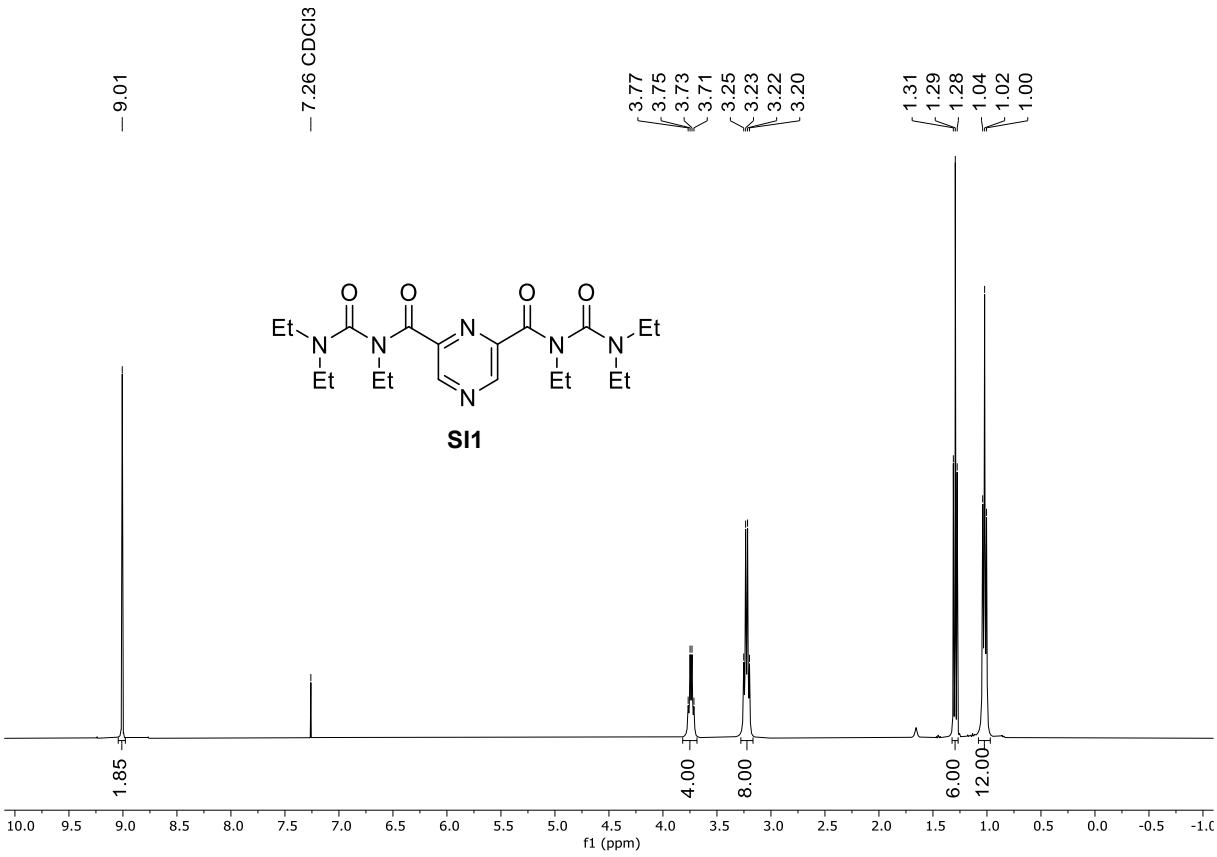
^{13}C NMR (101 MHz, 333 K, Chloroform-d [77.16 ppm], ppm) δ = 165.7, 156.5, 146.4, 145.9, 41.9, 13.3, 12.8.

MS (ESI): *m/z* (%) 443 ($[M+Na]^+$,100) 421 ($[M+H]^+$,30), 348 (27).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{20}H_{32}N_6O_4Na]^+$: 443.2377; found: 443.2377. (Error PPM: 0.0).

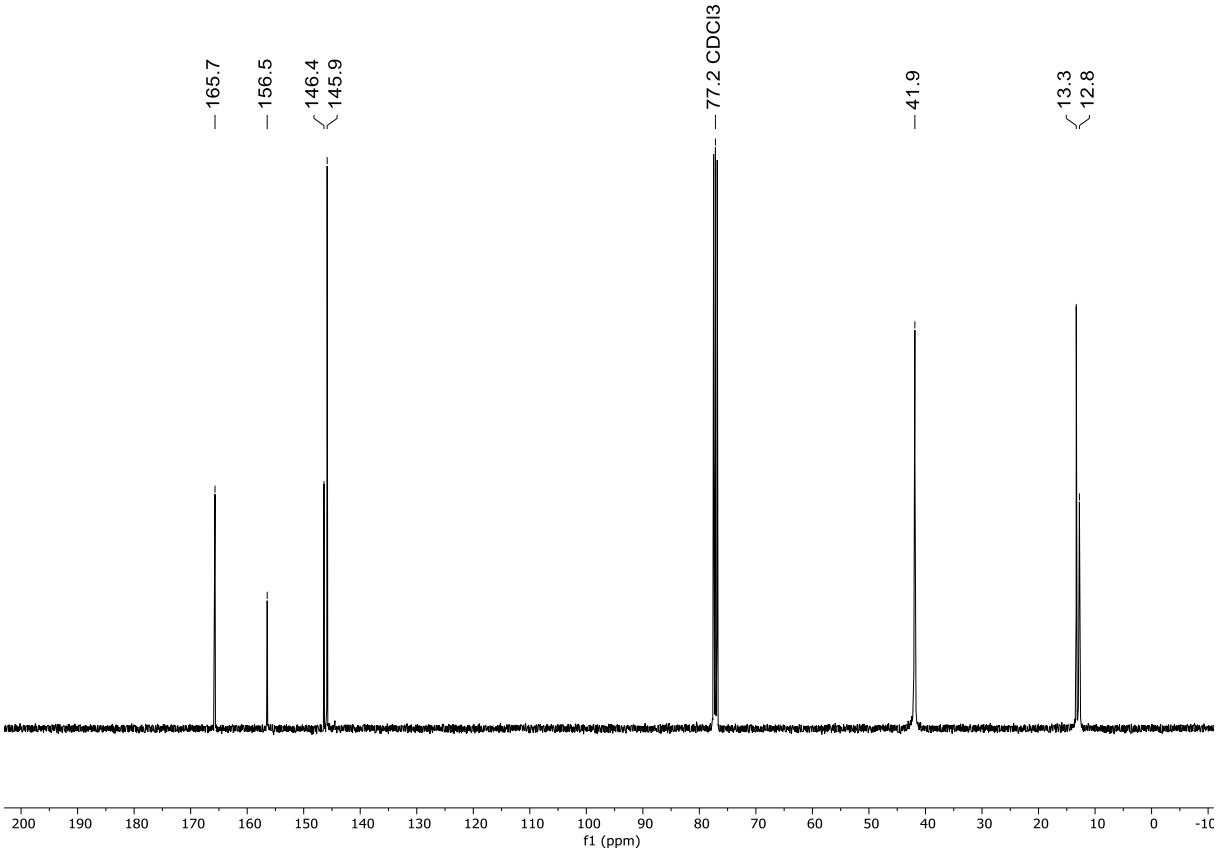
IR (ATR, ṽ) = 2977 (w), 2942 (w), 1668 (vs), 1650 (vs), 1466 (m), 1444 (m), 1417 (vs), 1399 (s), 1372 (vs), 1356 (s), 1342 (s), 1317 (s), 1268 (vs), 1213 (vs), 1191 (s), 1156 (m), 1126 (vs), 1097 (m), 1083 (m), 1068 (vs), 1017 (s), 981 (w), 964 (w), 944 (w), 911 (m), 860 (m), 848 (m), 805 (w), 791 (w), 779 (m), 763 (s), 732 (w), 657 (m), 581 (m), 530 (m), 516 (m), 500 (m), 461 (w), 442 (w), 420 (w), 404 (vs) cm^{-1}.

Notes: NMR spectra were measured at 333 K due to significant broadening of the signals at room temperature.



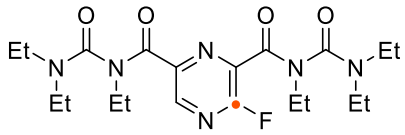
SI1

^{1}H NMR (400 MHz, 333 K, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (101 MHz, 333 K, Chloroform-d [77.16 ppm], ppm)

Synthesis of N^2,N^6-bis(diethylcarbamoyl)-N^2,N^6-diethyl-3-fluoropyrazine-2,6-dicarboxamide (SI1-F)



SI1-F

Following the general procedure GP3, using the corresponding pyrazine-2,6-dicarboxamide **SI1** (105 mg, 250 µmol, 1.00 equiv) with purification by column chromatography (*n*-pentane:ethyl acetate = 1:1) afforded the title compound SI1-F (57.6 mg, 131 µmol, 53% yield) as a colorless oil.

R_f = 0.4 (*n*-pentane:ethyl acetate = 1:1, UV)

^{1}H NMR (400 MHz, 333 K, Chloroform-d [7.26 ppm], ppm) δ = 8.76 (bs, 1H), 3.72 (bs, 4H), 3.47 – 3.11 (m, 8H), 1.29 (t, *J* = 7.1 Hz, 6H), 1.11 (t, *J* = 7.2 Hz, 6H), 1.02 (bs, 6H).

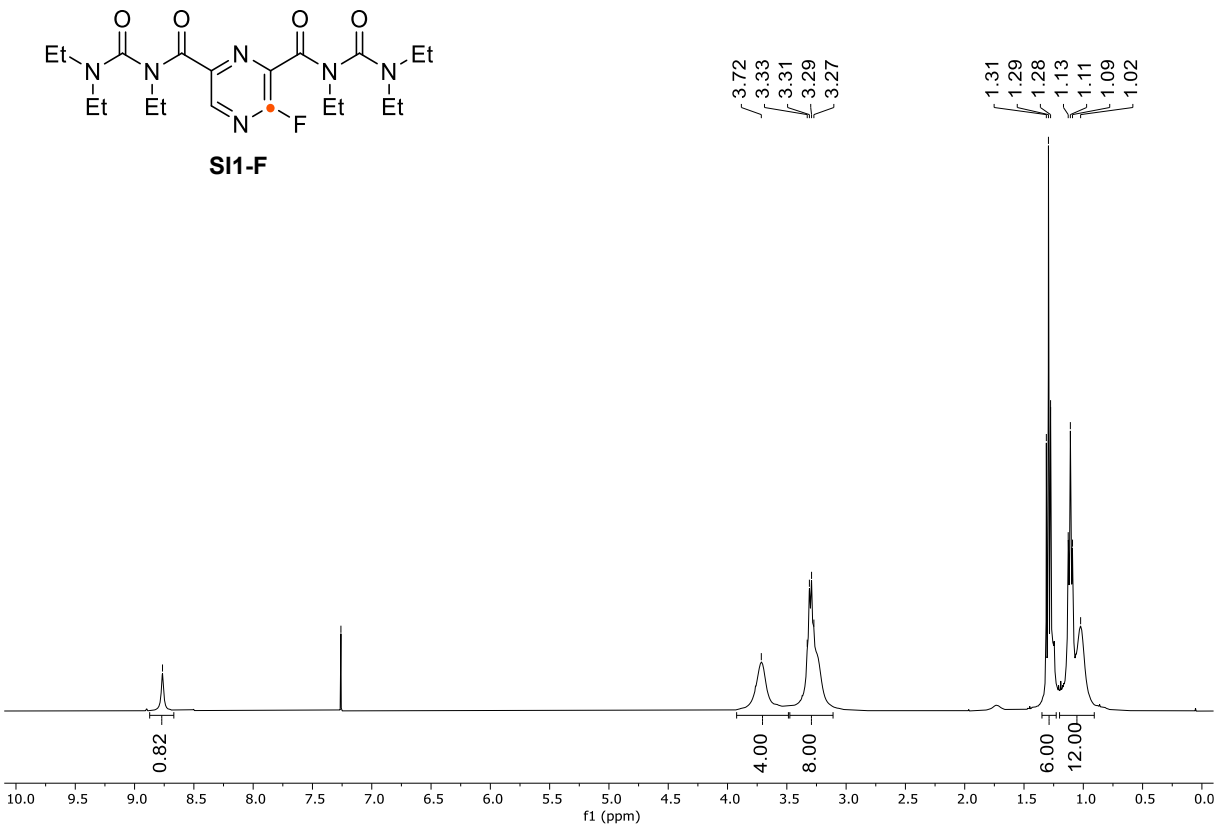
^{13}C NMR {^{19}F, ^{1}H } (101 MHz, 333 K, Chloroform-d [7.26 ppm], ppm) δ = 164.5, 162.8, 157.2, 156.7, 144.7, 144.3, 136.0, 42.2, 41.9, 13.4, 13.1, 12.8.

^{19}F NMR (376 MHz, Chloroform-d [7.26 ppm], ppm) δ = -72.45.

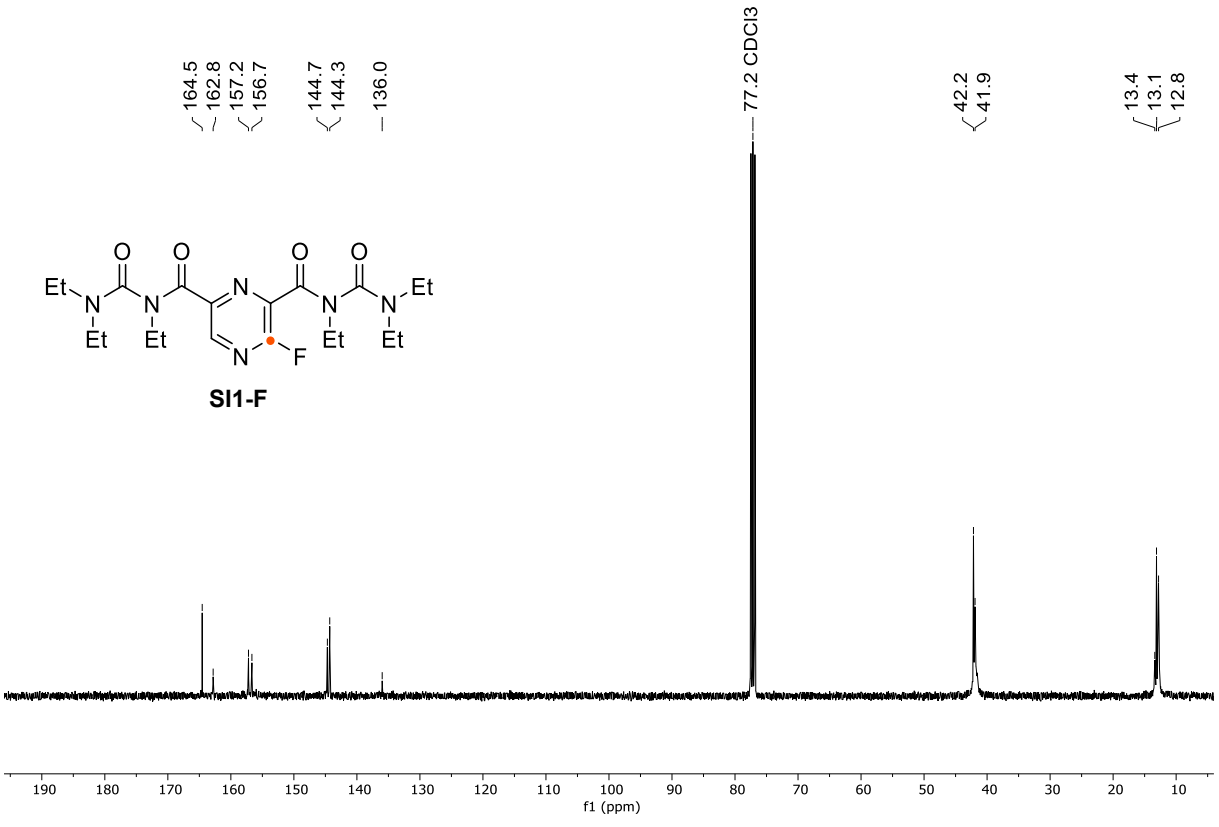
MS (ESI): *m/z* (%) = 461 (100, $[M+Na]^+$), 439 (89, $[M+H]^+$), 366 (32), 223 (19).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{20}H_{31}FN_6O_4Na]^+$: 461.2283; found: 461.2272 (Error PPM: -2.4).

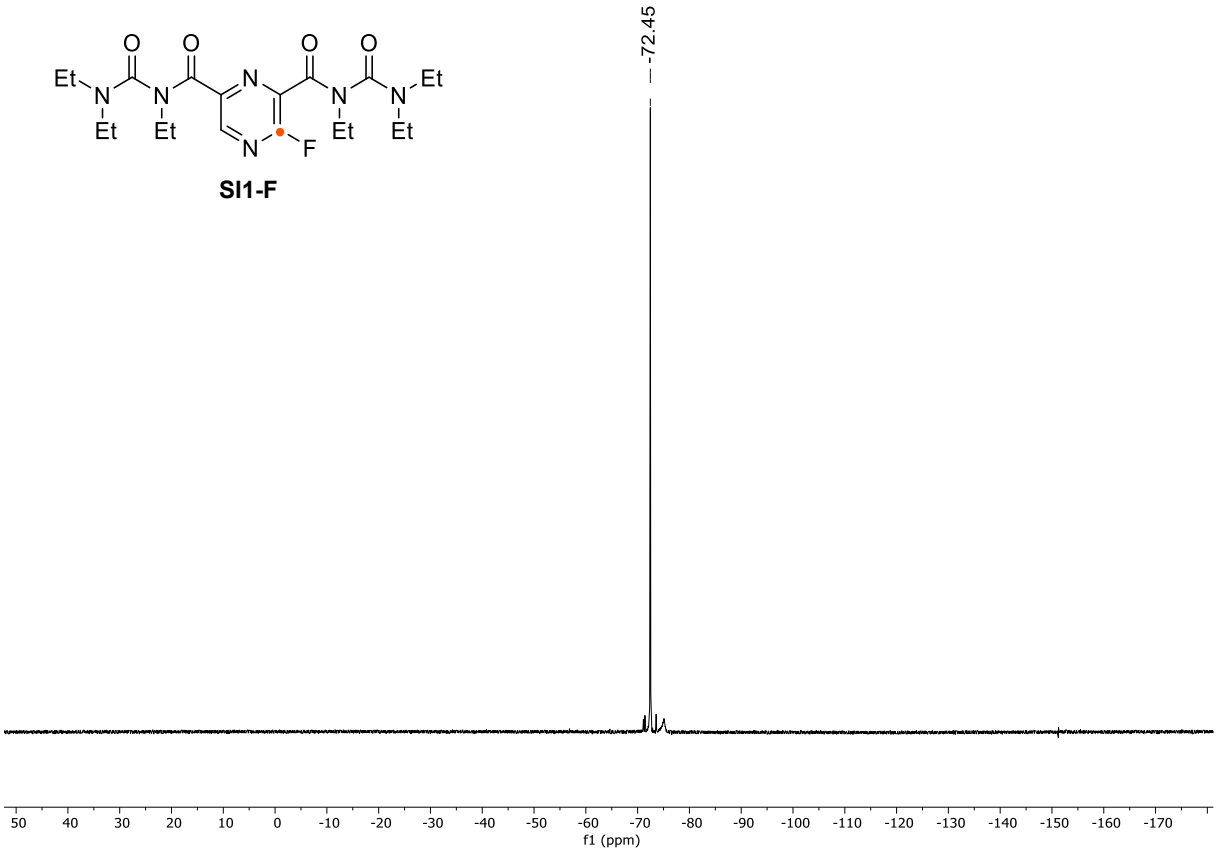
IR (ATR, neat, $\tilde{\nu}$) = 2977 (w), 2940 (w), 2877 (vw), 1666 (vs), 1578 (vw), 1541 (w), 1421 (vs), 1380 (s), 1348 (s), 1295 (s), 1260 (vs), 1215 (s), 1191 (m), 1128 (s), 1089 (m), 1064 (m), 1022 (w), 983 (w), 944 (w), 891 (vw), 869 (vw), 836 (w), 785 (w), 763 (w), 687 (w), 657 (w), 626 (w), 581 (w), 516 (w), 459 (w) cm^{-1}.



^{1}H NMR (400 MHz, 333 K, Chloroform-d [7.26 ppm], ppm)



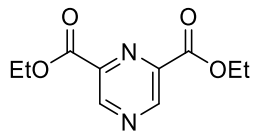
^{13}C NMR {^{19}F, ^{1}H } (101 MHz, 333 K,Chloroform-d [77.16 ppm], ppm)



SI1-F

^{19}F NMR (376 MHz, Chloroform-d, ppm)

Synthesis of diethyl pyrazine-2,6-dicarboxylate (SI2)



SI2

Pyrazine-2,6-dicarboxylic acid (504 mg, 3.00 mmol, 1.00 equiv.) was suspended in ethanol (3.00 mL, 1 M), and sulfuric acid (cat.) was added. The mixture was refluxed at 80 °C for 24 h. After the reaction cooled, the ethanol was removed *in vacuo,* and the remaining residue was picked up in 15 ml of EtOAc. The organic phase was subsequently washed with 3 × 10 ml of *aq*. $NaHCO_3$ solution. Afterwards, the organic phase was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed *in vacuo* using a rotary evaporator, yielding the title compound **SI2** (455 mg, 2.03 mmol, 68% yield) as a pale brown crystalline solid.

$\mathbf{R}_f$ = 0.28 (*n*-pentane:ethyl acetate = 2:1)

m.p. = 43-45 °C

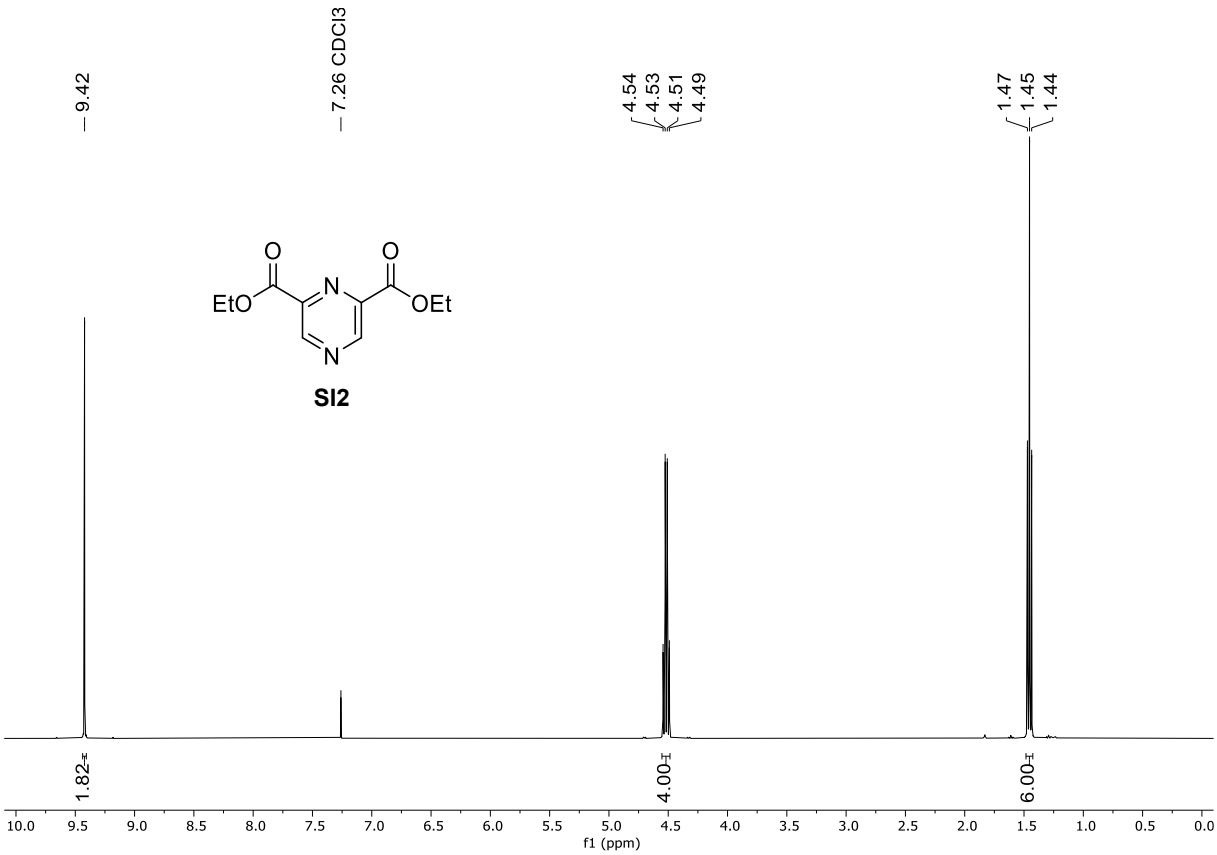
^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.42 (s, 2H), 4.52 (q, *J* = 7.2 Hz, 4H), 1.45 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 163.5, 148.5, 143.3, 62.9, 14.3.

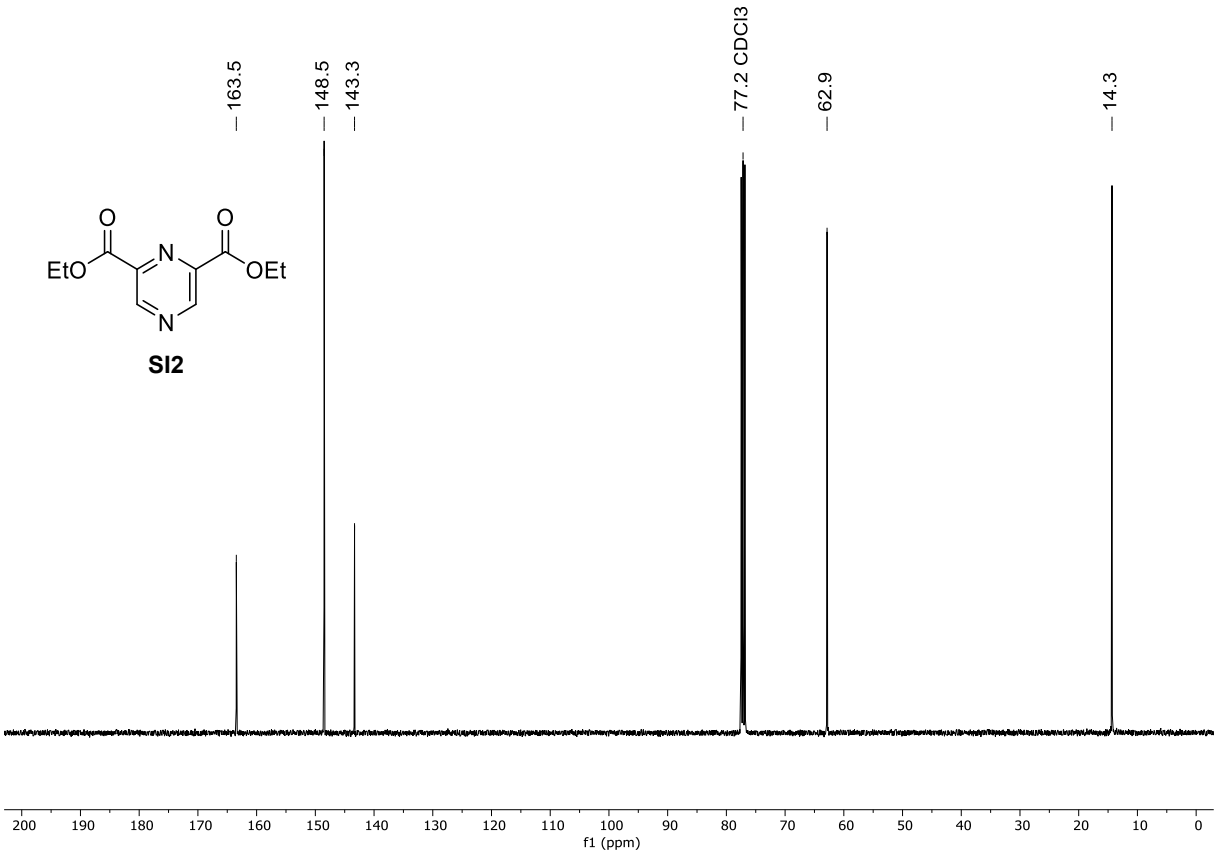
MS (EI): *m/z* (%) 224 (4 ,$[M]^+$), 180 (87), 152 (100), 124 (85), 106 (95), 95 (18), 80 (76), 78 (91).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{10}H_{12}N_2O_4Na]^+$: 247.0689; found: 247.0686 (Error PPM: -1.2).

IR (ATR, $\tilde{\nu}$) = 3059 (vw), 2981 (w), 2936 (w), 2910 (vw), 2873 (vw), 1715 (vs), 1711 (vs), 1654 (w), 1637 (vw), 1472 (w), 1456 (w), 1446 (w), 1438 (w), 1417 (w), 1397 (w), 1387 (w), 1370 (s), 1327 (vs), 1289 (vs), 1217 (w), 1154 (vs), 1123 (vs), 1111 (vs), 1013 (vs), 942 (m), 930 (m), 863 (s), 842 (w), 795 (w), 775 (vs), 746 (s), 705 (s), 642 (w), 602 (w), 563 (w), 434 (vs) cm^{-1}.

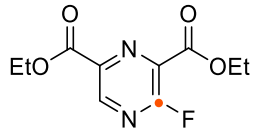


^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

Synthesis of diethyl 3-fluoropyrazine-2,6-dicarboxylate (SI2-F)



SI2-F

A flask was charged with diethyl pyrazine-2,6-dicarboxylate (**SI2**) (438 mg, 1.95 mmol, 1.00 equiv) and dissolved in MeCN (19.5 ml, 0.1 M). The solution was stirred, heated to 50 °C and AgF_2 (855 mg, 5.86 mmol, 3.00 equiv) wayed in in a glovebox, was added under argon counterflow. After 1 h the reaction mixture was transferred to separatory funnel containing 100 mL $NaHCO_3$ and extracted with 3 × 100 ml EtOAc. The combined organic phases were combined, dried over Na_2SO_4, concentrated *in vacuo*. Purification by column chromatography (*n*-pentane:ethyl acetate = 4:1) yielded the title compound **SI2-F** (177 mg, 730 µmol, 37% yield) as a transparent oil.

R_f = 0.35 (*n*-pentane:ethyl acetate = 5:1)

m.p. = N/A

^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.06 (d, *J* = 1.5 Hz, 1H), 4.53–4.47 (m, 4H), 1.45–1.41 (m, 6H).

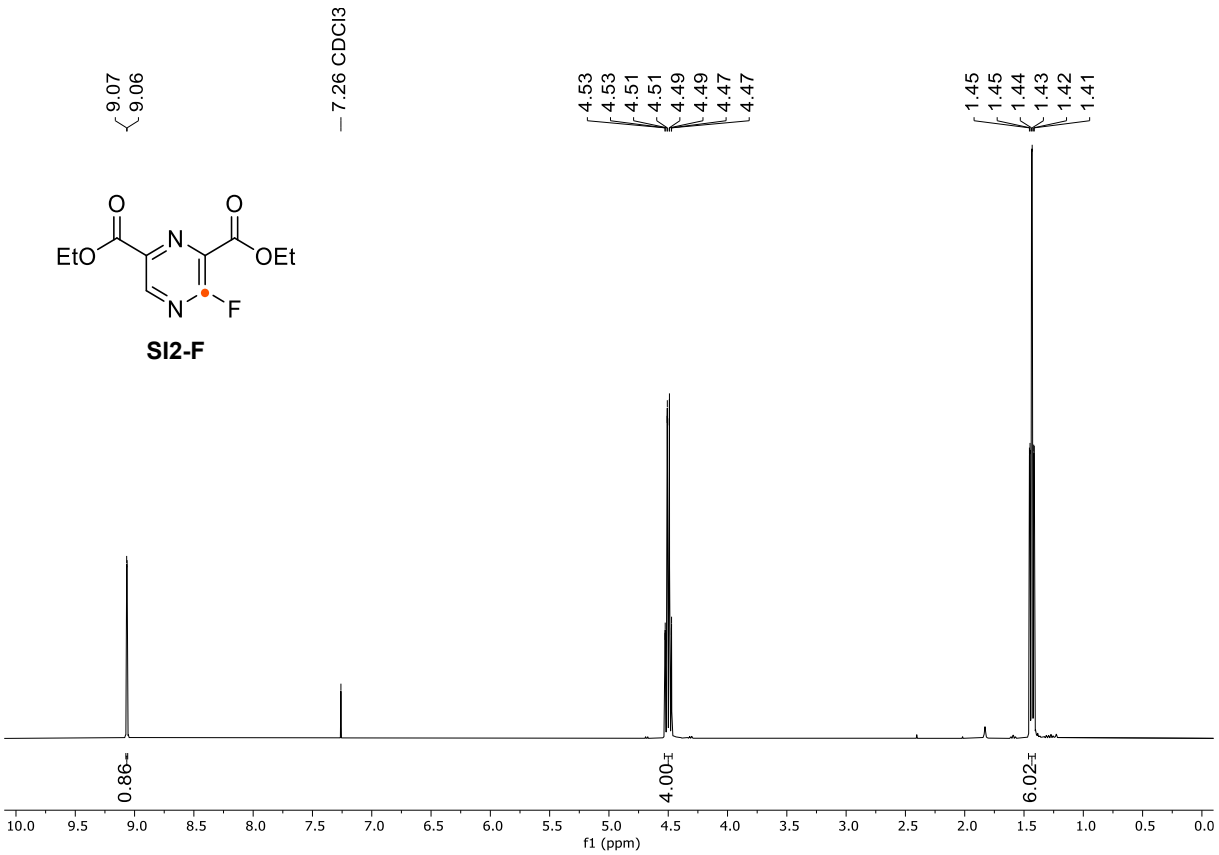
^{13}C NMR (100 MHz, Chloroform-d [77.2 ppm], ppm) δ = 162.2, 161.2 (d, *J* = 9.0 Hz), 159.7 (d, *J* = 268.3 Hz), 146.8 (d, *J* = 11.7 Hz), 141.1 (d, *J* = 5.4 Hz), 133.1 (d, *J* = 25.1 Hz), 63.1, 62.8, 14.2, 14.1.

^{19}F NMR (376 MHz, Chloroform-d, ppm) δ = -66.40 (s).

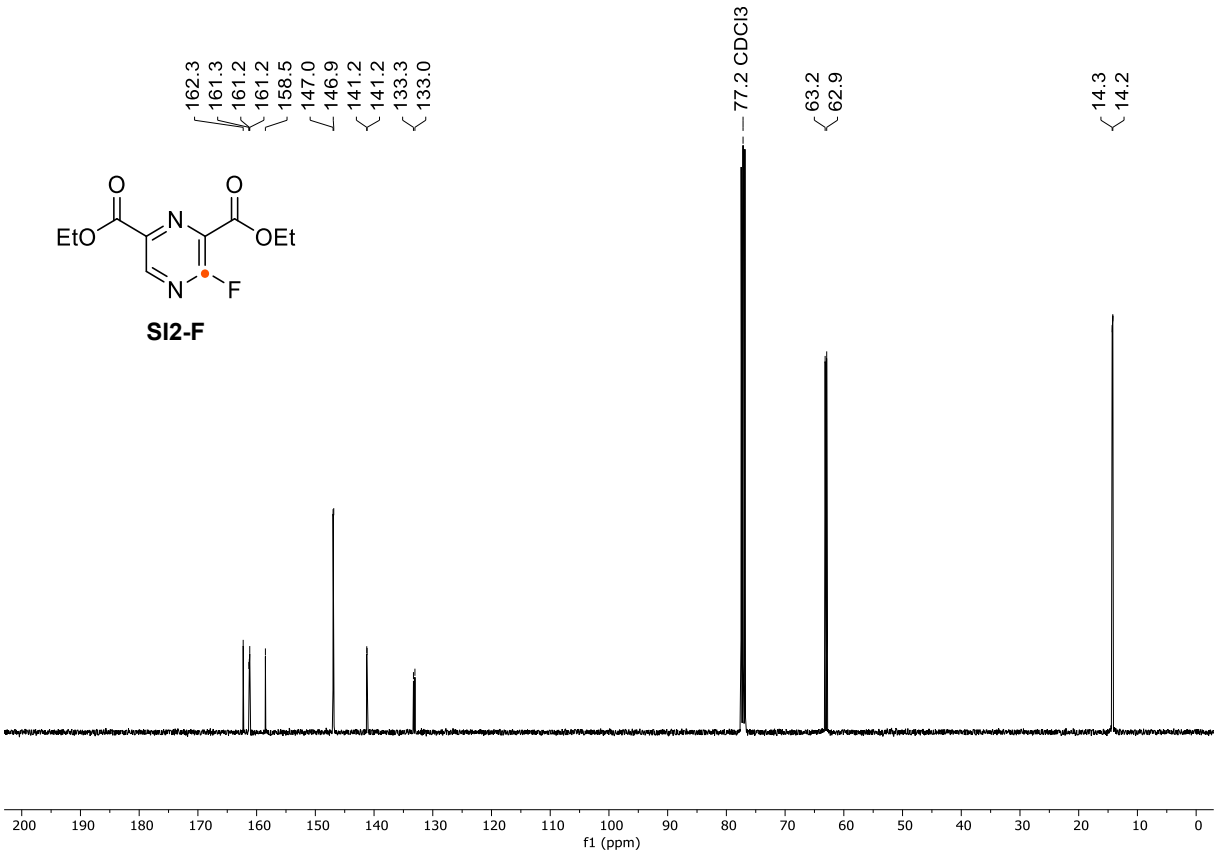
MS (EI): *m/z* (%) = 242 (4 $[M]^+$), 198 (99), 186 (10), 170 (100), 152 (12), 142 (95), 125 (73),113 (10), 98 (67), 76 (13), 69 (38), 51 (24).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{10}H_{11}FN_2O_4Na]^+$: 265.0595; found: 265.0595 (Error PPM: 0.0).

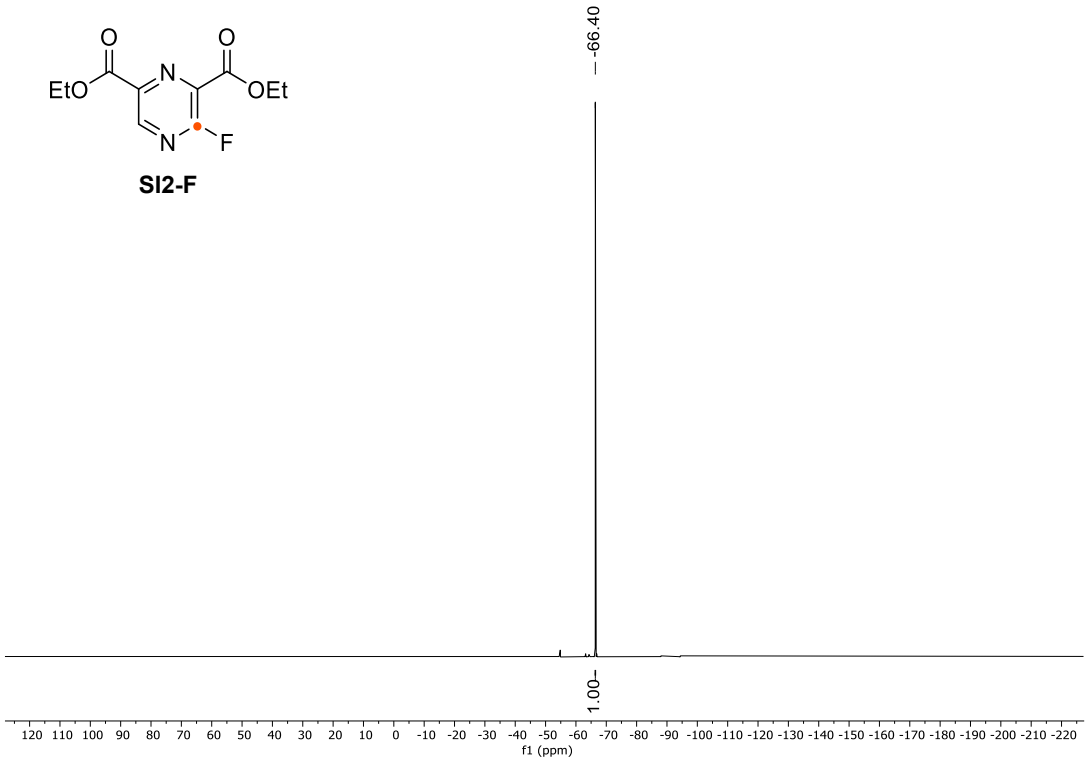
IR (ATR, ṽ) = 2985 (w), 2942 (vw), 1721 (vs), 1574 (w), 1552 (w), 1466 (w), 1444 (m), 1405 (w), 1384 (w), 1370 (m), 1321 (s), 1289 (s), 1238 (vs), 1193 (vs), 1177 (s), 1146 (s), 1099 (vs), 1017 (vs), 948 (w), 863 (m), 842 (w), 809 (m), 787 (w), 722 (w), 681 (w), 630 (w), 575 (vw), 548 (w), 489 (w), 459 (m), 434 (w), 420 (w) cm^{-1}.



^{1}H NMR (400 MHz, Chloroform-d [7.26 ppm], ppm)



^{13}C NMR (100 MHz, Chloroform-d [77.2 ppm], ppm)



^{19}F NMR (376 MHz, Chloroform-d, ppm)

6.3 Influence of fluoride ions on oxidation potential

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed at room temperature using a supporting electrolyte solution of 0.1 M $TBAPF_6$ in MeCN. A glassy carbon disk electrode (d = 2 mm) was used as working electrode, a platinum sheet as counter electrode, and Ag/AgCl/LiCl (EtOH) as reference electrode. All potentials are reported versus the ferrocene/ferrocenium couple (Fc^+/Fc), determined by addition of ferrocene as an internal standard after measurement of each compound measurement. The scan rate was 100 mV s^{-1}, and the concentration of all compounds was 10 mM. All potentials are reported and measured versus the ferrocene/ferrocenium couple (Fc^+/Fc) as the internal reference and can be referenced to the saturated calomel electrode (SCE) by addition of +0.38 V and to NHE by addition of +0.63 V to the obtained value.[16]

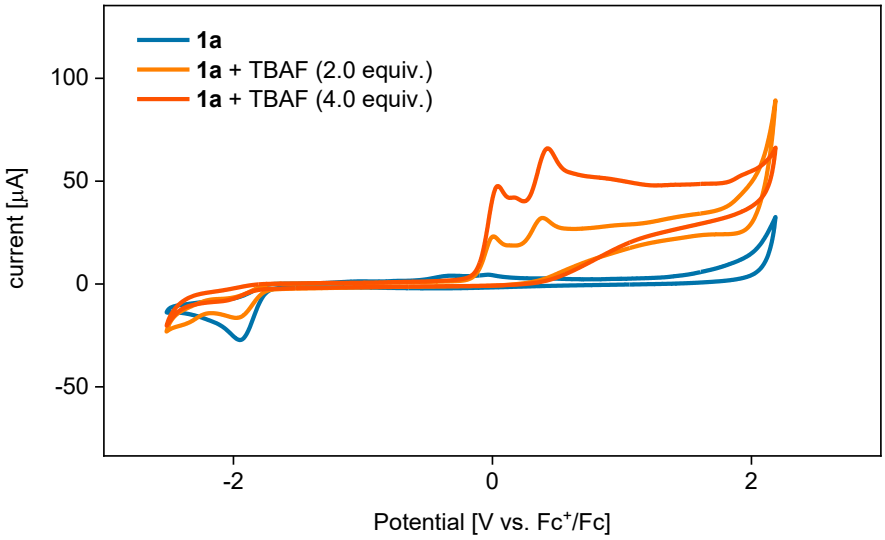


Figure S 19. . Cyclovoltammogramm of **1a**, 1**a**+TBAF (2.0 equiv.) and **1a**+TBAF (4.0 equiv.) in MeCN referenced to Fc^+/Fc.

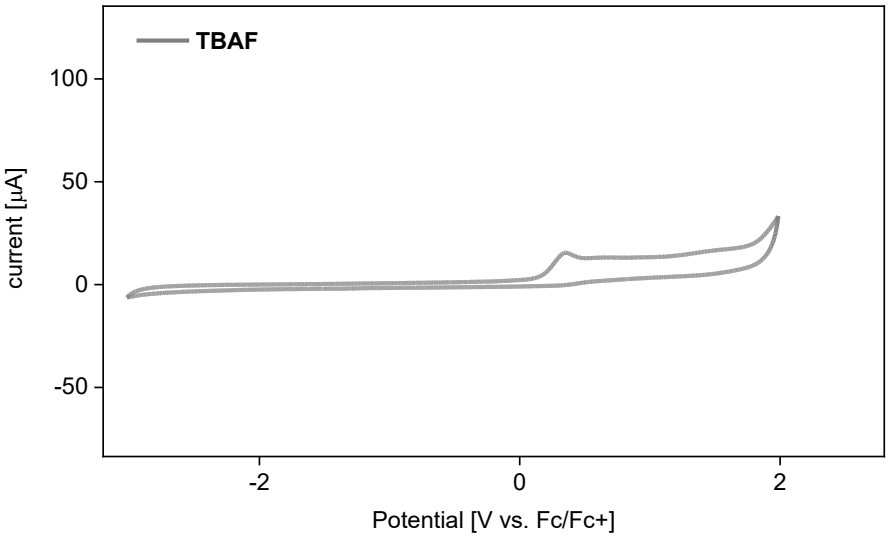
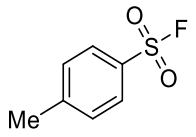


Figure S 20. Cyclovoltammogramm of TBAF in MeCN referenced to Fc^+/Fc.

6.4 Fluoride scavenging as *para*-tosyl fluoride (TsF)



TsF

To support the presence of fluoride anions in solution after completion of the reaction, efforts were made to trap the fluoride anion with 4-methylbenzenesulfonyl chloride (TsCl), based on a modified literature procedure.[17] The cyclization of standard substrate 1a was performed according to GP3 on a 0.1 mmol-scale. After completion of the 30 minutes reaction time, TsCl (86.3 mg, 453 µmol, 4.50 equiv.) was added and the reaction was stirred at 80 °C for 1 h. After 1 hour, 0.8 mL of tetrahydrofuran was added, to aid solubility, followed by an additional 0.8 mL 15 minutes later, resulting in a MeCN/THF solvent mixture (1:2). Subsequently, the reaction mixture was refluxed for additional 2 h before the crude reaction mixture was allowed to cool to ambient temperature and filtered over a cotton plug and washed with EtOAc (5 mL). The filtrate was evaporated to dryness *in vacuo* and 1,2-difluorobenzene (25.8 mg, 226 µmol, 2.25 equiv.) was added as internal standard. The yield of 4-methylbenzenesulfonyl fluoride (**TsF**) was calculated to be 88% according to ^{19}F NMR.

^{19}F NMR (376 MHz, Chloroform-d [7.26 ppm], ppm) δ = -65.75.

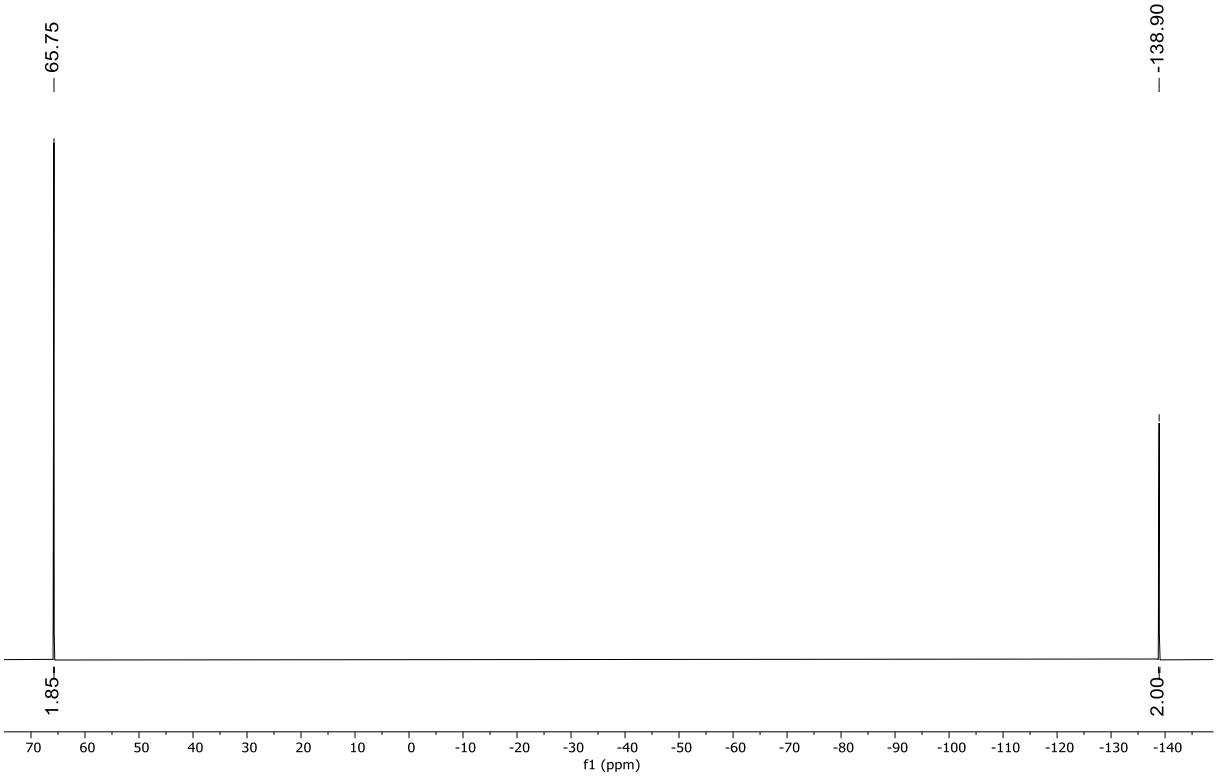
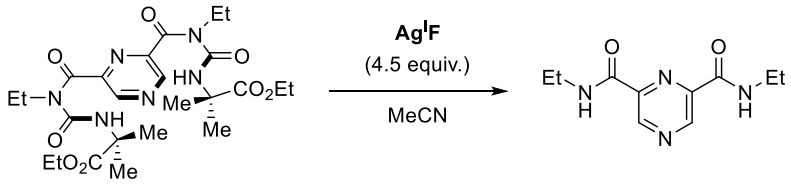


Figure S 21. ^{19}F NMR spectrum (282 MHz, Chloroform-d) of the fluorination of TsCl, obtained according to the procedure described *vide supra*; 1,2-difluorobenzene (δ = -138.90) was used as internal standard.

6.5 Degradation of carbamoyl-substituted pyrazine carboxamides by silver(I) fluoride



1a **16**

To evaluate the degradation caused by AgF, two experiments were conducted: In the first experiment, the conversion of **1a** was monitored over time. In the second experiment, the reaction was pushed to completion to isolate and characterize the double-degraded N^2,N^6-diethylpyrazine-2,6-dicarboxamide **16**.

6.5.1.1 Monitoring of conversion of substrate 1a in the presence of AgF

A 4 ml vial was charged with pyrazine dicarboxamide **1a** (80.3 mg, 150 µmol, 1.00 equiv.) and 1,3,5-trimethoxybenzene (12.6 mg, 75.0 µmol, 0.50 equiv), which were subsequently dissolved in MeCN (1.2 ml, 0.13 M). The solution was stirred, heated to 30 °C, and AgF (1.13 mmol, 4.5 equiv.) was added under argon counterflow. After 30, 60, and 120 minutes, and before the addition of AgF, 0.2 mL of the crude reaction mixture was removed using an Eppendorf pipette. Each sample was filtered over 150 mg of silica. and washed with EtOAc (10 mL). Subsequently, the solvent was removed *in vacuo*, and each sample was analyzed by ^{1}H NMR. The conversion of **1a** was measured by comparing the *Ar-H* signal of 1,3,5-trimethoxybenzene - which was found to be inert under the reaction conditions according to a separate experiment - to the *pyrazine-H* signal. The measurement before AgF addition was normalized to 100, resulting in the graph shown in Figure S 22, below.

The decay profile was analyzed using nonlinear least-squares fitting in OriginPro. The experimental data were fitted with a single-exponential decay function of the form

$$y = Ae^{-kx} + y_0$$

with

$$y = Ae^{-kx} + y_0 = 80.6900 \cdot e^{-7.99 \cdot 10^{-4} \cdot x} + 19.11915$$

which provided a satisfactory description of the observed decrease in substrate **1a** over time ($R^2 = 0.99464$). This model is consistent with a first-order decay process, characterized by an initially rapid decrease followed by a more gradual decline at longer times.

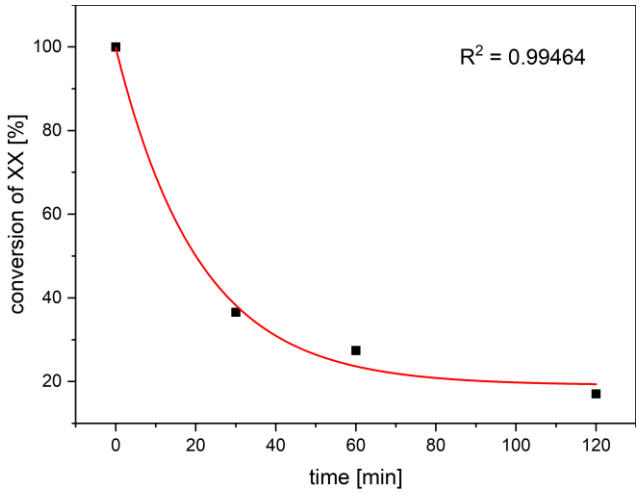


Figure S 22. Conversion of **1a** in the presence of AgF over 120 min.

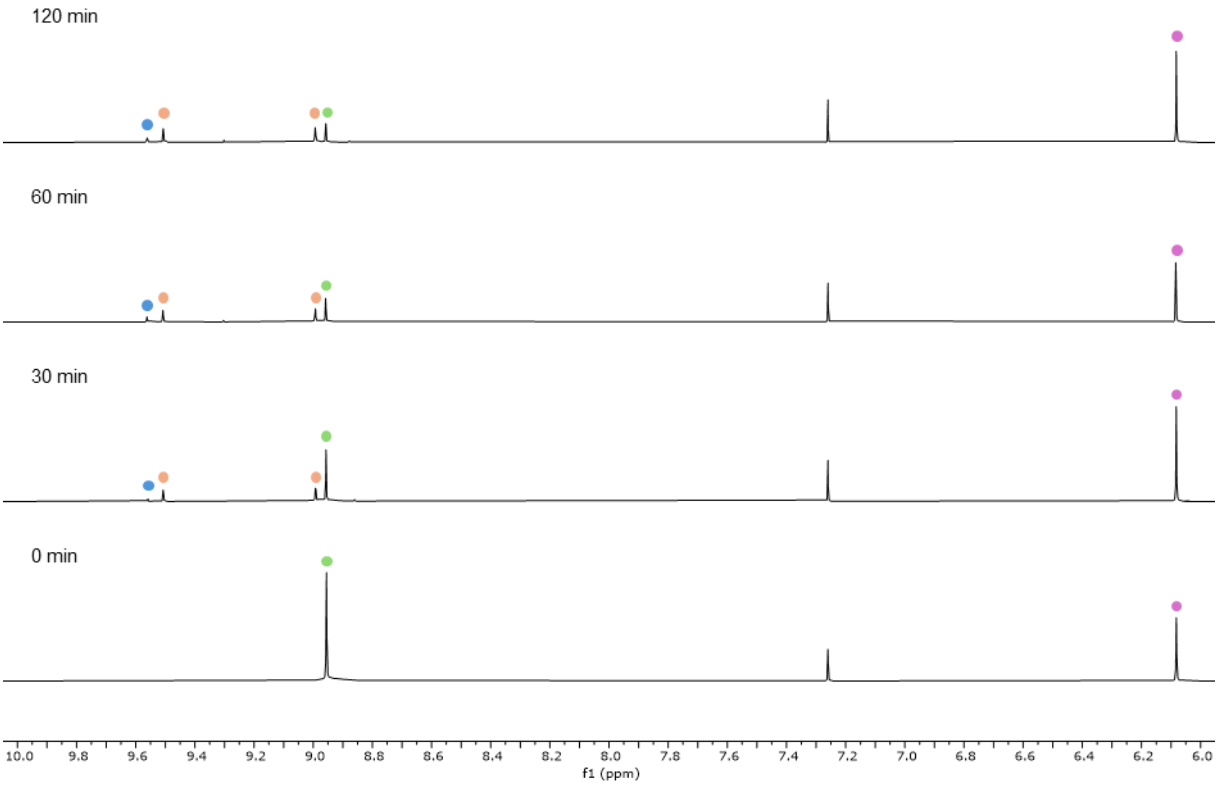
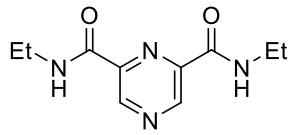


Figure S 23. ^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) of the degradation of **1a** (●) by AgF over time according to the procedure described *vide supra* at 0, 30, 60 and 120 minutes in the relevant range of 6.0 - 10 ppm. Evolution of semi-degraded ethylamide **14** (●) and double degraded bis(ethylamide) **15** (●) was observed and referenced to trimethoxybenzene (●) as internal standard (IS).

6.5.1.2 Synthesis of N^2,N^6-diethylpyrazine-2,6-dicarboxamide (15)



15

A 4 ml vial was charged with pyrazine dicarboxamide **1a** (134 mg, 250 µmol, 1.00 equiv.) which was subsequently dissolved in MeCN (2.0 ml, 0.13 M). The solution was stirred, heated to 30 °C, and AgF (143 mg, 1.13 mmol, 4.50 equiv.) was added under argon counterflow. After 30 min, the reaction temperature was increased to 85 °C and reaction mixture was stirred overnight (16 h). After 16 h, the reaction mixture was quenched with *aq*. $NaHCO_3$ and extracted with EtOAc (3 x 10 mL). The combined organic layer were combined, dried over Na_2SO_4, concentrated *in vacuo,* and purified by column chromatography (*n*-pentane:EtOAc = 1:2, UV) yielding the title compound **15** as light orange solid (18.2 mg, 81.9 µmol, 33% yield).

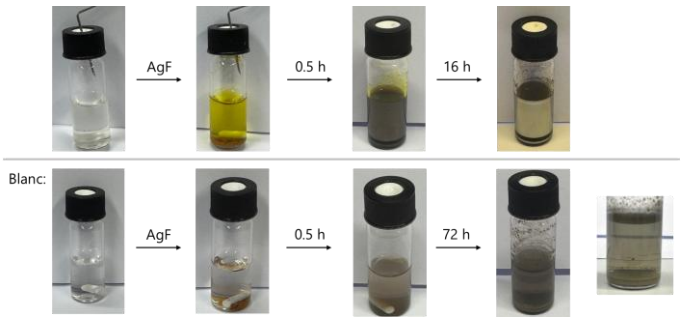


Figure S 24. Comparing the reaction against a reaction without pyrazine dicarboxamide **1a** (Blanc) a clear silver mirror is formed on the inside of the reaction vial:

R_f = 0.14 (*n*-pentane:ethyl acetate = 1:2, UV)

m.p. = 205-207 °C

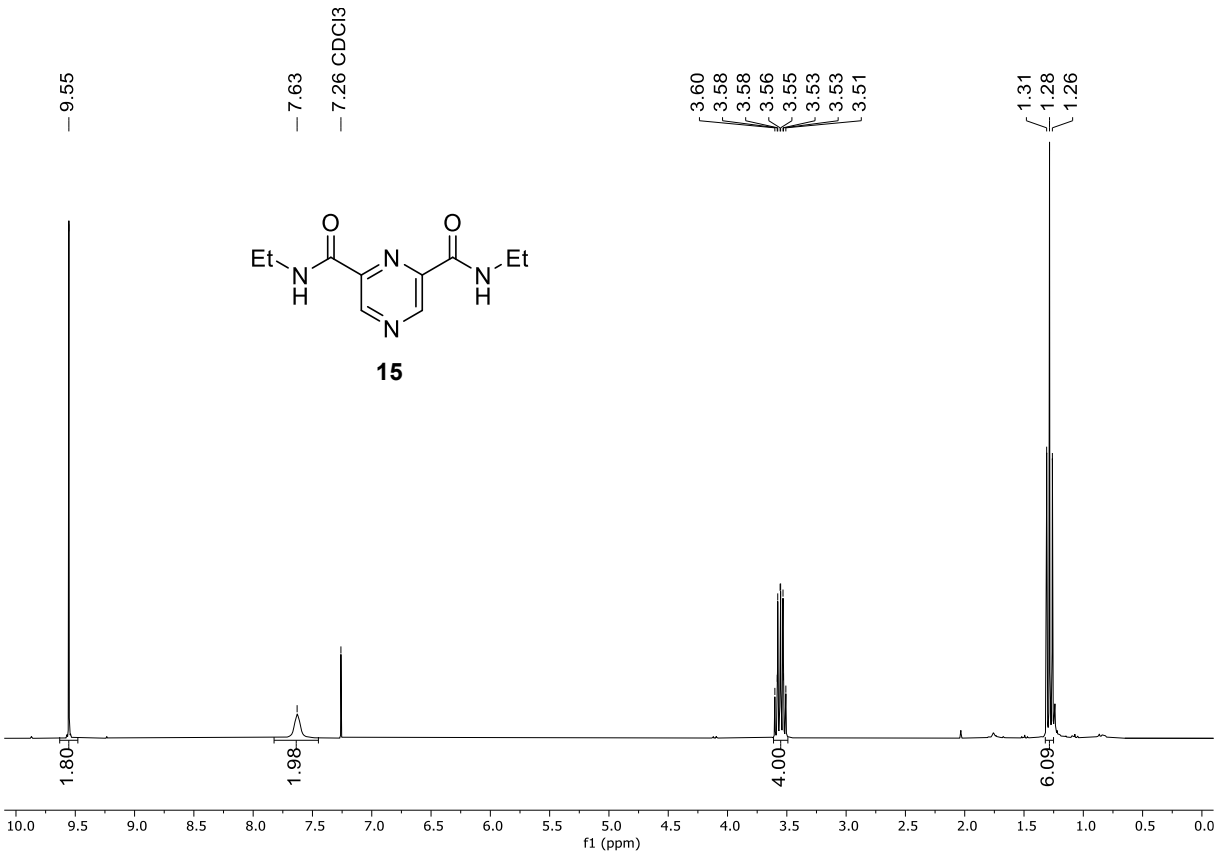
^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 9.55 (s, 2H), 7.63 (s, 2H), 3.55 (qd, *J* = 7.2, 6.0 Hz, 4H), 1.28 (t, *J* = 7.3 Hz, 6H).

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 162.3, 147.0, 142.6, 34.7, 15.0.

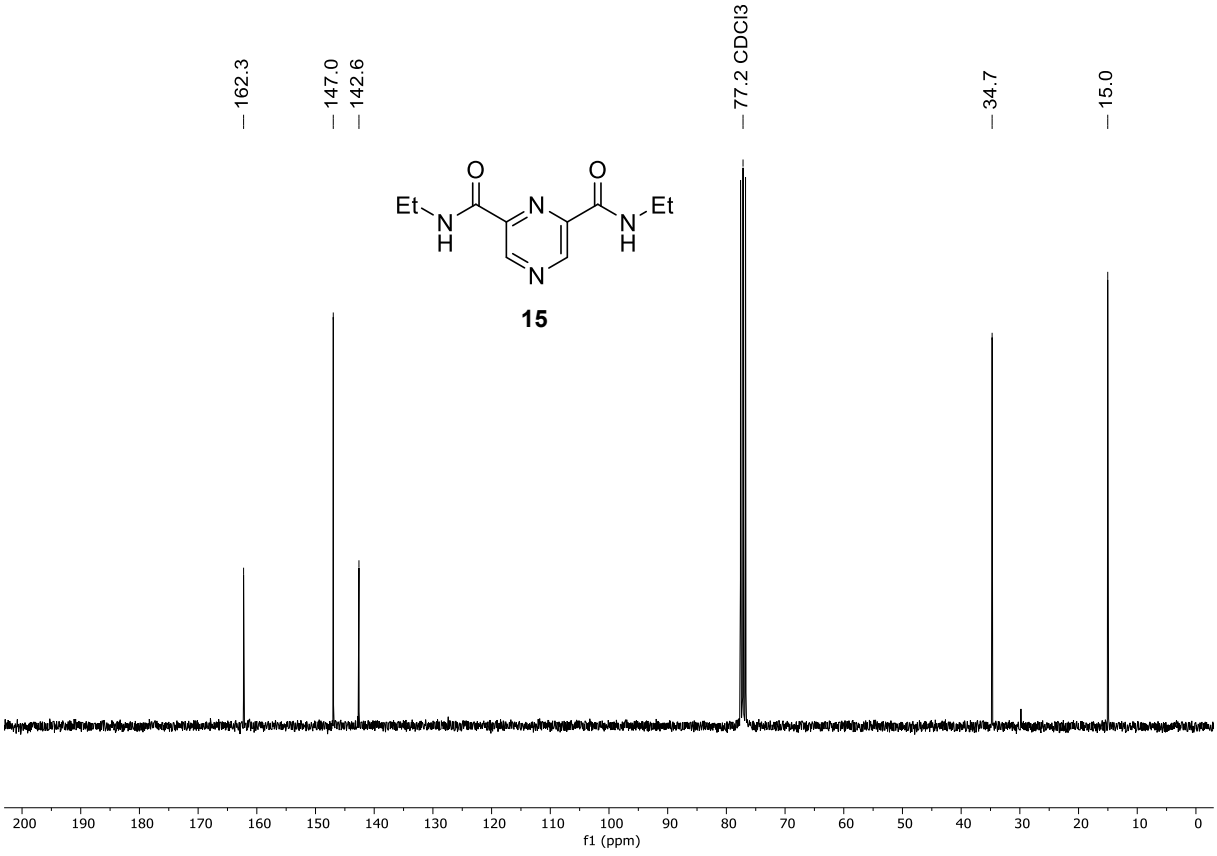
MS (ESI): *m/z* (%) 245 ([M+Na]$^+$, 100), 223 ([M+H$^+$], 62).

HRMS (ESI-TOF): *m/z* [M+Na]$^+$ calcd. for $[C_{10}H_{14}N_4O_2Na]^+$: 245.1009; found: 245.1015 (Error PPM: 2.4).

IR (ATR, $\tilde{\nu}$) = 3305 (w), 2977 (w), 2928 (w), 2877 (w), 2855 (w), 1672 (vs), 1650 (vs), 1511 (vs), 1450 (s), 1401 (m), 1376 (s), 1352 (m), 1317 (m), 1283 (m), 1268 (m), 1230 (m), 1181 (vs), 1148 (vs), 1119 (m), 1095 (m), 1022 (vs), 944 (w), 920 (w), 899 (w), 852 (m), 822 (w), 732 (vs), 669 (vs), 553 (s), 491 (w), 445 (vs) cm^{-1}.



^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)

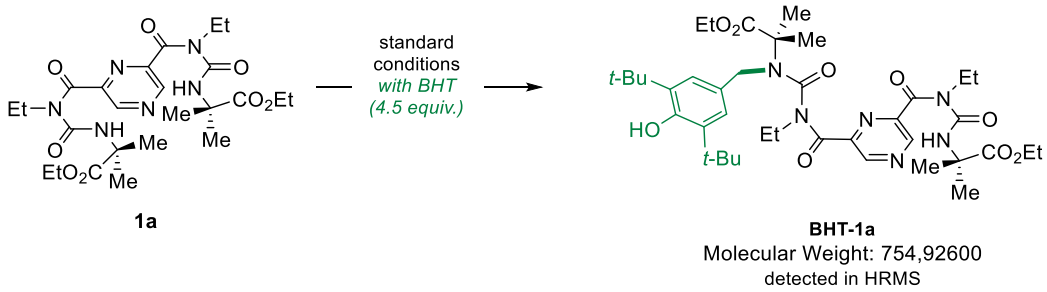


^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

6.6 Radical trapping Experiments

6.6.1 Butylated hydroxytoluene (BHT) as radical scavenger

Synthesis of ethyl 2-(6-(((3,5-di-tert-butyl-1-methyl-4-oxocyclohexa-2,5-dien-1-yl)(1-ethoxy-2-methyl-1-oxopropan-2-yl)carbamoyl)(ethyl)carbamoyl)-3-ethyl-2,4-dioxo-3,4-dihydropteridin-1(2*H*)-yl)-2-methylpropanoate (BHT-1a)



A 4.0 ml-vial was charged with substrate **1a** (107 mg, 200 µmol, 1.0 equiv.) and dissolved in MeCN (1.6 ml, 0.13 M). The solution was heated to 30 °C and AgF_2 (131 mg, 900 µmol, 4.50 equiv.) weighed in a glovebox, was added under argon counterflow. After 30 seconds of stirring, butylated hydroxytoluene (BHT) (198 mg, 900 µmol, 4.50 equiv.) was added. The reaction mixture was stirred for 30 min and was subsequently filtered over ca. 200 mg of celite washed with 10 ml of MeCN and subjected to LC-MS and ESI-HRMS. Whilst minor traces of product **2a** were detectable in the LC-MS, no product could be isolated. The ESI-HRMS revealed the **BHT-1a** $[M+H]^+$ and $[M+Na]^+$:

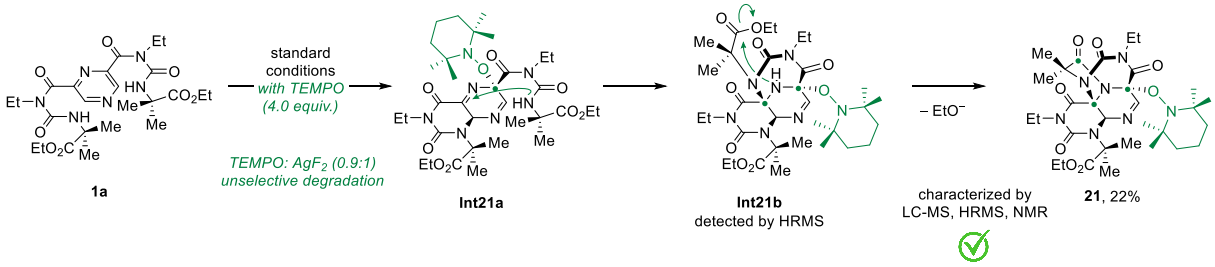
HRMS (ESI-TOF): *m/z* $[M+H]^+$ calcd. for $[C_{39}H_{59}N_6O_9]^+$: 755.4338; found: 755.4360 (Error PPM: 2.9).

HRMS (ESI-TOF): *m/z* $[M+Na]^+$ calcd. for $[C_{39}H_{58}N_6O_9Na]^+$: 777.4157; found: 777.4161 (Error PPM: 0.5).

Similar BHT adducts to the structure of **BHT-1a** were found a transformation also involving amidyl radicals.[18]

6.6.2 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) as radical scavenger

Synthesis of ethyl 2-(3,13-diethyl-6,6-dimethyl-2,4,7,12,14-pentaoxo-9-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)-3,4,6,7,9,11a-hexahydro-5,9-(methanoiminomethano)imidazo[2,1-*e*]pteridin-1(2*H*)-yl)-2-methylpropanoate (21)



A 4.0 ml-vial was charged with substrate **1a** (81.0 mg, 151 µmol, 1.0 equiv.) and 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) (94.3 mg, 604 µmol, 4.0 equiv) and dissolved in MeCN (1.2 ml, 0.13 M). The solution was stirred, heated to 30 °C and AgF_2 (131 mg, 900 µmol, 4.50 equiv.) weighed in in a glovebox, and was added under argon counterflow. The reaction mixture was stirred for 30 min and directly purified by column chromatography (*n*-pentane:EtOAc = 5:1 → 1:2) yielding the title compound **21** (21.7 mg, 33.6 µmol, 22%) as a colorless oil. Furthermore, **Int21b** was detected by HRMS in the crude reaction mixture.

R_f = 0.35 (*n*-pentane: EtOAc = 4:1, UV)

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm) δ = 8.07 (d, *J* = 2.9 Hz, 1H), 5.92 (d, *J* = 2.9 Hz, 1H), 4.30 – 4.15 (m, 2H), 3.67 – 3.49 (m, 4H), 1.64 – 1.42 (m, 16H), 1.33 (s, 3H), 1.33 – 1.16 (m, 14H), 1.14 (s, 3H), 1.00 (s, 3H).

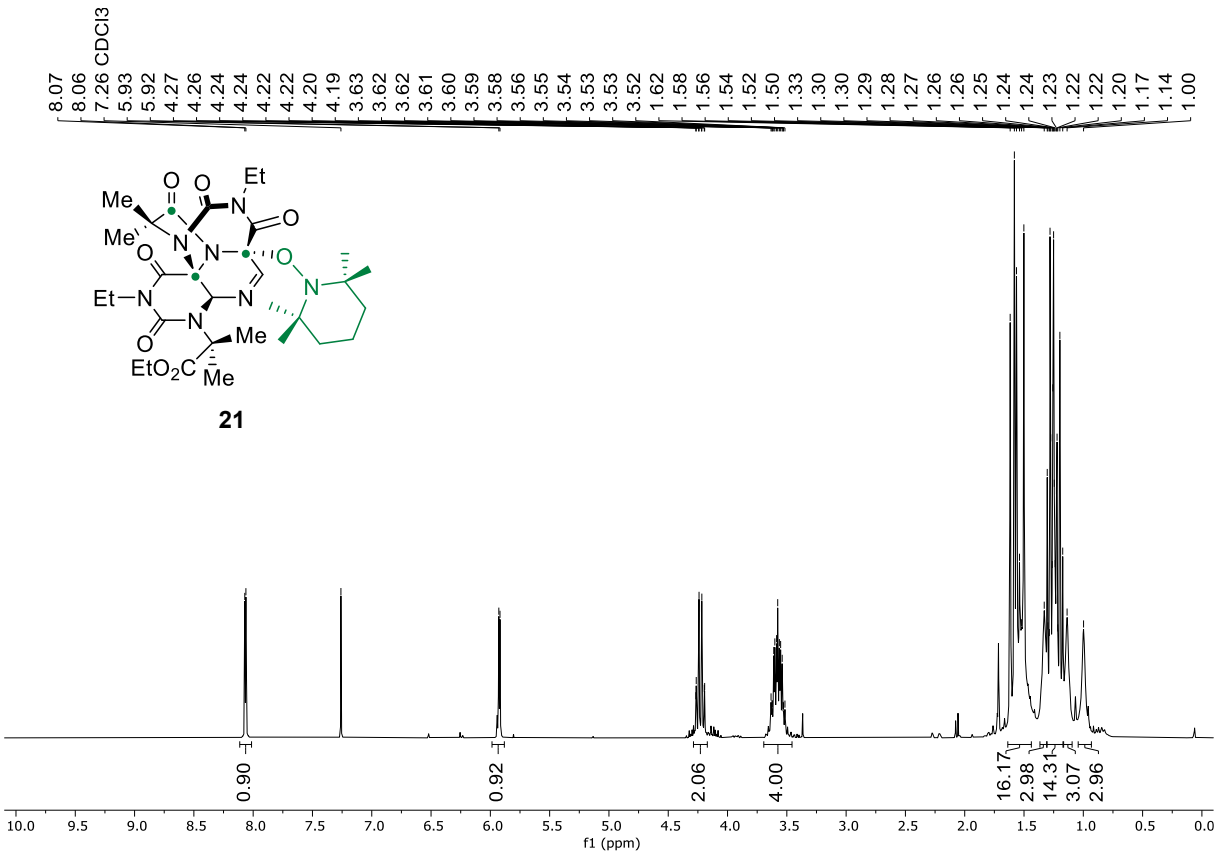
^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm) δ = 180.2, 173.6, 168.7, 167.1, 157.3, 155.2, 154.3, 93.7, 75.7, 75.5, 62.0, 61.9, 60.9, 60.2, 40.3, 35.4, 34.9, 33.6, 26.2, 25.4, 25.2, 22.8, 21.3, 20.8, 17.2, 14.2, 13.5, 13.1.

HPLC (Agilent, SB-C18, Water (0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI)**:** tR = 28.7

21 MS (ESI): *m/z* (%) = 646 (100, [M+H$^+$]).

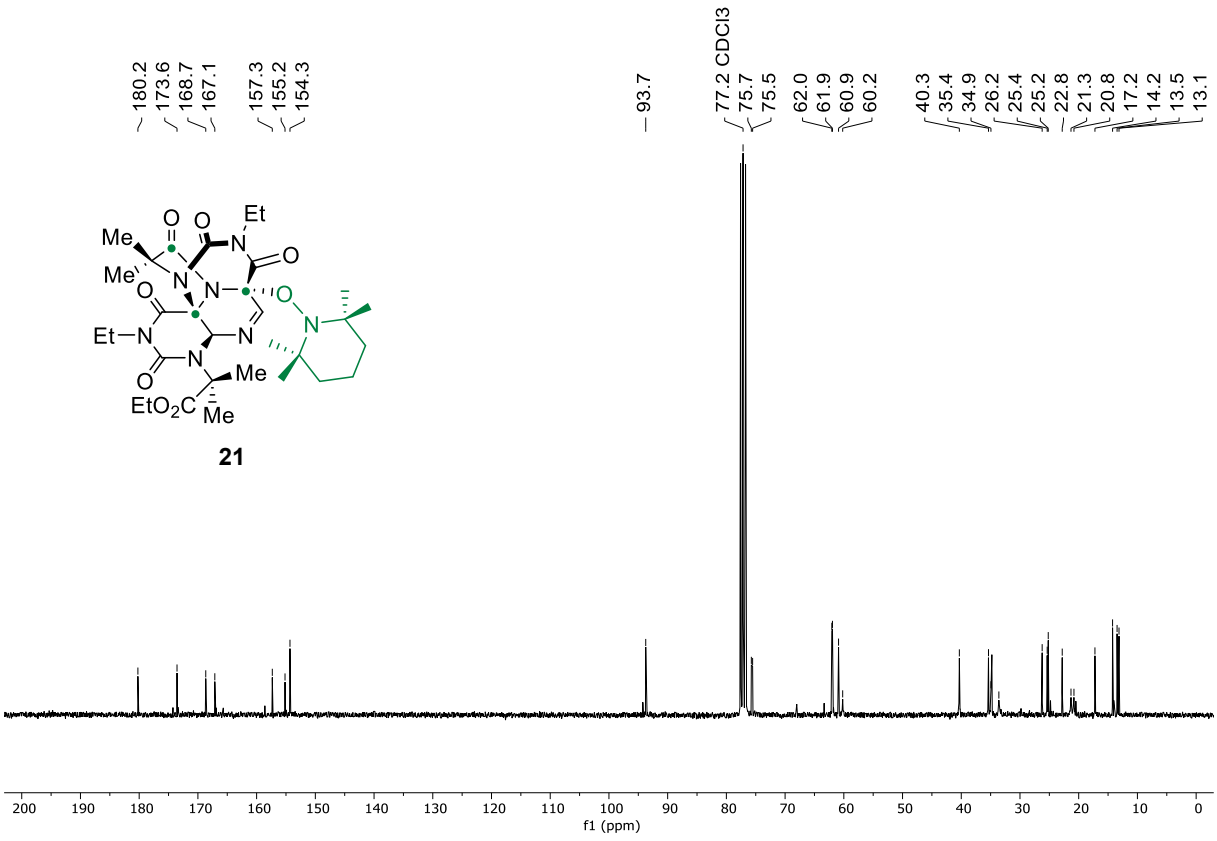
HRMS 21 (ESI-TOF): *m/z* [M+H]$^+$ calcd. for [$C_{31}H_{48}N_7O_8$]$^+$: 646.3559; found: 646.3566 (Error PPM: 0.9).

HRMS 21b (ESI-TOF): *m/z* [M+H]$^+$ calcd. for [$C_{33}H_{54}N_7O_9$]$^+$: 692.3978; found: 692.3989 (Error PPM: 1.6).



21

^{1}H NMR (300 MHz, Chloroform-d [7.26 ppm], ppm)



21

^{13}C NMR (75 MHz, Chloroform-d [77.16 ppm], ppm)

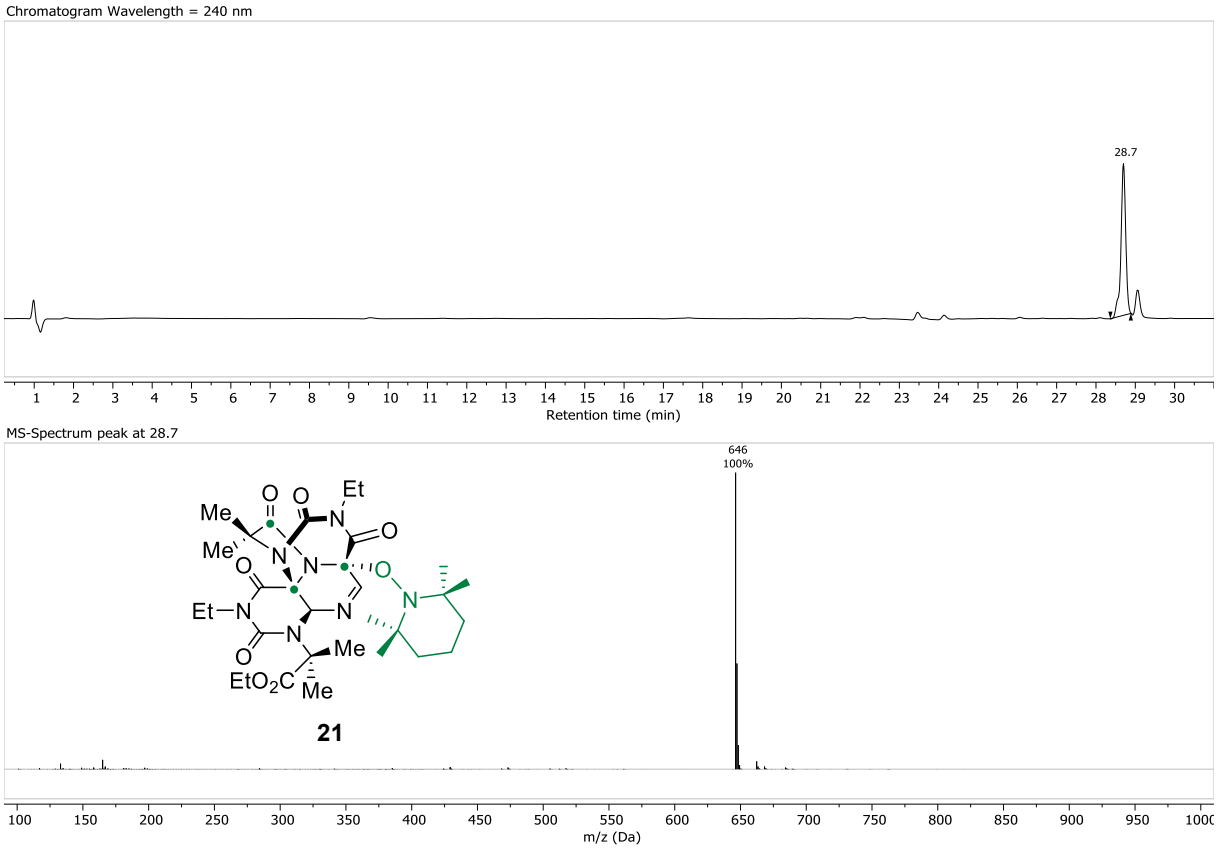


Figure S 25. Chromatogram and corresponding mass spectrum of isolated compound **21**; (Agilent, SB-C18, Water (0.1 formic acid)/MeOH 95:5 → 0:100, 30 min, 0.3 ml/min, ESI).

Note: For the depiction of the molecule, the structure was drawn in ChemDraw and optimized by MM2 using Chem3D. The structure file was saved as mol-file (.mol2) and visualized in MERCURY using customized layout settings.

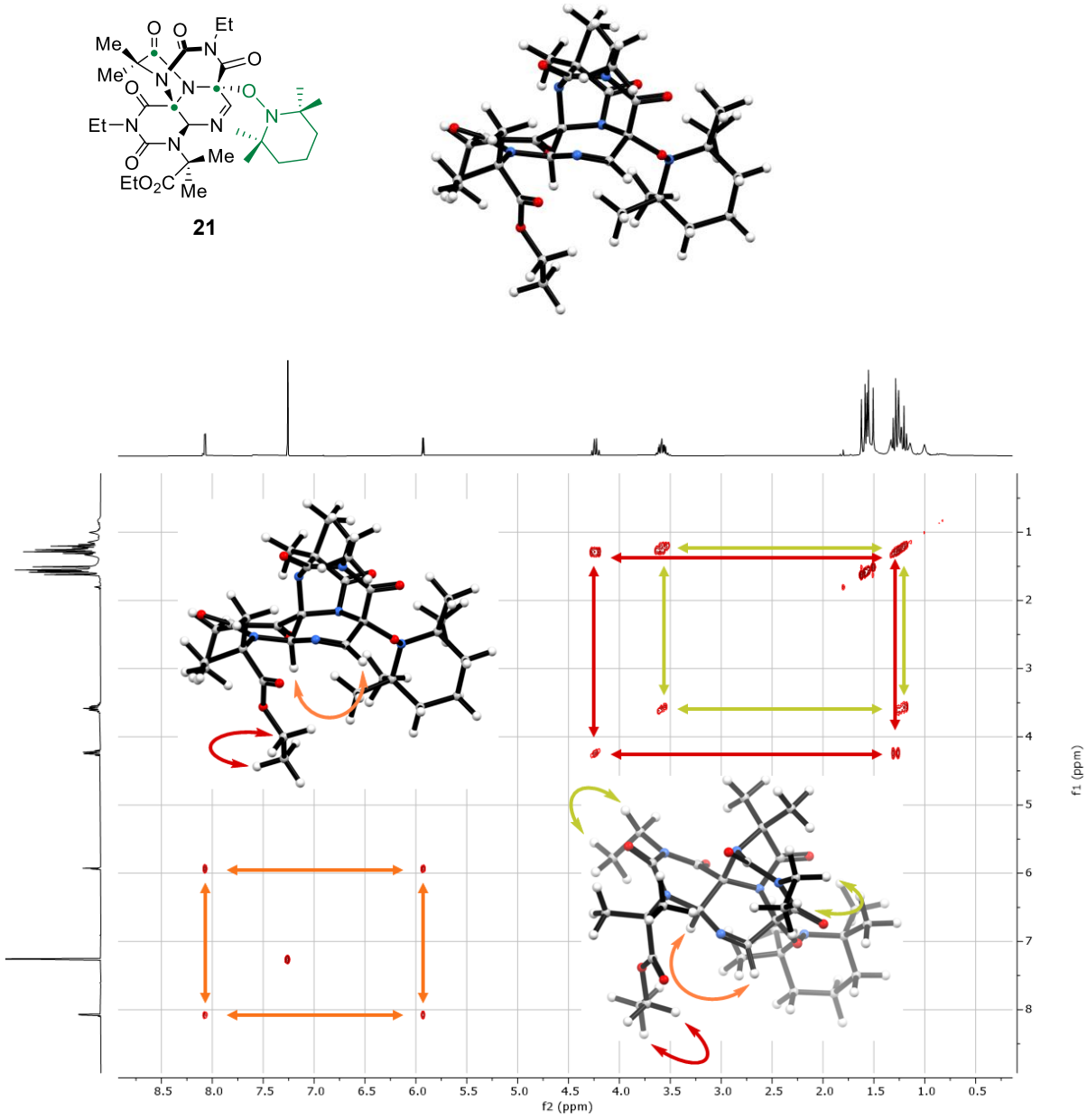


Figure S 26. 1H COSY-45 of **21** in $CDCl_3$.

7 Bond dissociation enthalpies (BDEs) and free-energies (BDFEs)

Bond dissociation enthalpies (BDEs) and bond dissociation free energies (BDFEs) were estimated using the **ALFABET BDE Predictor**, a machine-learning model developed by the Paton Lab and available as a Hugging Face Space application. Molecular structures were provided as SMILES strings. The computed BDEs and BDFEs served as a qualitative guide for identifying the most labile bonds and rationalizing observed reactivity patterns. The model was used to predict homolytic bond strengths across all relevant bonds (N–H and C(pyrazine)–H bonds). A summary is listed in **Table S 6**, below.

Software: ALFABET BDE Predictor - a Hugging Face Space by patonlab

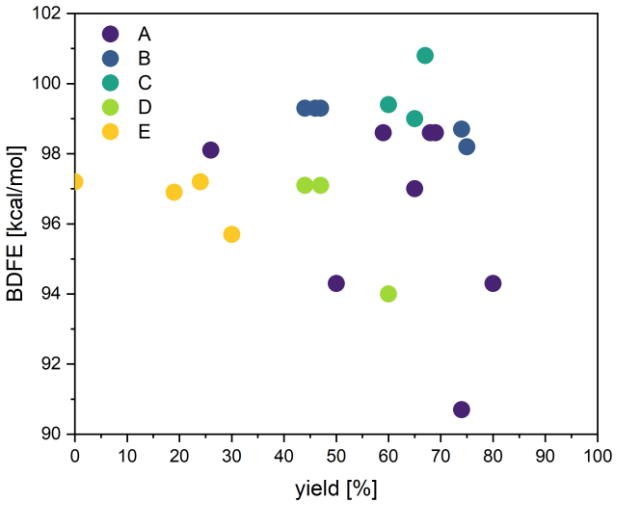


Figure S 27. Plot of yield in percentage versus BDFE in kcal/mol shows no strong correlation between BDFE and expected yield.

Table S 6. Computed BDEs and BDFEs of compounds **1a-1y**.

				N–H bond			C(sp^2) bond		
Entry	**Compound**	**R**	**# of disting. Bonds**	**BDE**	**BDFE**	**rel. Strength**	**BDE**	**BDFE**	**rel. Strength**
1	**1a**	Aib	19	107.4	98.6	18	109.4	100.7	19
2	**1c**	Acp	18	99.2	90.7	11	109.4	100.7	18
3	**1d**	Acb	19	103.0	94.3	18	109.4	100.7	19
4	**1e**	Acp	19	105.6	97.0	18	109.4	100.7	19
5	**1f**	Ach	20	106.4	98.1	19	109.4	100.7	20
6	**1g**	Gly	18	107.8	99.3	17	109.4	100.7	18
7	**1i**	GlyOBn	20	107.8	99.3	16	109.4	100.7	17
9	**1k**	Val	22	107.6	98.7	21	109.4	100.7	22
10	**1j**	Leu	24	106.6	98.2	23	109.4	100.7	24
11	**1n**	Phe	24	107.5	99.0	20	109.4	100.7	21
12	**1m**	TyrOBn	26	107.2	99.4	24	109.4	100.7	25
13	**1l**	AspOMe	24	109.1	100.8	23	109.4	100.7	24
14	**1u**	Et	14	106.0	96.9	13	109.4	100.7	14
15	**1v**	Pr	18	106.6	97.2	17	109.4	100.7	18

16	**1x**	tBu	13	106.5	97.2	12	109.4	100.7	13
17	**1w**	NHEt/iPr	14	104.7	95.7	13	109.3	100.6	14
18	**1t**	NHPh/Et	28	98.6	89.8	13	109.4	100.7	21
	1t'	NHEt/Ph		103.4	94.0	18	109.2	100.6	20
19	**2p**	MeBn	18	105.9	97.1	14	109.4	100.6	18
20	**5**	AibPro	26	107.4	98.6	24	109.4	100.7	26
21	**S21**	Aib(mono)CO2Et					109.1	100.5	25

8 Theoretical investigations

8.2 Computational details

A conformational search was carried out to identify low-energy geometries of the substrate–oxidant assemblies using Aib-derived carbamoyl-substituted pyrazine carboxamides **1a** as the standard substrate and AgF_2. The conformational space of the initial structures was explored using CREST (Version 2.12)[19,20] in combination with the semiempirical tight-binding method GFN2-xTB. Default CREST settings were employed to ensure a broad sampling of the accessible minima. The lowest-energy conformers, and if relevant several, low-lying conformers within a small energy window, were subsequently reoptimized at the DFT level.

All DFT calculations were performed with the ORCA program package (version 5.0.3).[21] Pre- and post-processing steps were carried out using in-house scripts. Geometry optimizations and harmonic frequency calculations were conducted with the B3LYP hybrid exchange-correlation functional in conjunction with the def2-SVP basis set and Grimme's D3 dispersion correction with Becke–Johnson damping (D3BJ). For Ag, the corresponding def2 effective core potential (def2-ECP) was employed to replace the core electrons while treating the valence electrons explicitly. To accelerate the calculations, the RIJCOSX approximation was used together with the corresponding def2/J auxiliary basis set. Solvent effects were described using the conductor-like polarizable continuum model (CPCM) with acetonitrile as the solvent. Final electronic energies were refined by single-point calculations with the def2-TZVP basis set on the DFT-optimized geometries.[22–32]

Transition states were located by performing relaxed potential energy surface scans along the relevant reaction coordinate(s) to generate a suitable initial assessment, followed by transition-state optimizations. Harmonic frequency calculations at the same level of theory were used to characterize all stationary points. Minima exhibited no imaginary frequencies, whereas transition states showed exactly one imaginary frequency corresponding to motion along the reaction coordinate. Intrinsic reaction coordinate (IRC) calculations were carried out to confirm the connectivity between reactants, transition states, and products, using the default convergence settings in ORCA.[33]

In addition, electronic-structure analyses were performed to examine charge and spin distributions for key intermediates and transition states. Where appropriate, NBO analysis was used to assess changes in bonding and the redistribution of charge and spin density along the proposed pathways.[34] Cartesian coordinates of all optimized structures are provided in XYZ format in a separate Excel file.

8.3 Mechanistic modeling strategy

To investigate the cyclisation mechanism, several substrate–oxidant assemblies were considered in order to reflect the experimentally relevant presence of AgF_2 and the possibility of distinct coordination motifs.[35] Three classes of mechanistic models could be identified for complete cyclisation (Figure S1).

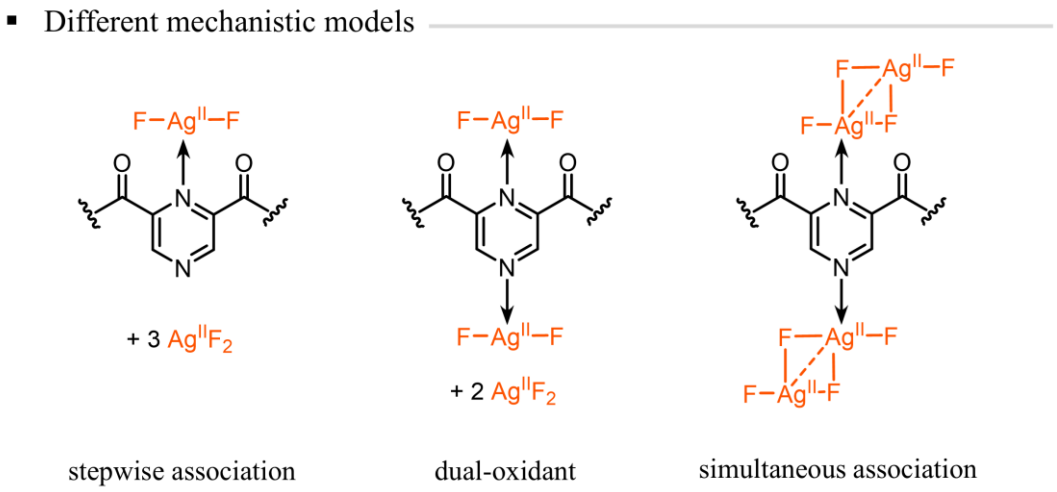


Figure S 28. Schematic representation of the mechanistic models considered for the AgF_2-mediated cyclisation: stepwise association, dual-oxidant, and simultaneous association with 4 AgF_2 models. The simultaneous association model was not pursued further due to its high computational cost.

First, **a stepwise association model** was considered, in which a single AgF_2 unit coordinates to the substrate and promotes N–H activation, leading to formation of an *N*-centered amidyl radical prior to C–N bond formation. This step is followed by ring closure, subsequent coordination of a second AgF_2 equivalent at N4, and final hydrogen abstraction/rearomatisation. An analogous sequence was assumed for the second cyclisation.

Second, a **dual-oxidant model** was investigated, in which two AgF_2 units participate simultaneously in each cyclisation event, with one AgF_2 unit coordinated to each pyrazine nitrogen atom, N1 and N4. An analogous sequence was again assumed for the second cyclisation.

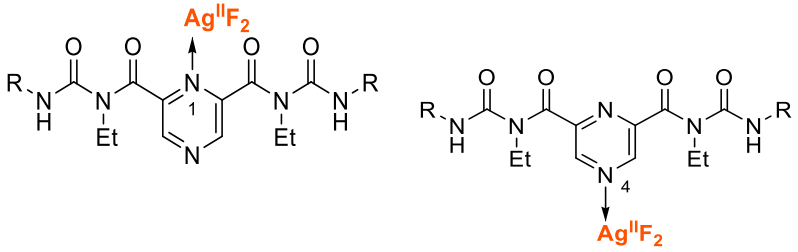
Third, a more elaborate model involving the simultaneous participation of four AgF_2 units over the course of the full reaction toward the final tricyclic product was considered. In this representation, a bimetallic $[AgF_2]_2$ assembly is coordinated to each pyrazine nitrogen atom.[35] Owing to the substantial computational expense, this model was not investigated further.

Because the substrate offers multiple potential coordination sites for AgF_2, including the pyrazine nitrogen atoms and carbonyl oxygen atoms, alternative coordination patterns were explored systematically. In particular, assemblies were generated in which AgF_2 was coordinated at different sites of the substrate. Given the considerable conformational flexibility of the substituents and the multiple possible coordination geometries, CREST conformational searches were performed within each mechanistic and coordination class to identify low-energy adduct structures. The most plausible starting

geometries for reaction profiling were selected based on their relative stability and their productive arrangement.

In the stepwise model, the initial coordination of the first AgF_2 equivalent was examined explicitly to identify the preferred binding site. The calculations showed that coordination at N1 is energetically favored, consistent with the arrangement required for amide N–H activation, the subsequent PCET-like process, and the following ring-closure step (Table S1 and Figure S2).

Table S 7. Relative Gibbs free energies of the two possible AgF_2-bound substrate complexes for different substituents. For each substituent, the lower-energy coordination mode shown on the left was taken as the reference structure (ΔG = 0.00 kcal mol^{-1}). Energies are reported in kcal.mol^{-1} at 303.15 K.



Entry	R	ΔG [kcal/mol], 303.15 K	
1	EtOAib	0.00	5.53
2	EtOAcb	0.00	4.89
3	Et	0.00	12.07
7	Ph	0.00	11.68

▪ Optimized structures of the substrate–AgF_2 complexes

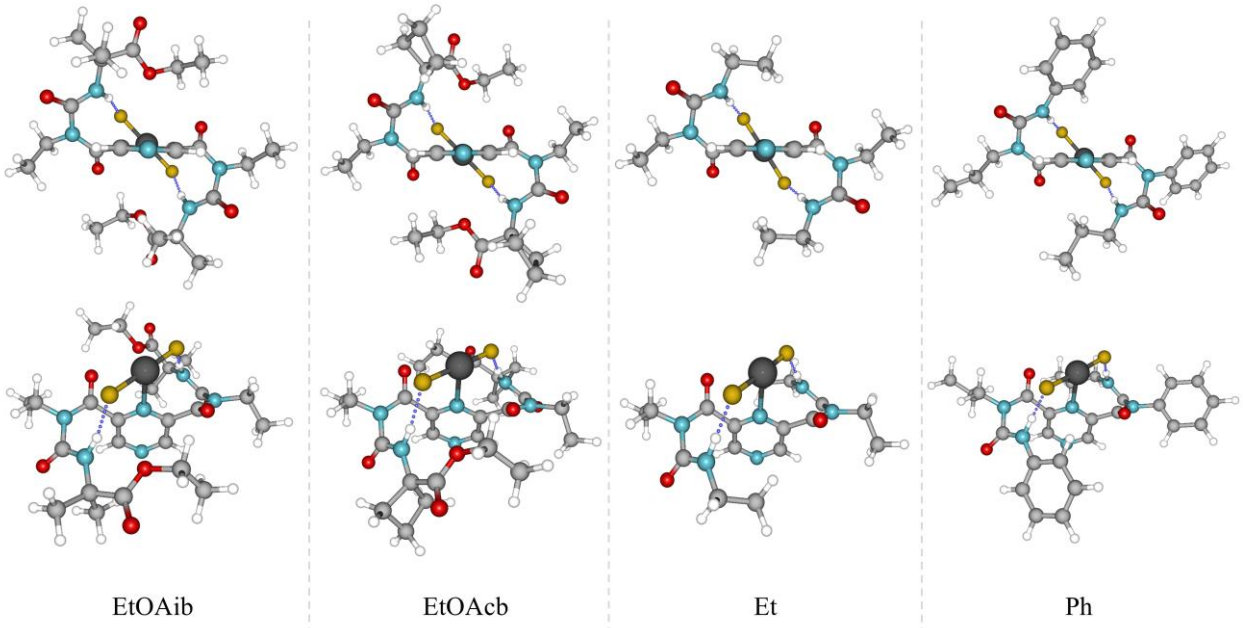


Figure S 29. Optimized structures of the substrate–AgF_2 complexes considered for the initial coordination step in the stepwise mechanistic model for different amide substituents: EtOAib, EtOAcb, Et, and Ph. Coordination of AgF_2 at the N1 position of the pyrazine ring was found to be the most favourable arrangement for all investigated substrates, consistent with the geometry required for subsequent amide N–H activation and ring closure. Structures were optimized at the UB3LYP-D3BJ/def2-SVP level of theory with def2-ECP for Ag and CPCM(acetonitrile). Two views are shown for each complex.

For the AgF_2-mediated cyclisation mechanism, open-shell electronic structures must be considered because AgF_2 contains a formally Ag(II) centre with a d^9 electronic configuration and one unpaired electron ($S = 1/2$). The electronic situation depends on the mechanistic model considered. In the stepwise association model, the first AgF_2 equivalent promotes N–H activation through proton transfer from the amide N–H group to fluoride, coupled with electron transfer to the Ag(II) centre. This process generates a substrate-centred radical while reducing the first Ag(II) centre. Upon coordination of the second AgF_2 equivalent, the system therefore contains two unpaired electrons: one located mainly on the organic radical fragment and one associated with the Ag(II) unit. In the dual-oxidant model, two AgF_2 equivalents are coordinated to the substrate simultaneously; therefore, two Ag(II)-based unpaired electrons are already present at the beginning of the reaction.

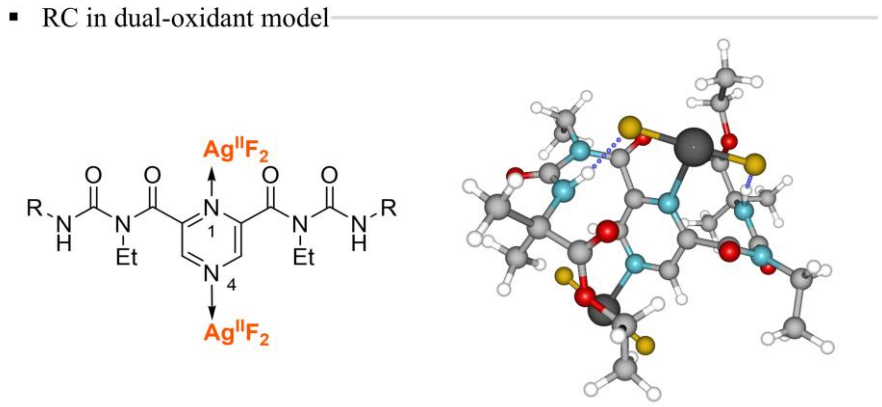


Figure S 30. Schematic representation and optimized structure of the reactant complex (RC) in the dual-oxidant model, optimized at the UB3LYP-D3BJ/def2-SVP level of theory with def2-ECP for Ag and CPCM (acetonitrile).

In both situations, two unpaired electrons can reside on different fragments. These unpaired electrons may couple ferromagnetically to give an overall triplet state (S=1), or antiferromagnetically to give an open-shell singlet state (S=0). To account for these possible spin couplings, the relevant intermediates and transition states were treated using unrestricted Kohn–Sham DFT (UKS-DFT).[36] While the triplet state is generally well represented by a single determinant, the singlet pathway, which is the preferred pathway for formation of the final product, often exhibits pronounced open-shell character with two antiferromagnetically coupled unpaired electrons. Under these conditions, a closed-shell restricted description is not appropriate. Accordingly, the broken-symmetry (BS) formalism was employed to model the open-shell singlet surface.[37]

Because the BS solution is not a pure spin eigenfunction and commonly exhibits spin contamination (i.e., $\langle S^2\rangle_{BS} > 0$), an approximate spin-projection correction was applied using the Yamaguchi scheme.[38,39] The singlet–triplet energy gap was estimated as

$$\Delta E_{ST} = \frac{2(E_{BS} - E_T)}{\langle S^2\rangle_T - \langle S^2\rangle_{BS}}$$

where E_{BS} and E_T are the energies of the broken-symmetry singlet and the triplet states, respectively, and $\langle S^2 \rangle_T$_$\langle S^2 \rangle_{BS}$ are the corresponding spin angular momentum expectation values (with $\langle S^2 \rangle_T \approx 2$ for an ideal triplet). This procedure provides an estimate of the energetics associated with the pure open-shell singlet state while reducing the bias introduced by spin contamination in the BS determinant.

8.4 HOMO and LUMO analysis of 1a, [1a-H]$^+$, [1a][AgF_2], [1a][AgF_2]$_2$

The frontier molecular orbitals of **1a**, [**1a**–H]$^+$, [**1a**][AgF_2], and [**1a**][AgF_2]$_2$ were analysed to evaluate the electronic effect of AgF_2 coordination (Figure S4). Compared with the neutral substrate **1a**, protonation/deprotonation-related modification in [**1a**–H]$^+$ lowers both the HOMO and LUMO energies and reduces the HOMO–LUMO gap from 0.1765 to 0.1397. However, coordination of AgF_2 has a much stronger effect on the frontier orbital energies, particularly on the LUMO, which is significantly stabilized upon formation of [**1a**][AgF_2]. As a result, the HOMO–LUMO gap decreases to 0.0864. Addition of a second AgF_2 unit further stabilizes the acceptor orbitals and narrows the gap to 0.0751. This progressive decrease in the HOMO–LUMO gap indicates that AgF_2 coordination increases the electronic activation of the substrate–oxidant complex and facilitates charge-transfer in the subsequent N–H activation step. The spatial distribution of the frontier orbitals also shows an increasing contribution from the AgF_2 fragment upon coordination, supporting the role of AgF_2 as the oxidizing unit in the PCET-like N–H activation process.

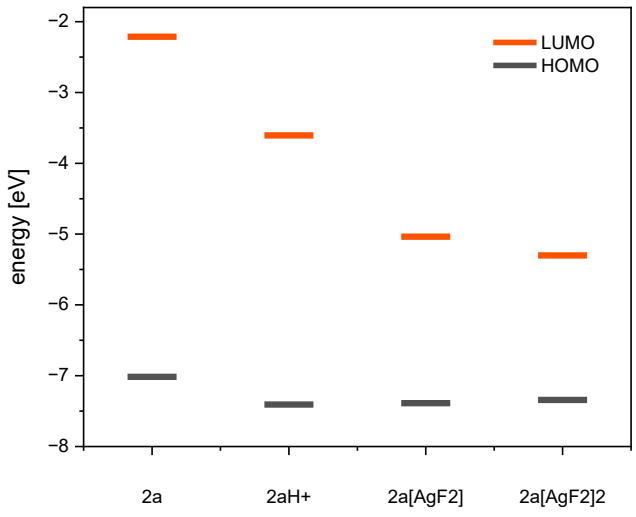
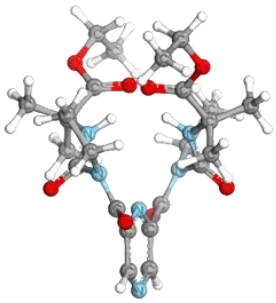
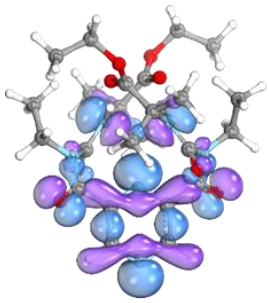
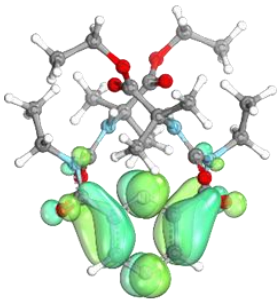
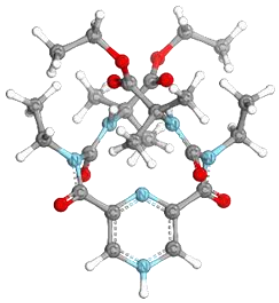
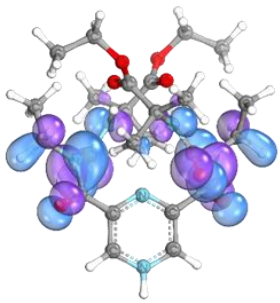
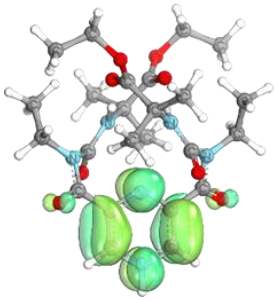
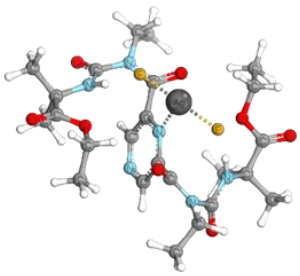
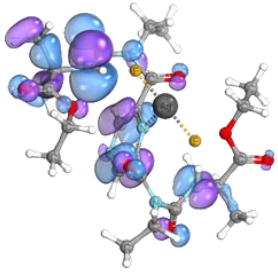
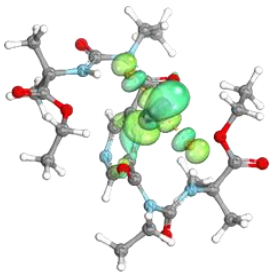
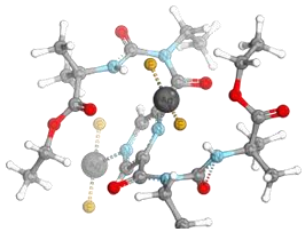
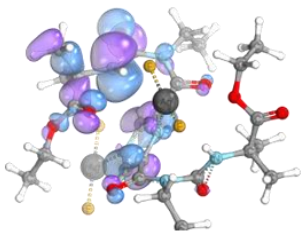
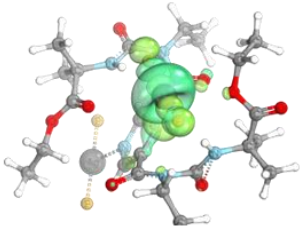


Figure S 31. Variation of the HOMO-LUMO gap with LUMO-lowering protonation and AgF_2 coordination, respectively.

Table S 8. Frontier molecular orbital plots of **1a**, [**1a**–H]$^+$, [**1a**][AgF_2], and [**1a**][AgF_2]$_2$. HOMO and LUMO energies, together with the corresponding HOMO–LUMO gaps, are given below each orbital representation. Energies are reported in eV. For the open-shell AgF_2-containing complexes, the relevant α and β HOMO energies are reported. The calculations show a progressive decrease in the HOMO–LUMO gap upon substrate activation and coordination to AgF_2.

▪ HOMO and LUMO

Compound	HOMO	LUMO	H-LGap
			
2a	–7.0138	–2.2127	**4.8011**
			
[**2a**-H]$^+$	–7.4070	–3.6056	3.8014
			
[**2a**][AgF_2]	–7.3879 (α), –7.3875 (β)	–5.0365	2.351
			
[**2a**][AgF_2]$_2$	–7.3427 (α), –7.3444 (β)	–5.3007	**2.042**

8.5 Mechanistic analysis and reaction energy profile

In both mechanistic scenarios investigated, the first elementary step involves coordination of AgF_2 to the substrate at the N1 site, followed by amide N–H activation and generation of an *N*-centered amidyl radical prior to C–N bond formation. The subsequent ring-closure step leads to a pyrazine-ring-centered radical intermediate. The first cyclisation sequence then terminates through hydrogen abstraction from the newly formed N–C(H) moiety by a fluorine ligand of the AgF_2 unit coordinated to the neighboring N4 site, followed by rearomatisation and formation of the monocyclized product. An analogous three-step sequence on the opposite side of the pyrazine ring then furnishes the final tricyclic product. These elementary steps are discussed in detail below for the two mechanistic models.

The complete energy profiles of the two mechanistic models are shown in Figure S1. For a direct comparison, the relative Gibbs free energies of all stationary points were referenced to a common starting point, defined as the separately optimized substrate and four AgF_2 units (SR). Because the number of molecular species changes upon association, standard-state corrections were included to convert the gas-phase 1 atm standard state to the 1 M solution standard state. Accordingly, each stationary point was corrected by a term $\Delta nRT \ln(24.88)$ at 303.15 K, where $\Delta n = N_i - N_{SR}$ denotes the change in the number of molecular species relative to SR. The key structural parameters of all transition states are also shown in Figure S2, and the corresponding activation barriers are reported below each transition-state structure relative to the preceding local minimum intermediate.

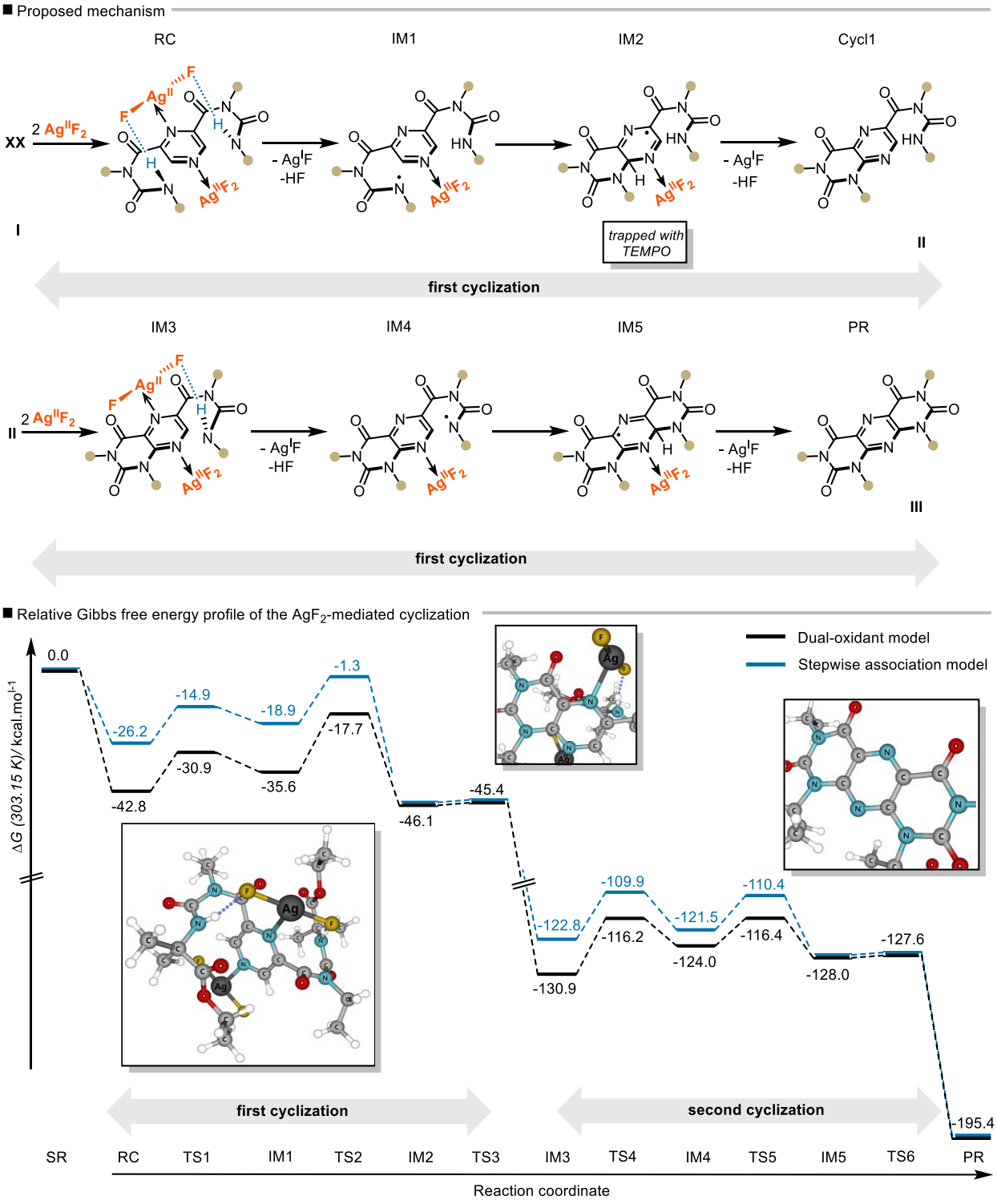


Figure S 32. Proposed mechanism and relative Gibbs free energy profile for the AgF_2-mediated double cyclization, calculated at the UB3LYP-D3BJ/def2-TZVP level of theory with CPCM (acetonitrile). Energies are given in kcal mol^{-1} relative to the separated reactants at 303.15 K. Black and blue lines represent the dual-oxidant and stepwise association models, respectively. SR, RC, TS, IM, and PR denote separated reactants, reactant complex, transition state, intermediate, and product, respectively.

As described above, the reaction is initiated by the coordination of AgF_2 to the substrate at the N1 site. This coordination arrangement enables N7–H activation, which is assisted by hydrogen-bonding interactions between the amide N–H units and fluoride ligands on both sides of the pyrazine plane

(Figure S1). The first N–H activation step can be described as a PCET-like process, in which proton transfer to fluoride is coupled to electron transfer to the Ag(II) center. This step proceeds through TS1 with relatively low activation barriers of ca. 11.4 and 12.0 kcal.mol^{-1} in the two mechanistic models, respectively, leading to the formation of an N-centered amidyl radical. The natural spin densities for this and the subsequent intermediates are collected in Table S2, as obtained from NBO analysis. Moreover, to obtain a more direct visual representation of the electronic rearrangement during this and the next key elementary steps (namely the PCET-like N7–H activation and the subsequent C3–N7 bond-forming ring closure), IBO analyses were performed along the corresponding IRC pathways and are shown in Figure S3.

Inspection of the NBO spin densities together with the IBO analysis indicates that transfer of the proton from the amide N atom to fluoride is accompanied by electron transfer from the amide N lone pair to Ag(II)F_2, resulting in reduction of Ag(II) to Ag(I) and formation of HF. The remaining unpaired electron is localized predominantly on the amide nitrogen, N7, giving rise to the N-centered radical character of the intermediate, which is stabilized by delocalization into the π-system of the pyrazine ring. This electronic arrangement promotes intramolecular radical cyclisation through the formation of the N7–C3 bond. The ring-closure step generates a pyrazine-ring-centered radical intermediate, IM2, in which the unpaired electron is delocalized over the aromatic framework but has the largest natural spin-density contribution at C6, approximately 0.4. This spin distribution is consistent with the experimental TEMPO-trapping results, which indicate interception of the transient radical intermediate at the position of highest radical character. The relatively higher activation barrier for this step, ca. 18.0 kcal mol^{-1} in both mechanistic models, suggests that C–N bond formation is the kinetically most demanding step of the first cyclisation sequence.

The final step of the first cyclisation sequence is common to both mechanistic scenarios. In this step, hydrogen abstraction from C3–H by a fluorine ligand of the AgF_2 unit coordinated to the neighboring N4 site restores aromaticity in the pyrazine ring and leads to the formation of the monocyclized product. The very small computed activation barrier of ca. 0.7 kcal mol^{-1}, together with the pronounced thermodynamic stabilization of the monocyclized product by ~ >80 kcal mol^{-1}, indicates that this rearomatization step is expected to proceed rapidly once the pyrazine-ring-centered radical intermediate has been formed.

Overall, the calculated energy profiles indicate that the initial N–H activation is facile, whereas the subsequent C–N bond-forming ring closure represents the kinetically most demanding step. Comparison of the stepwise and dual-oxidant models further suggests that pre-coordination of the second AgF_2 equivalent does not substantially alter the early part of the mechanism, either geometrically or energetically (Figure S2). Instead, the second AgF_2 unit provides the oxidizing fluoride ligand required for the final hydrogen-abstraction/rearomatization step. Thus, the AgF_2 unit coordinated at N1 is directly involved in the initial N–H activation, whereas the second AgF_2 equivalent becomes important during radical oxidation and product formation.

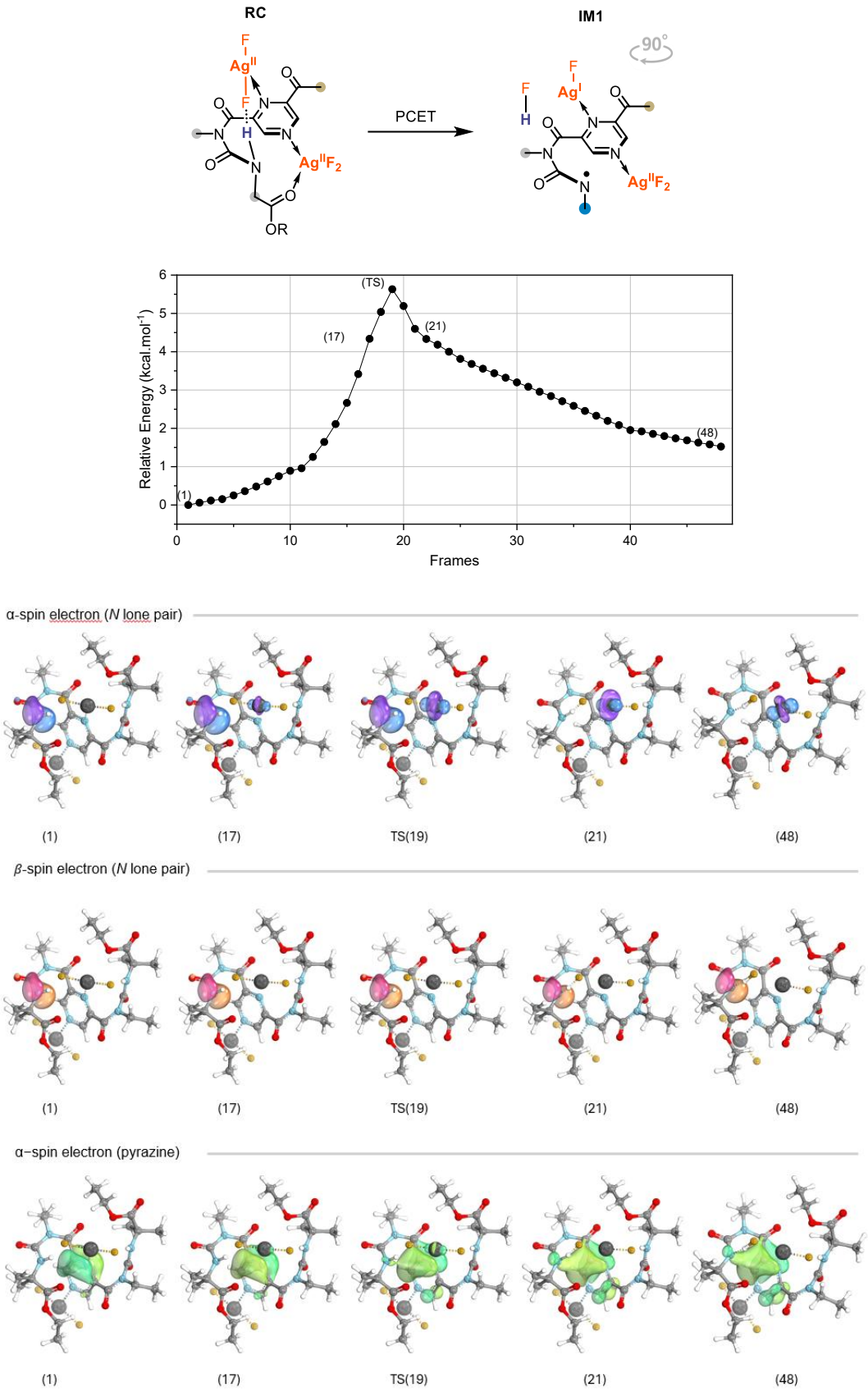


Figure S 33. IRC analysis of the investigated reaction pathway and corresponding intrinsic bonding orbital (IBO) evolution. The IRC was calculated from the transition state **T1** toward both reactant and product minima to confirm the connectivity of the stationary points on the potential energy surface. Representative IBOs along the IRC illustrate the electronic reorganization associated with the N–H bond cleavage.

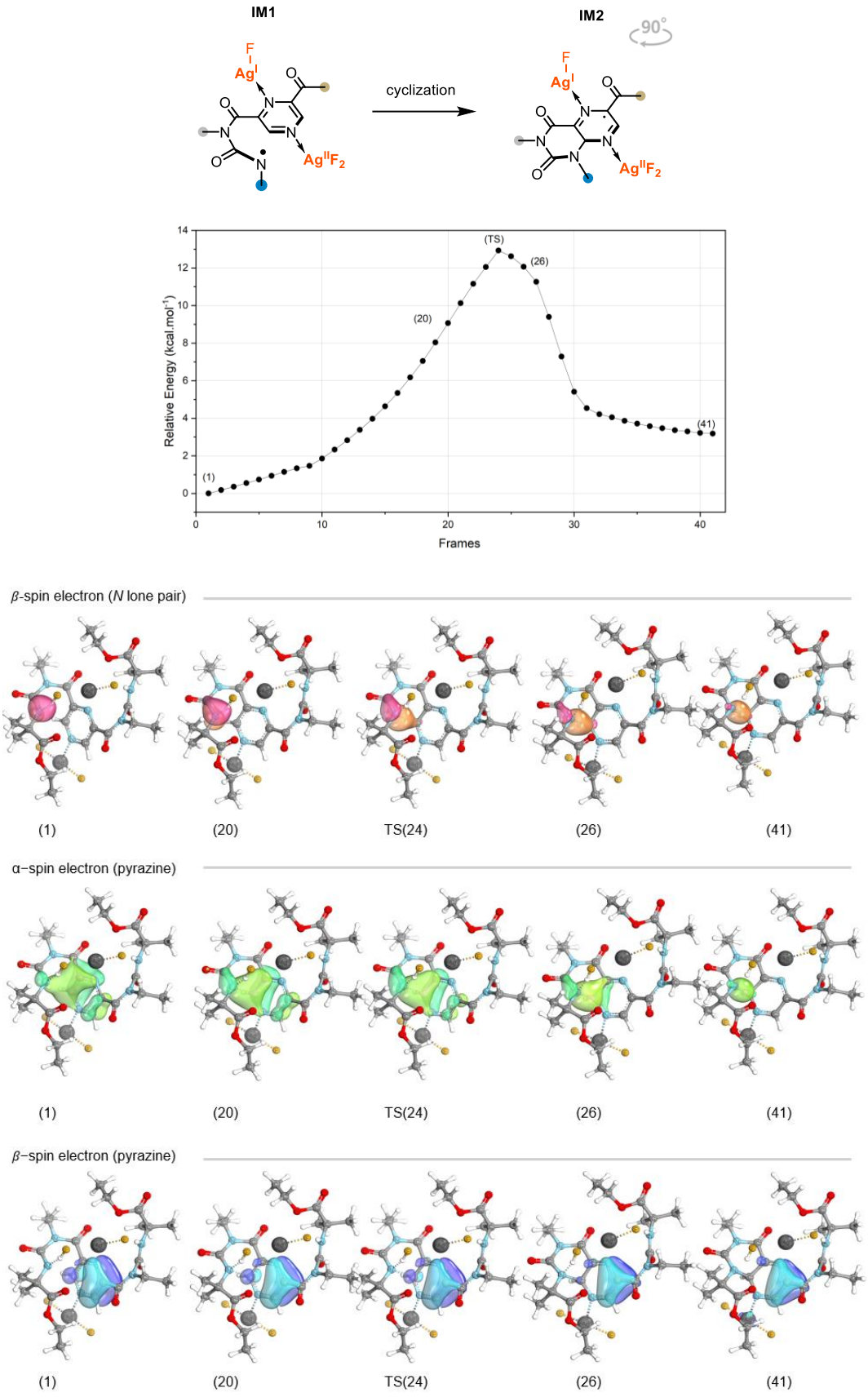


Figure S 34. IRC analysis of the investigated reaction pathway and corresponding IBO evolution. The IRC was calculated from the transition state **T2** toward both reactant and product minima to confirm the connectivity of the stationary points on the potential energy surface. Representative IBOs along the IRC illustrate the electronic reorganization associated with the radical amidation.

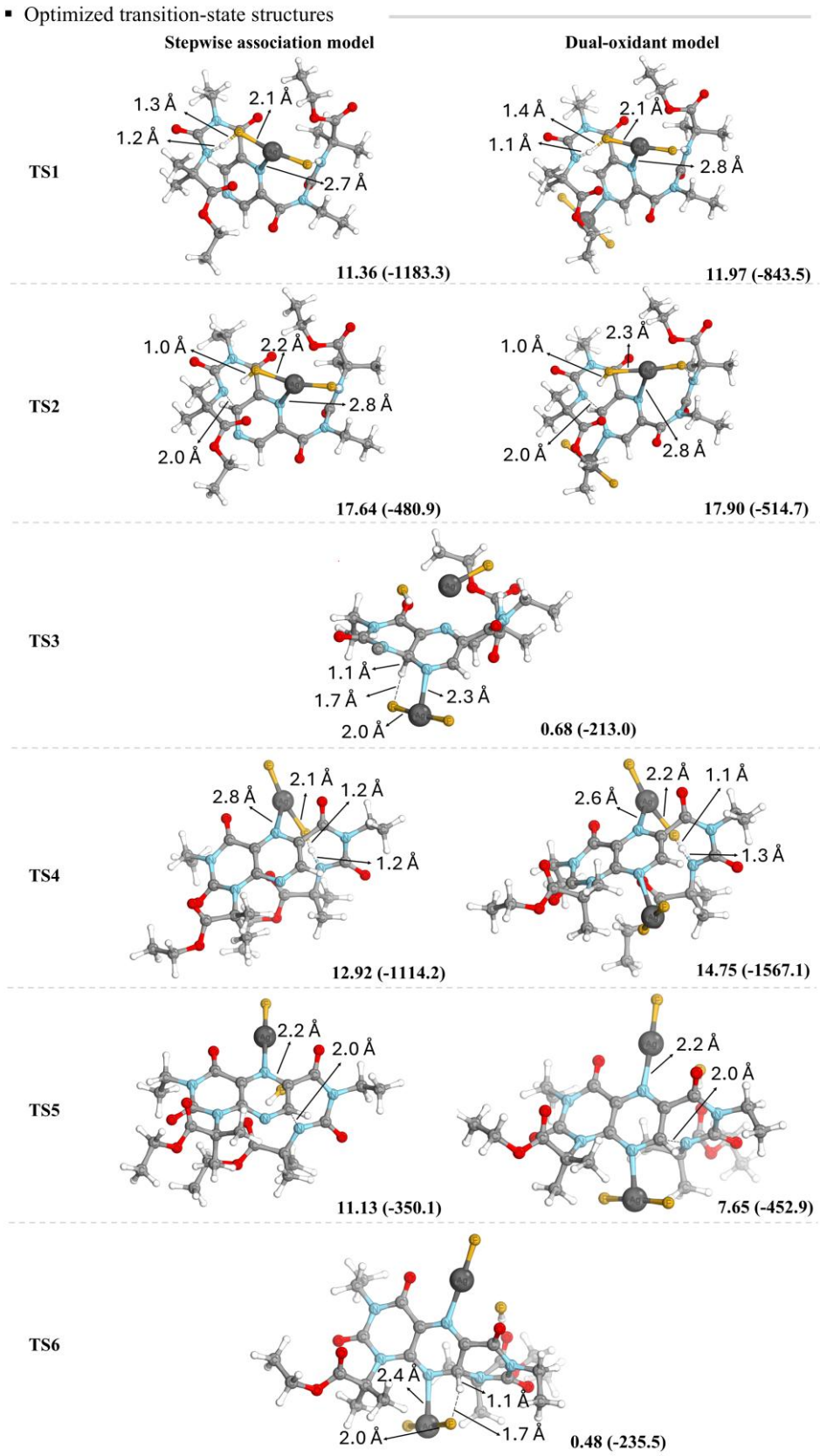
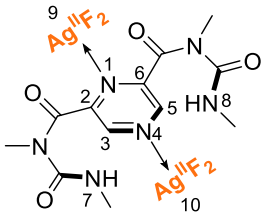
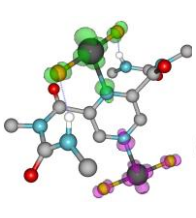
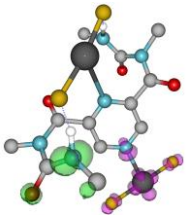
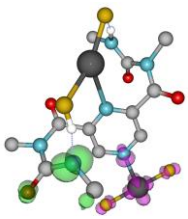
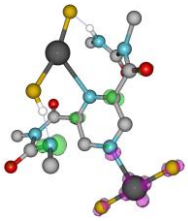
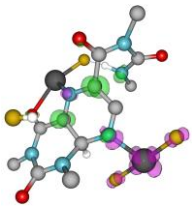
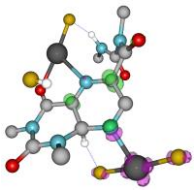
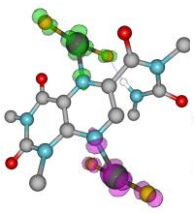
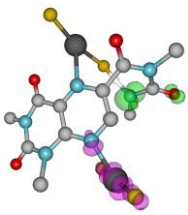
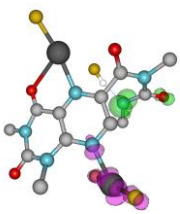
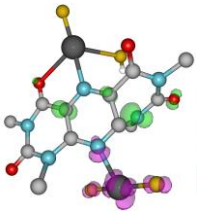
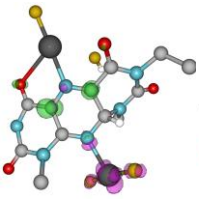
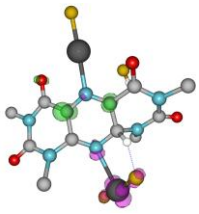


Figure S 35. Optimized transition-state structures for the stepwise association and dual-oxidant mechanistic models at the UB3LYP-D3BJ/def2-SVP level of theory with def2-ECP for Ag and CPCM(acetonitrile). Characteristic interatomic distances are indicated in Å. The activation barriers reported below each transition state are given in kcal.mol^{-1} relative to the preceding local minimum intermediate. Imaginary frequencies for the TS structures are reported in parentheses in cm^{-1}.

Table S 9. Natural spin-density plots and atom-resolved spin populations for the stationary points along the reaction pathway calculated at UB3LYP-D3BJ/def2-TAVP with CPCM (acetonitrile). The natural spin populations are reported for selected atoms, labeled according to the molecular structure shown above the table. For clarity, the side chains are omitted from the spin-density plots, and for each stationary point, the atoms with the largest absolute spin populations are highlighted in bold.



Natural Spin Density						
Atom	**RC**	**TS1**	**IM1**	**TS2**	**IM2**	**TS3**
						
N 1	-0.18631	-0.00417	0.01807	0.09054	0.10649	0.07423
C 2	0.02457	0.00398	-0.02364	-0.22412	-0.25378	-0.17027
C 3	-0.02634	-0.04558	0.04272	0.08443	0.01446	0.00201
N 4	0.20137	0.21246	0.21299	0.06178	0.01942	-0.01935
C 5	-0.02599	-0.06203	0.05883	-0.00583	0.02941	0.04215
C 6	0.02331	-0.00977	-0.00792	-0.30341	**-0.36014**	**-0.29021**
N 7	0.00086	**-0.59986**	**-0.73580**	**-0.34418**	-0.02389	-0.01784
N 8	0.00069	0.00008	-0.00022	0.00109	-0.09914	0.00066
Ag 9	**-0.52954**	-0.05384	0.00005	0.00089	-0.00052	0.00055
Ag10	**0.53638**	**0.53300**	**0.53444**	**0.53334**	**0.45177**	**0.33558**

Natural Spin Density						
Atom	**IM3**	**TS4**	**IM4**	**TS5**	**IM5**	**TS6**
						
N 1	-0.12419	0.00551	-0.00854	0.02326	0.11795	0.08005
C 2	0.01092	-0.03290	-0.01442	-0.23464	**-0.47857**	**-0.34997**
C 3	-0.00438	-0.02290	-0.02059	0.00077	0.04091	0.04429

N 4	0.13591	0.17118	0.17181	0.12426	0.04957	0.03029
C 5	-0.01086	-0.03764	-0.02421	0.04478	0.01709	0.00657
C 6	0.01237	-0.02047	0.00671	-0.12027	-0.33521	-0.22838
N 7	0.00467	-0.02656	-0.01124	-0.02492	-0.00929	0.00979
N 8	0.00055	**-0.66998**	**-0.74601**	**-0.43550**	-0.02857	0.00294
Ag 9	**-0.56056**	0.00074	-0.00174	-0.00758	0.00266	0.00488
Ag 10	**0.54022**	**0.53648**	**0.53993**	**0.52965**	**0.51450**	**0.34288**

Next, the effect of the substituent attached to the amide group was further investigated. For this purpose and based on the results obtained for the acyclic ester, the elementary steps of the first cyclisation sequence were calculated for different substituents, including cyclobutyl ester, ethyl, and phenyl groups, using the stepwise mechanistic model. The corresponding energy profiles are shown in Figure S4, while selected structural parameters of the transition states and their imaginary frequencies are summarized in Table S3.

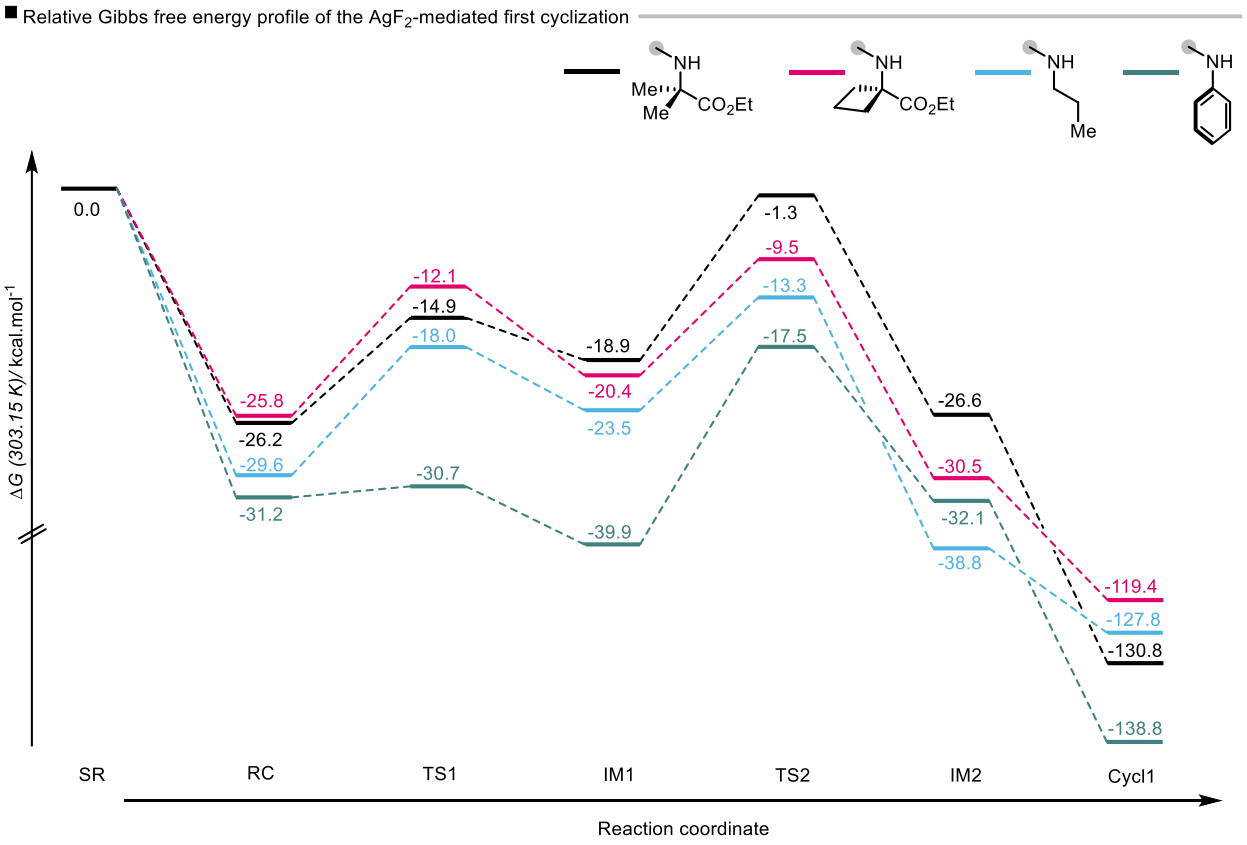


Figure S 36. Relative Gibbs free energy profiles for the AgF_2-mediated first cyclization of substrates bearing different amide substituents, calculated at the UB3LYP-D3BJ/def2-TZVP level of theory with CPCM (acetonitrile). Energies are given in kcal mol^{-1} relative to the separated reactants at 303.15 K. The different colored profiles correspond to the substituents shown above the energy diagram. SR, RC, TS, IM, and Cyc1 denote separated reactants, reactant complex, transition state, intermediate, and first cyclization product, respectively.

Overall, the same qualitative reaction pattern is obtained for all investigated substrates. Formation of the initial reactant complex is exergonic in all cases, indicating favorable association of AgF_2 with the substrate prior to N–H activation. The first transition state, TS1, corresponds to N–H activation through proton transfer from the N–H group to the fluoride ligand, coupled with electron transfer to the Ag(II) centre. For all substituents, TS1 remains below the separated reactants, showing that this step is energetically accessible and is not expected to be rate-determining.

The structural parameters of TS1 further support this conclusion. For most substituents, the N–H and H···F distances indicate an advanced proton-transfer geometry from the N–H group to the fluoride ligand. In contrast, the phenyl-substituted substrate shows a markedly earlier, more reactant-like TS1 structure. In this case, the N–H bond remains relatively short, whereas the H···F distance is comparatively long, indicating that proton transfer to the fluoride ligand is less advanced at the transition state. This structural feature is consistent with the very small TS1 barrier obtained for the phenyl derivative.

The particularly facile N–H activation in the phenyl-substituted substrate can be rationalized by stabilization of the developing N-centred radical character through conjugative interaction with the phenyl π-system. This interpretation is also supported by the calculated N–H bond dissociation free energies, which follow the same qualitative trend as the TS1 barriers. The calculated N–H BDFE values are 98.6, 94.3, 96.9, and 89.8 kcal mol^{-1} for the acyclic ester, cyclobutyl ester, ethyl, and phenyl derivatives, respectively. Thus, the N–H BDFE of the phenyl-substituted substrate is lower by ca. 8.8 kcal mol^{-1} compared with the acyclic ester and by ca. 7.1 kcal mol^{-1} compared with the ethyl derivative. This finding indicates that formation of the corresponding N-centred radical is thermodynamically more favourable for the phenyl-substituted system, in agreement with the very low TS1 barrier.

The main energetic difference among the substituents is observed for the subsequent cyclisation step. In all cases, TS2 corresponds to the C–N bond-forming step and represents the highest-energy point along the first cyclisation sequence. Therefore, this step is identified as the kinetically most demanding elementary step for the first cyclisation. The calculated profiles show that the amide substituent mainly modulates the relative stability of the intermediates and the energy of the C–N bond-forming transition state, rather than changing the overall mechanistic picture.

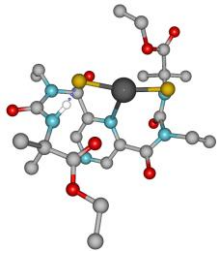
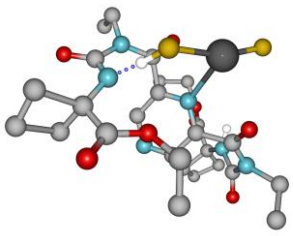
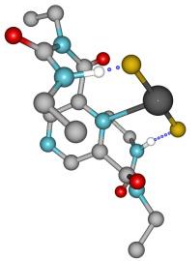
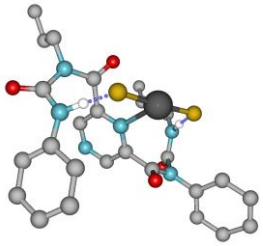
The structural data for TS2 are also consistent with the calculated trends. The N···C distances are in a similar range for all substituents, indicating comparable C–N bond-forming transition states. However, the phenyl-substituted substrate shows a slightly shorter N···C distance, suggesting a somewhat more advanced cyclisation geometry. Despite these differences, TS2 remains the key transition state for comparing the substituent effect on the first cyclisation sequence.

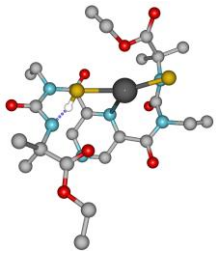
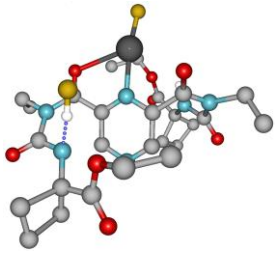
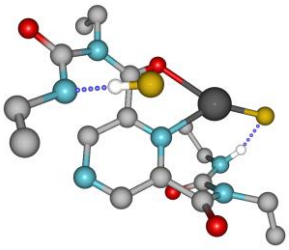
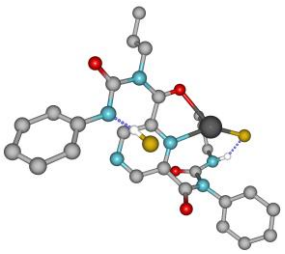
After IM2, the final step leading to the first cyclised product is associated with a very shallow energy region and an expected very small barrier. Therefore, explicit localization of the corresponding TS3 was

not pursued for this substituent comparison. Instead, IM2 was connected directly to the first-cyclisation product, Cyc1. This simplification does not affect the comparison of the main energetic trends, since TS2 remains the highest and most relevant transition state along the first cyclisation sequence for all investigated substrates.

Taken together, these results indicate that changing the amide substituent does not alter the general mechanism of the AgF_2-mediated first cyclisation. Instead, the substituent mainly affects the stability of the radical/intermediate region and the accessibility of the C–N bond-forming transition state. The phenyl substituent shows a particularly small barrier for the initial N–H activation step, which is consistent with its early TS1 geometry, conjugative stabilization of the developing N-centred radical, and the lower calculated N–H BDFE.

Table S 10. Relative electronic energies (ΔEel, kcal.mol^{-1}) and solution-phase Gibbs free energies (ΔG, kcal.mol^{-1}) for the AgF_2-mediated first cyclization of substrates bearing different amide substituents, calculated at the UB3LYP-D3BJ/def2-TZVP level of theory with CPCM(acetonitrile). Energies are referenced to the separated radicals (SR) set to 0.0. RC, TS, IM, and Cyc1 denote reactant complex, transition state, intermediate, and first cyclization product, respectively. Imaginary frequencies for the TS structures are reported in cm^{-1}. Selected optimized geometries of TS1 and TS2 are shown, together with key structural parameters: N–H and H···F distances for TS1, and N···C distances for TS2 in Å. The calculated N–H bond dissociation free energies (BDFEs) are reported in kcal mol^{-1}.

	EtOAib			**EtOAcb**			**Et**			**Ph**		
	ΔE_{el}	ΔG	Freq.	ΔE_{el}	ΔG	Freq.	ΔE_{el}	ΔG	Freq.	ΔE_{el}	ΔG	Freq.
SR	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0	
RC	-33.2	-26.2		-33.5	-25.8		-37.8	-29.6		-38.2	-31.2	
TS1	-17.3	-14.9	-1183.3	-13.8	-12.2	-1200.2	-20.5	-18.0	-1396.3	-36.2	-30.7	-288.2
IM1	-23.3	-18.9		-24.0	-20.4		-28.8	-23.5		-44.5	-39.9	
TS2	-6.4	-1.3	-480.9	-13.3	-9.5	-523.7	-19.1	-13.3	-464.6	-22.7	-17.5	-590.0
IM2	-33.1	-26.6		-37.0	-30.5		-47.1	-38.8		-37.3	-32.1	
Cycl 1	-147.1	-130.8		-135.1	-119.4		-141.2	-127.8		-152.2	-138.8	
TS1												
N···H	1.15			1.40			1.23			1.07		
F···H	1.30			1.07			1.18			1.48		
BDFE	98.6			94.3			96.9			89.8		

TS2				
N···C	1.98	1.99	2.03	1.93

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