ELECTRONIC SUPPORTING INFORMATION

Constitutional Adaptation to pKa Modulation by Remote Ester Hydrolysis

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Table of contents

1. Experimental section

Materials and Methods

All **AAOMe**/$^{+}$**AA**$^{-}$, aldehydes and components of buffer solutions were obtained from commercial suppliers and used without further purification. Unless otherwise noted, the (*L*) enantiomers of **AAOMe** were used. Acetylcholine esterase (**AChE**), **trypsin** and **chymotrypsin** were obtained from SigmaAldrich. A Mettler Toledo SevenCompact pH Meter S220 was used to monitor the pD of the solutions, adjusted with either NaOD or DCl solutions as appropriate. All reactions were performed at room temperature, unless otherwise noted. Imination reactions were carried out in 50 mM deuterated phosphate buffer pD 7.0 unless otherwise indicated. For the imination reactions at > 20 mM concentration of reagents, a 500 mM deuterated phosphate buffer pD 7.0 was used instead to assure that the pD was constant. The product abundances (%) and concentrations (mM) were determined by relative integration of the signals in the 500 MHz ^{1}H-NMR spectra (Bruker Ascend Spectroscope Advance Neo-500 MHz; 500 MHz for ^{1}H and 125 MHz for ^{13}C{^{1}H}). First, the spectrum was referenced, phased, and the baseline was corrected. Afterwards, the signals of interest were accurately integrated to minimise the residual error, and finally, the numerical values are processed for calculating product abundances.[1] The hydrate detected was included in the aldehyde concentration. Error bars correspond to 5% of the measurement. An example of the procedure is illustrated in Fig. S33. The presence of the imine species was also confirmed by HRMS. Data analysis was performed using MestReNova (version 14.2.3) and OriginPro (version 9.8.0.200). HRMS-electro-spray ionization (HRMS-ESI) mass spectra were recorded using a ThermoFisher Exactive Plus EMR Orbitrap mass spectrometer.

Protonation degree and pKa estimation

All the pKa and -NH_2 protonation degrees were estimated using MarvinSketch software from ChemAxon. After drawing the desired molecule on the software, the pKa and microspecies distribution -in terms of protonation and pH- were calculated using the "protonation -- pKa" tool. The macro mode with dynamic acid/base prefix was selected, for pKa values ranging between $-2 \leq x \leq 35$. The temperature for the calculation was set at 298 K, and the correction library was used. For the microspecies distribution, a pH step size of 0.2 was used. This allowed for estimation of the degree of -NH_2 protonation at different pH. See Tables S1, S2, S12, and S13 for obtained results.

AAOMe hydrolysis kinetic experiments

The kinetic profiles for the **AAOMe** hydrolysis were monitored by ^{1}H NMR spectroscopy (500 MHz, 298 K, D_2O, pD = 7.0, PBS 50 mM). The abundance of the species was determined by relative integration of **AAOMe,** $^{+}$**AA**$^{-}$ and CH_3OH. To corroborate a correct assessment of the hydrolysis profile, the integration of the proton of the chiral C* of $^{+}$**AA**$^{-}$ was compared to that of the -CH_3 group of methanol, observing in all cases the expected 1 : 3 ratio (see Fig. S34 and S35 for an example concerning the hydrolysis of **LysOMe**). The reaction rates were estimated using the slopes (linear fitting with fixed Y-intercept = 0) determined for the linear range in each case. Comparison between slopes was used

to qualitatively study the rates of hydrolysis, both in the presence and absence of the enzyme.

Enantiomeric excess and *s*-factor determination by ^{1}H NMR

The abundance of each of the **LysOMe** and $^+$**Lys**$^-$ enantiomers in the kinetic resolution of racemic **LysOMe** (5 mM) in the presence of **A1** (5 mM) and **AChE** (0.01 mol%) was estimated by ^{1}H NMR spectroscopy (500 MHz, 298 K, D_2O, pD = 7.0, PBS 50 mM). Studying the separated hydrolysis of **(*L*)LysOMe** and **(*D*)LysOMe** in the presence of **AChE** (0.01 mol%), relative slopes for the linear ranges of 3.5 and 1.2 were noted, respectively. During the kinetic resolution, an initial relative $slope_1$ of 3.07 was observed (0-16 h), after which the kinetic profile for the hydrolysis suffered a drastic deacceleration ($slope_2$ = 0.79, 16-72 h). Thus, one can notice that the concentration of **(*L*)LysOMe** at 16 h is close to 0, and thus the concentration of **(*D*)LysOMe** can be estimated by the relative integration of its characteristic protons in the ^{1}H NMR spectrum. Once the concentration of these species is known, the abundance of the hydrolysed species $^+$**(*L*)Lys**$^-$ and $^+$**(*D*)Lys**$^-$ can also be determined through relative integration (taking into account the aldehyde CHO and imine CH integration). The enantiomeric excess could be thus determined at different times with the relative concentration of each species, using the formula -example for **(*L*)LysOMe**-: ee**(*L*)LysOMe** (%) = ([**(*L*)LysOMe**]-[**(*D*)LysOMe**])/([**(*L*)LysOMe**]+[**(*D*)LysOMe**])*100. The *s*-factor could also be calculated with the formula: *s*-factor = (ln (1-Y)(1-ee))/(ln (1-Y)(1+ee));[2] where Y is the hydrolysis yield and ee is the enantiomeric excess for a certain species at a certain time.

Note: the slight difference in slope -decrease in rates- when comparing the racemic resolution and each separated hydrolysis of the pure enantiomers of **LysOMe** was assigned to the presence of **A1**, as also observed in the case of **ArgOMe** (see Fig. S36 and Table S13).

Assessment of micelle formation by fluorescent probe

Fluorescent measurements were done on a CLARIOstar Plus microplate reader using a Costar 96 wells plate. Nile red is a fluorescent probe which display fluorescence only in hydrophobic environment, hence used to prove the formation of micelles.[3] 1 µL of a 0.75 mM solution of Nile red in ethanol was deposited in a well and dried at room temperature for 15 min in the dark. 150 µL of the sample to analyze was added and the mixture was equilibrated for 30 min, and the fluorescence was measured afterwards. Nile red excitation and emission were respectively fixed at 515 nm and 585 nm. The resulting fluorescence intensity was normalized by dividing it by the intensity of the control sample: 150 µL of a 50 mM solution of SDS (above its CMC of 7-10 mM). The fluorescence of the buffer solution was also measured as blank.

DLS analyses

Dynamic Light Scattering (DLS) experiments were done on a Malvern Zetasizer Ultra instrument. 1 mL of the sample was filtered with a syringe filter (0.2 µm pore, PTFE) into the plastic cuvette (1 cm path) for analysis.

Synthesis of the pseudopeptidic amines

Synthesis of AlaNHC5. BocAlaOSuc (1 eq., 0.518 g, 1.809 mmol) was dissolved in DCM (10 ml) and 1-pentylamine (1 eq., 0.157 g, 0.210 mL, 1.809 mmol) was added to the solution. The mixture was stirred at r.t. for 16 h. Basic H_2O (10 mL, pH = 9) was then added and the organic phase was separated by decantation. This process was repeated 3 additional times (3 x 10 mL of H_2O). The organic phase was dried with Na_2SO_4. The solid was filtered-off, and 2 mL of TFA (excess) were added to the DCM solution (≈ 10 mL). The mixture was stirred at r.t. for 3 h to assure complete Boc deprotection. Thereafter, the solvent was removed under reduced pressure and the resultant yellowish oil was redissolved in DCM (10 mL). Liquid-liquid extractions were then carried out with basic water (3 x 5 mL, pH = 9). The organic phase was dried with Na_2SO_4 and the solvent was removed under reduced pressure, yielding pure **AlaNHC5** as a colourless viscous liquid (0.077 g, 0.488 mmol, 27% yield). Characterization: FTIR (ATR): 3320(bs), 2939, 1645, 1520, 1279. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.15 (s, 1H), 3.42 (q, *J* = 7.0 Hz, 1H), 3.17 (td, *J* = 7.3, 5.9 Hz, 2H), 1.47 – 1.43 (m, 2H), 1.26 (d, *J* = 7.0 Hz, 7H), 0.83 (t, *J* = 7.0 Hz, 3H). ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$) δ (ppm) 175.5, 50.8, 39.0, 29.3, 29.1, 22.4, 21.9, 14.0. HRMS-QTOF(+) calcd for $C_8H_{18}ON_2$: 159.1492, found 159.1489. See Fig. S37-S39 and S46 for spectra.

Synthesis of AlaNHC6. This compound was synthesized following the same procedure as described for **AlaNHC5** (0.230 g, 1.336 mmol, 47%, colourless viscous liquid). Characterization: FTIR (ATR): 3298(bs), 2966, 1641, 1520, 1249. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.19 (s, 1H), 3.42 (q, *J* = 7.0 Hz, 1H), 3.17 (td, *J* = 7.3, 5.9 Hz, 2H), 1.46 – 1.38 (m, 2H), 1.30 – 1.19 (m, 9H), 0.87 – 0.78 (m, 3H). ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$) δ (ppm) 175.5, 50.8, 39.0, 31.5, 29.6, 26.6, 22.6, 21.9, 14.0. HRMS-QTOF(+) calcd for $C_8H_{20}ON_2$: 173.1648, found 173.1646. See Fig. S40-S42 and S47 for spectra.

Synthesis of AlaNHC7. This compound was synthesized following the same procedure as described for **AlaNHC5** (0.317 g, 1.703 mmol, 62%, colourless viscous liquid). Characterization: FTIR (ATR): 3311(bs), 2943, 1635, 1531, 1275. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm) 7.10 (s, 1H), 3.42 (d, J = 6.9 Hz, 1H), 3.24 – 3.10 (m, 2H), 1.44 (t, J = 7.2 Hz, 2H), 1.31 – 1.19 (m, 11H), 0.81 (t, J = 6.7 Hz, 3H). ^{13}C{^{1}H}-NMR (125 MHz, $CDCl_3$) δ (ppm) 175.5, 50.8, 39.0, 31.8, 29.6, 29.0, 26.9, 22.6, 21.9, 14.1. HRMS-QTOF(+) calcd for $C_8H_{22}ON_2$: 187.1805, found 187.1801. See Fig. S43-S45 and S48 for spectra.

2. Main text supporting Figures and Tables

Table S1. Calculated pKa and protonation degree values for the $N_{\alpha/\beta}$ amino groups of the different amino acid derivatives studied.

$^{+}$**AA**$^{-}$	pKa **AAOMe**[a]	pKa $^{+}$**AA**$^{-}$ [a]	Protonation degree (%) **AAOMe**[a,b]	Protonation degree (%) $^{+}$**AA**$^{-}$ [a,b]
$^{+}$**Arg**$^{-}$	7.0	9.1	29	98
$^{+}$**Lys**$^{-}$	7.4	9.4	50	> 99
$^{+}$**Val**$^{-}$	7.5	9.6	54	> 99
$^{+}$**Phe**$^{-}$	6.8	9.5	21	> 99
$^{+}$**Ala**$^{-}$	7.3	9.7	47	> 99
$^{+}$**β-Ala**$^{-}$	9.3	10.3	98	> 99

AAOMe: amino acid methyl ester; $^{+}$**AA**$^{-}$: amino acid
[a]Values calculated using MarvinSketch
[b]Values determined at pD = 7.0 (pH = 7.4).

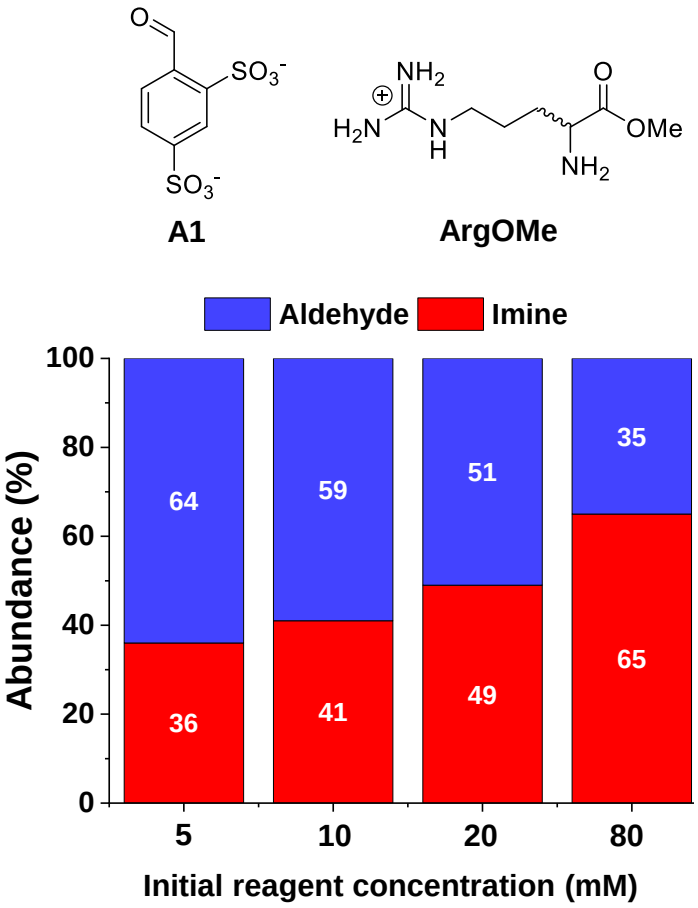

Fig. S1. Component abundances obtained after 1 h equilibration. The hydrate detected was included in the aldehyde composition (%). All compositions were calculated using ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, 293 K). PBS concentration was of 50 mM for reactions at 5 mM of reagents and of 500 mM at 20 and 80 mM reagent concentrations.

Table S2. Calculated protonation degree values for the N_α amino group of arginine derivatives at different pD values.

pD	pH	Protonation degree (%) **ArgOMe**[a]	Protonation degree (%) $^{+}$**Arg**$^{-}$ [a]
6.2	6.6	72	> 99
7.0	7.4	29	98
7.8	8.2	6	90

[a]Values calculated using MarvinSketch

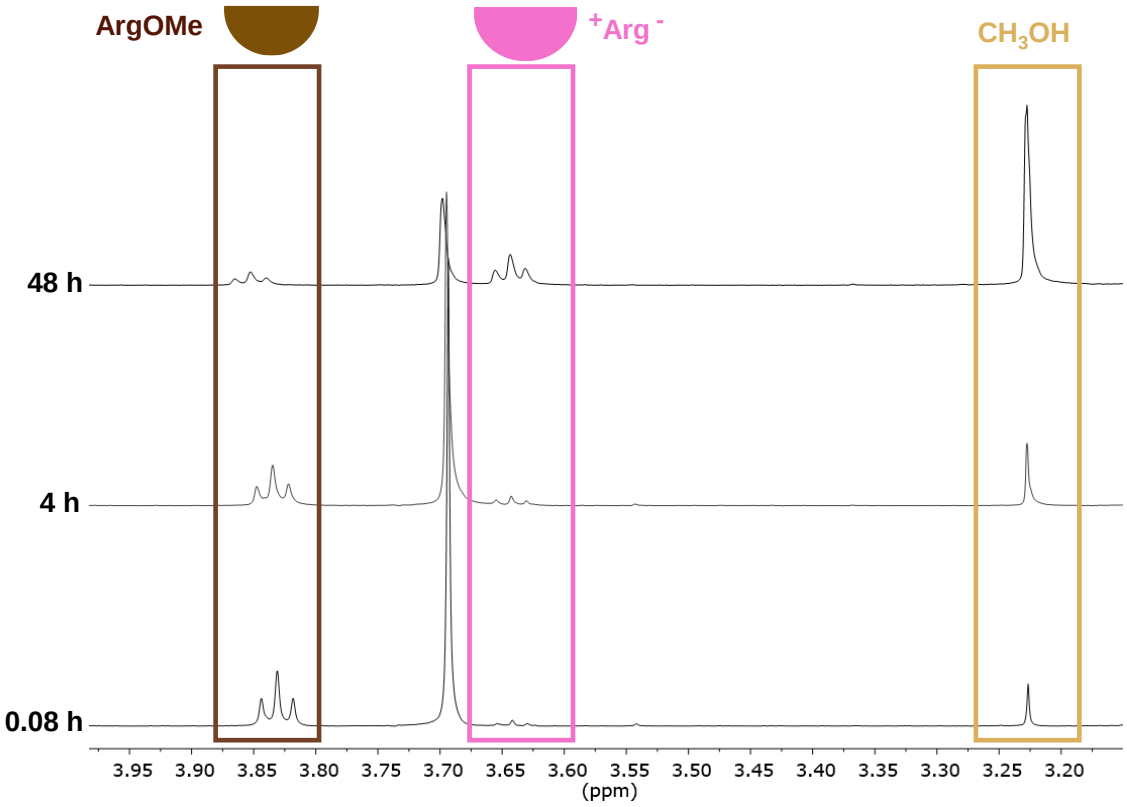

Fig. S2. Partial ^{1}H NMR spectra (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) monitoring the time evolution of the **ArgOMe** (dark brown half-ball) hydrolysis to yield $^{+}$**Arg**$^{-}$ (light brown half-ball) and CH_3OH (highlighted in green). Initial concentration **ArgOMe**: 5 mM.

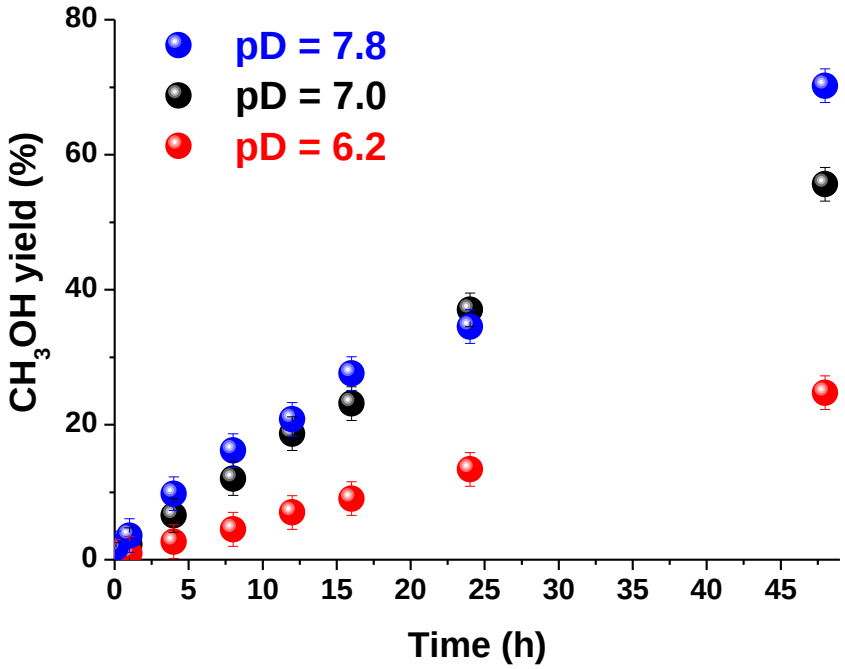

Fig. S3. Kinetic profiles for the hydrolysis of **ArgOMe** (initial concentration: 5 mM) at different pD values. Yields determined by ^{1}H NMR integration (500 MHz, D_2O, PBS buffer 50 mM, 293 K) taking into account the concentration of CH_3OH produced over time. Error bars correspond to 5% of the measurement.

Table S3. Linear fitting parameters (linear range, 0-24 h) for the pD effect in the ArgOMe hydrolysis rates (Fig. S3).

pD	pH	Slope	Normalized slope	R^2
6.2	6.6	0.57	1.0	0.9981
7.0	7.4	1.52	2.7	0.9988
7.8	8.2	1.60	2.8	0.9786

[a]**ArgOMe** initial concentration: 5 Mm.
[b]Values calculated using a linear fitting with a fixed Y-intercept value of 0.

Table S4. A1ArgOMe and $A1^+Arg^-$ yields dependence on pD values.

pD	pH	**A1ArgOMe** (%)[a,b]	**$A1A^+Arg^-$** (%)[a,c]
6.2	6.6	15	< 1
7.0	7.4	36	< 1
7.8	8.2	44	3

[a] Yields determined by 1H NMR spectroscopy (500 MHz, D_2O, PBS buffer 50mM, 293 K) after 1 h of equilibration at room temperature.
[b] **ArgOMe** and **A1** initial concentration: 5mM.
[c] $^+$**AA**$^-$ and **A1** initial concentration: 5mM

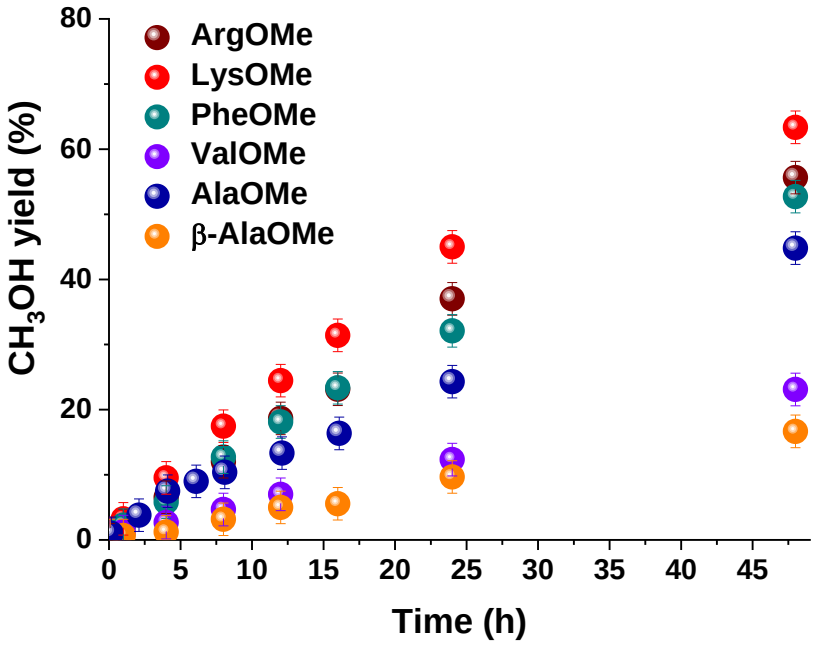

Fig. S4. Kinetic profiles for the hydrolysis of **AAOMe** (5 mM initial concentration). Yields determined by ^{1}H NMR integration (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) taking into account the concentration of CH_3OH produced over time. The (*L*) enantiomers of **AAOMe** were used in all cases. Error bars correspond to 5% of the measurement.

Table S5. Linear fitting parameters (linear range, 0-24 h) for the side chain effect in the AAOMe hydrolysis rates (Fig. S4).

AAOMe[a]	Slope[b]	Normalized slope	R^2
Arg	1.52	3.9	0.9988
Lys	1.95	5.0	0.9962
Val	0.54	1.4	0.9897
Phe	1.41	3.6	0.9955
Ala	1.02	2.6	0.9526
β-Ala	0.39	1.0	0.9910

[a]**AAOMe** initial concentration: 5 Mm.

[b]Values calculated using a linear fitting with a fixed Y-intercept value of 0.

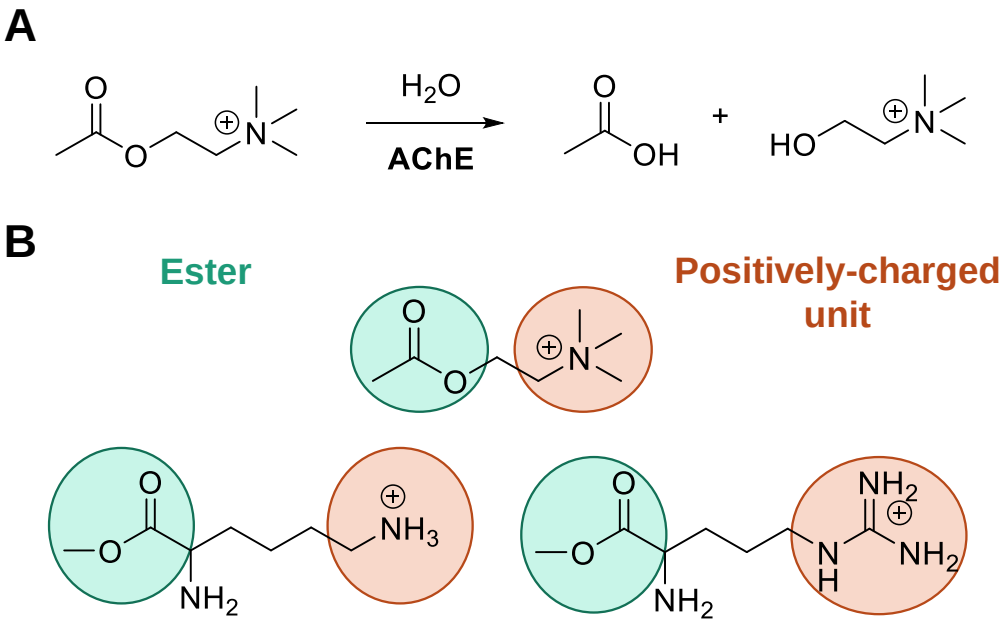

Fig. S5. (A) Hydrolysis of acetylcholine to choline and acetic acid catalysed by acetylcholine esterase (**AChE**). (B) Chemical structures of acetylcholine (above), **LysOMe** (left, below) and **ArgOMe** (right, below) presenting similar scaffolds: an ester unit (highlighted in light blue) and a positively-charged group (highlighted in orange).

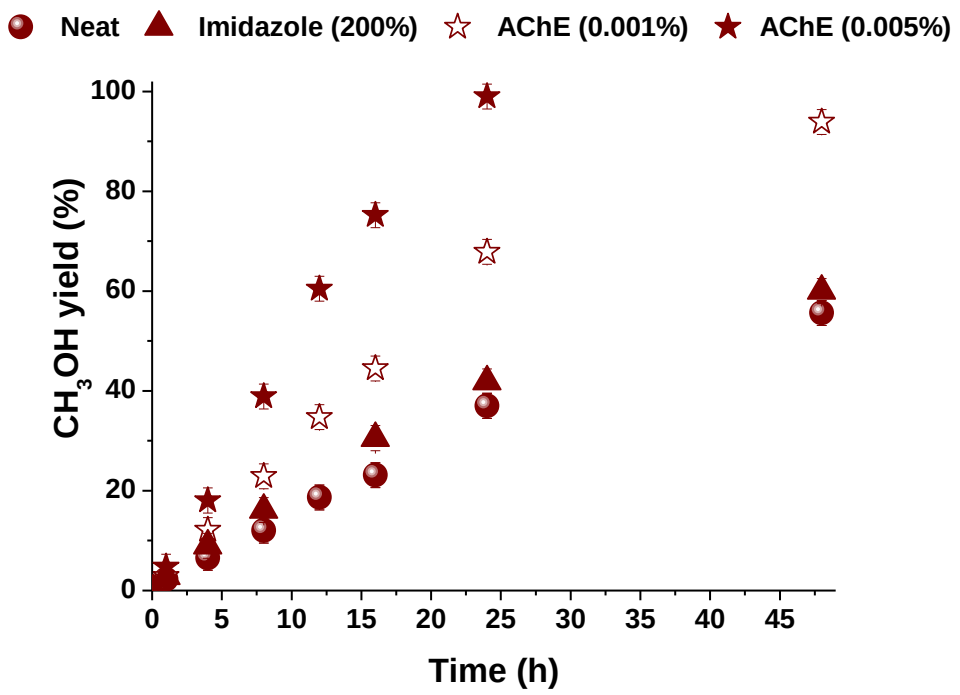

Fig. S6. Kinetic profiles for the hydrolysis of **ArgOMe** (5 mM initial concentration) in the absence and presence of different amounts of imidazole (triangles) and **AChE** (stars). The percentages in brackets correspond to the mol% of imidazole and **AChE** added. Yields determined by ^{1}H NMR integration (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) taking into account the concentration of CH_3OH produced over time. The (*L*) enantiomer of **ArgOMe** was used in all cases. Error bars correspond to 5% of the measurement.

Table S6. Linear fitting parameters (linear range, 0-24 h) for the catalyst effect in the ArgOMe hydrolysis rates (Fig. S6).

Catalyst[a]	mol%	Slope[b]	Normalized slope	R^2
-	-	1.52	1.0	0.9988
imidazole	200	1.82	1.2	0.9997
AChE	0.001	2.83	1.9	0.9949
AChE	0.005	4.82	3.2	0.9798

[a]**ArgOMe** initial concentration: 5 Mm.
[b]Values calculated using a linear fitting with a fixed Y-intercept value of 0.

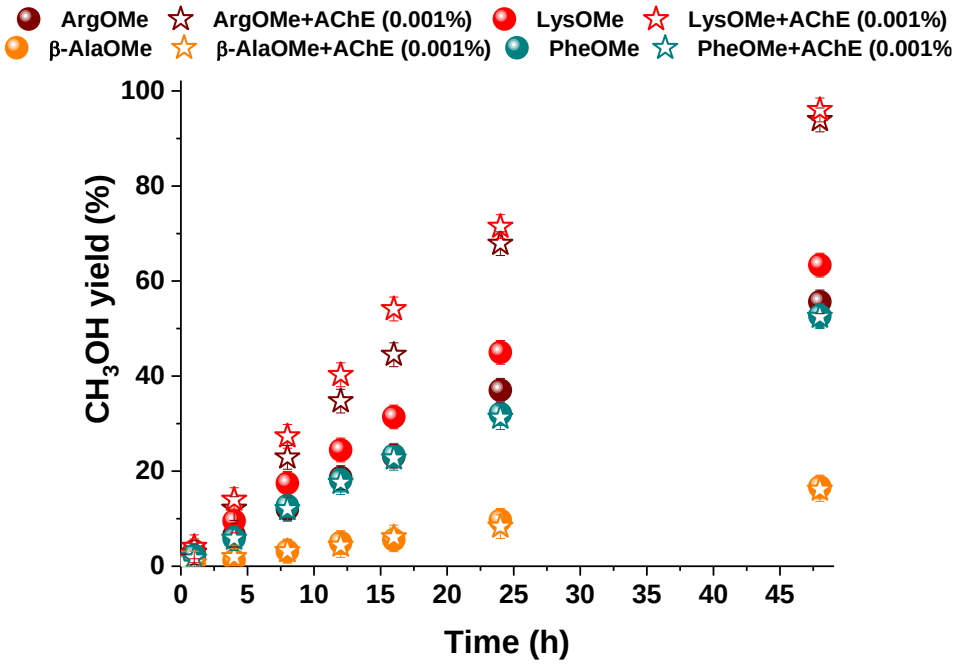

Fig. S7. Kinetic profiles for the hydrolysis of **AAOMe** (5 mM initial concentration) in the absence (spheres) and presence of **AChE** (stars). The percentages in brackets correspond to the mol% of imidazole and **AChE** added. Yields determined by ^{1}H NMR integration (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) taking into account the concentration of CH_3OH produced over time. The (*L*) enantiomers of **AAOMe** were used in all cases. Error bars correspond to 5% of the measurement.

Table S7. Linear fitting parameters (linear range, 0-24 h) for the AChE selectivity depending on the sidechain of the AAOMe (Fig. S7).

AAOMe [a]	Catalyst[b]	Slope[c]	Normalized slope	R^2
ArgOMe	-	1.52	1.0	0.9988
	AChE	2.83	1.9	0.9949
LysOMe	-	1.95	1.0	0.9962
	AChE	3.16	1.6	0.9902
PheOMe	-	1.41	1.0	0.9955
	AChE	1.37	1.0	0.9991
β-AlaOMe	-	0.39	1.0	0.9910
	AChE	0.36	1.0	0.9989

[a]**AAOMe** initial concentration: 5 Mm.
[b]**AChE** catalytic loading: 0.001 mol%.
[c]Values calculated using a linear fitting with a fixed Y-intercept value of 0.

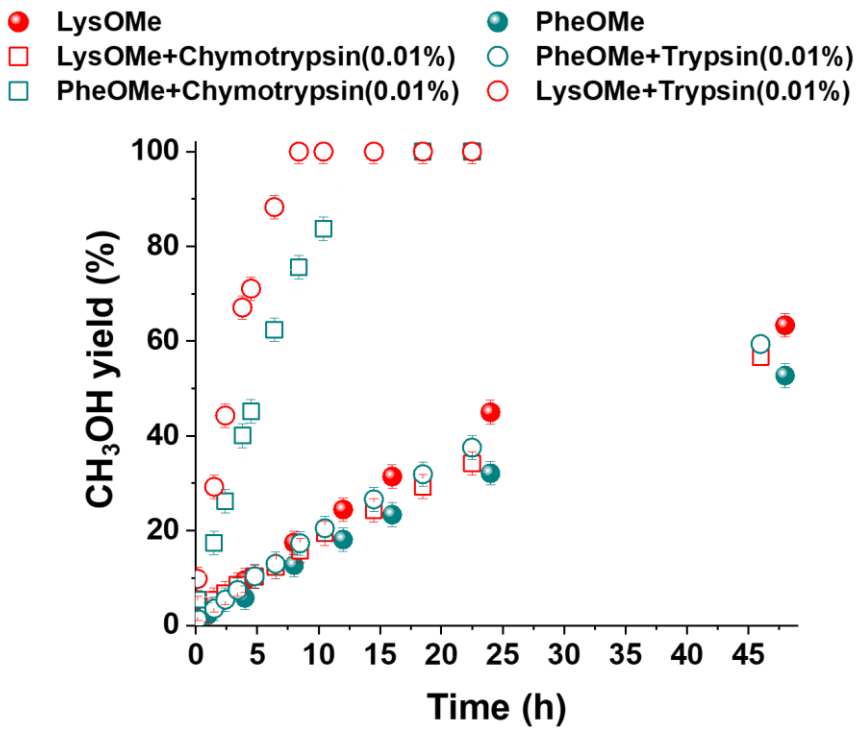

Fig. S8. Kinetic profiles for the hydrolysis of **AAOMe** (5 mM initial concentration) in the absence (filled circles) and presence of **trypsin** (empty circles) and **chymotrypsin** (empty squares). Yields determined by ^{1}H NMR integration (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) taking into account the concentration of CH_3OH produced over time. The (*L*) enantiomers of **AAOMe** were used in all cases. Error bars correspond to 5% of the measurement.

Table S8. Linear fitting parameters (linear range, 0-24 h) for the trypsin and chymotrypsin catalysed hydrolysis of LysOMe and PheLysOMe (Fig. S8).

AAOMe [a,b]	Catalyst (mol%)	Slope[c]	Normalized slope	R^2
LysOMe	-	1.95	1.0	0.9962
	Trypsin (0.01)	19.09	9.28	0.9925
	Chymotrypsin (0.01)	2.02	1.1	0.9609
PheOMe	-	1.41	1.0	0.9955
	Trypsin (0.01)	1.57	1.1	0.9949
	Chymotrypsin (0.01)	10.09	7.2	0.9963

[a]**AAOMe** initial concentration: 5 Mm.
[b]The (*L*) enantiomers of **AAOMe** were used in all cases
[c]Values calculated using a linear fitting with a fixed Y-intercept value of 0.

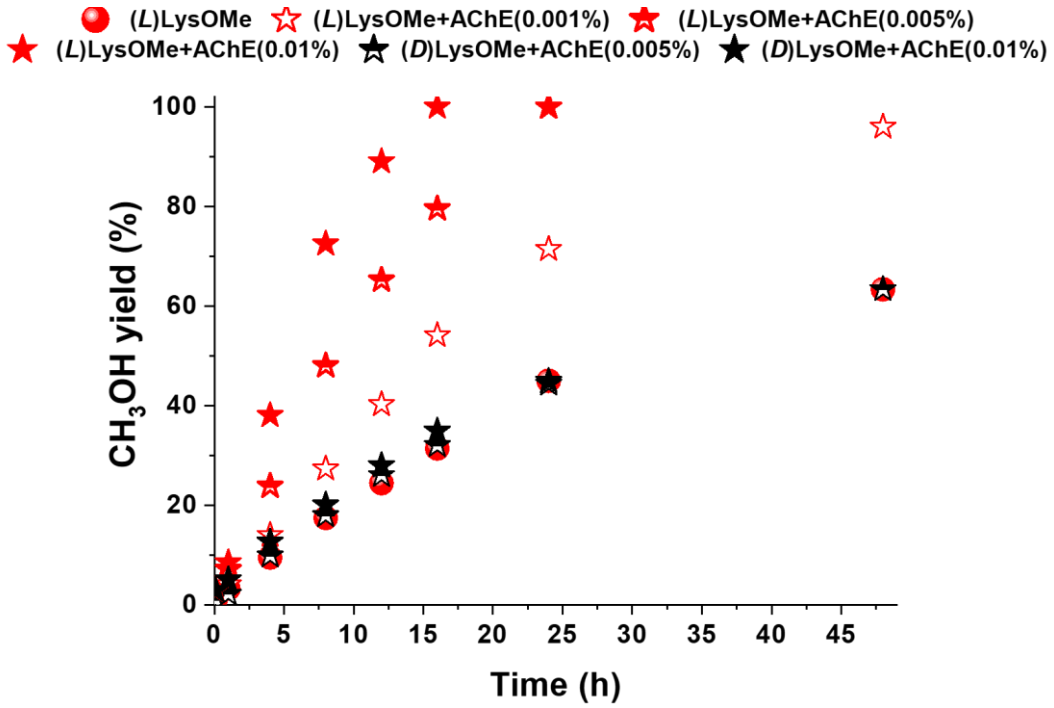

Fig. S9. Kinetic profiles for the hydrolysis of **(*D*)LysOMe** and **(*L*)LysOMe** (5 mM initial concentration) in the absence and presence of different amounts of **AChE**. The percentages in brackets correspond to the mol% of **AChE** added. Yields determined by ^{1}H NMR integration (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) taking into account the concentration of CH_3OH produced over time. Error bars correspond to 5% of the measurement.

Table S9. Linear fitting parameters (linear range, 0-24 h) for the AChE catalysed hydrolysis of (*D*)LysOMe and (*L*)LysOMe (Fig. S9).

AAOMe [a]	Catalyst (mol%)	Slope[b]	Normalized slope	R^2
***(L)*LysOMe**	-	1.95	1.0	0.9962
	AChE (0.001)	3.16	1.6	0.9902
	AChE (0.005)	5.29	2.7	0.9825
	AChE (0.01)	7.09	3.5	0.9645
***(D)*LysOMe**	**AChE** (0.005)	2.03	1.0	0.9920
	AChE (0.01)	2.31	1.2	0.9954

[a]**AAOMe** initial concentration: 5 Mm.

[b]Values calculated using a linear fitting with a fixed Y-intercept value of 0.

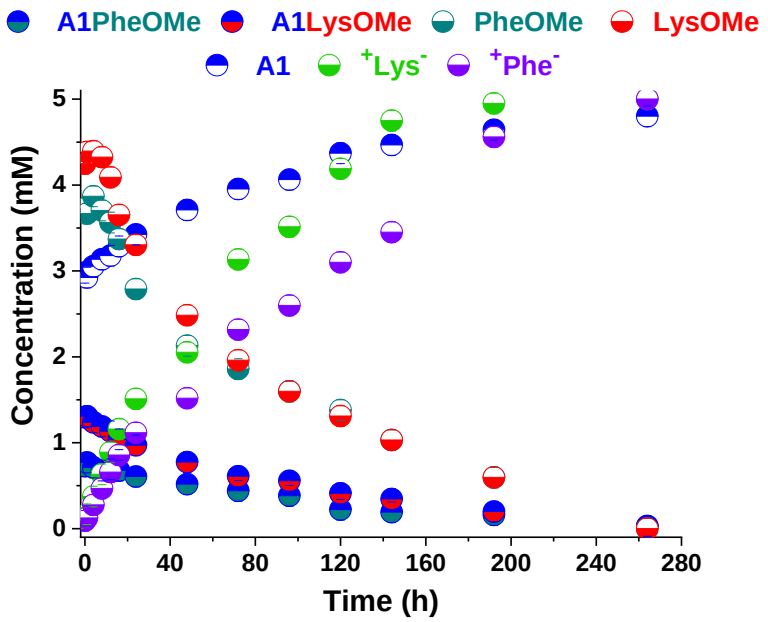

Fig. S10. Concentration profile for the different species detected in solution over 264 h for the reaction between **A1, LysOMe**, and **PheOMe**. Concentrations determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K). The hydrate detected was included in the aldehyde concentration (mM). Initial concentration of reagents: 5 mM. Error bars correspond to 5% of the measurement. The (*L*) enantiomers of **AAOMe** were used in all cases.

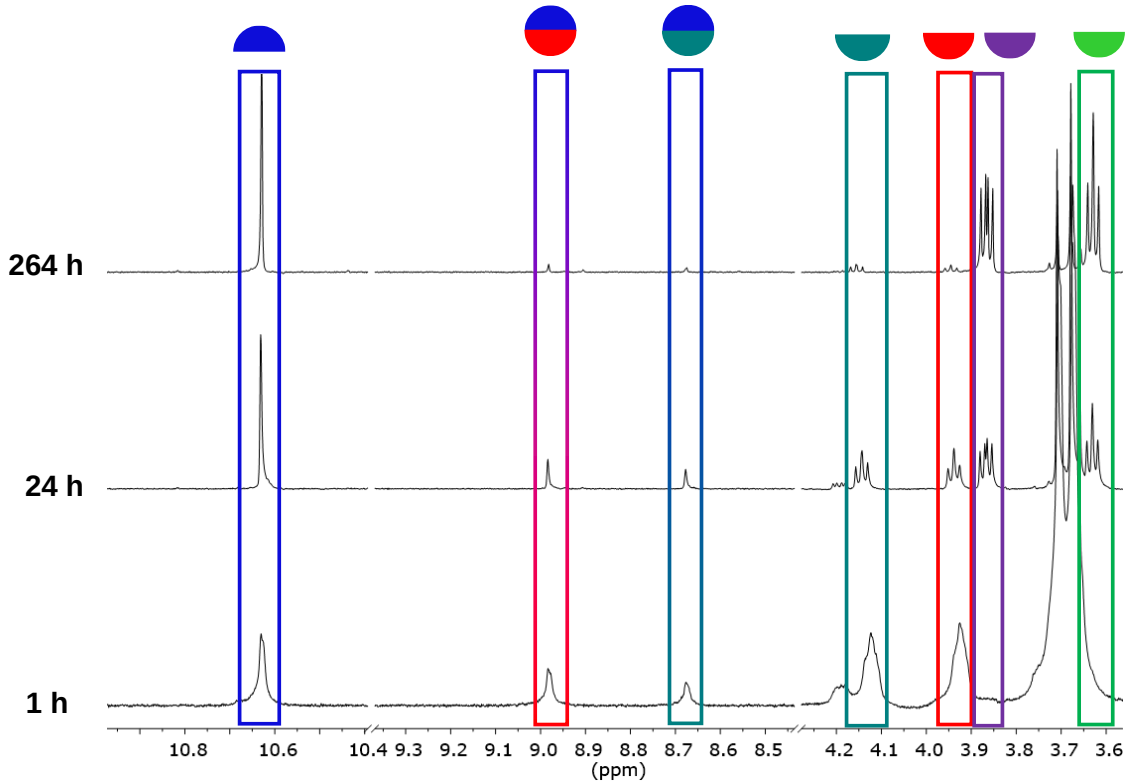

Fig. S11. Partial ^{1}H NMR spectra (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) monitoring the time evolution of the reaction between **A1, LysOMe**, and **PheOMe.** Initial concentration: 5 mM. Colours correspond to the ones of Fig. S10.

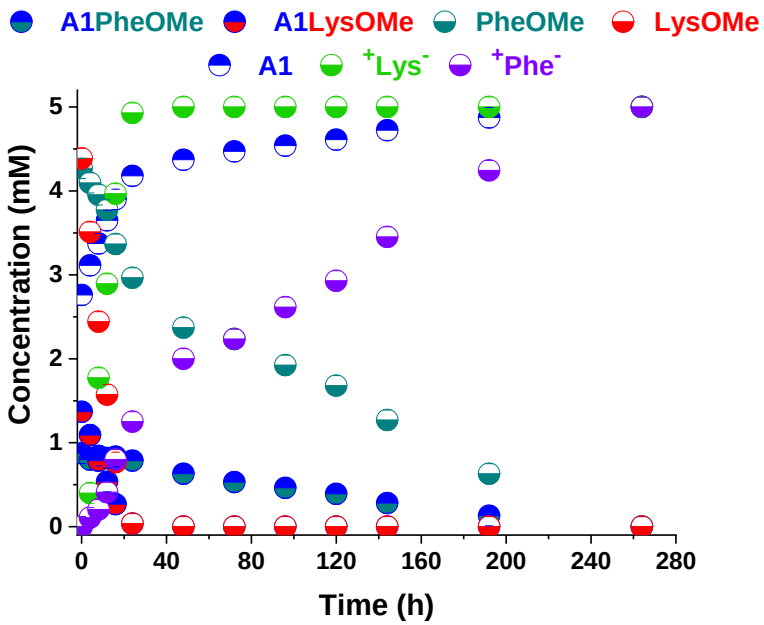

Fig. S12. Concentration profile for the different species detected in solution over 264 h for the reaction between **A1, LysOMe**, and **PheOMe** in the presence of **AChE** (0.01 mol%). Concentrations determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K). The hydrate detected was included in the aldehyde concentration (mM). Initial concentration of reagents: 5 mM. Error bars correspond to 5% of the measurement. The (*L*) enantiomers of **AAOMe** were used in all cases.

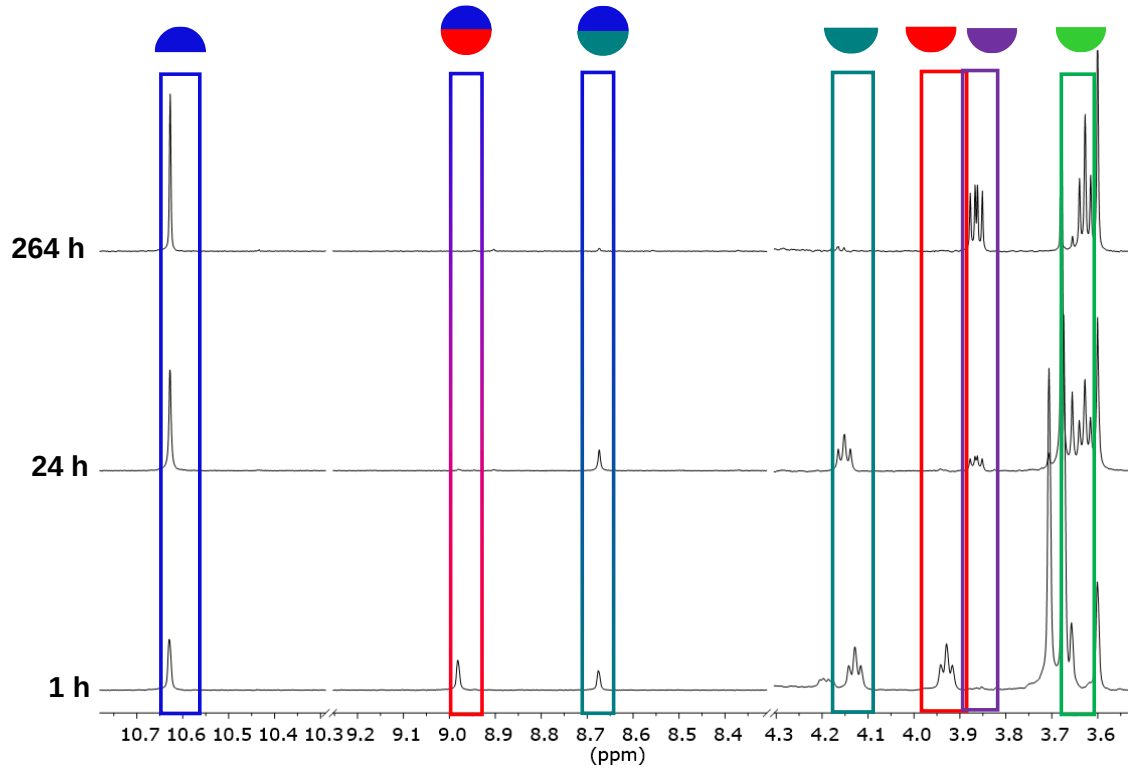

Fig. S13. Partial ^{1}H NMR spectra (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) monitoring the time evolution of the reaction between **A1, LysOMe**, and **PheOMe** in the presence of **AChE** (0.01 mol%). Initial concentration: 5 mM. Colours correspond to the ones of Fig. S12.

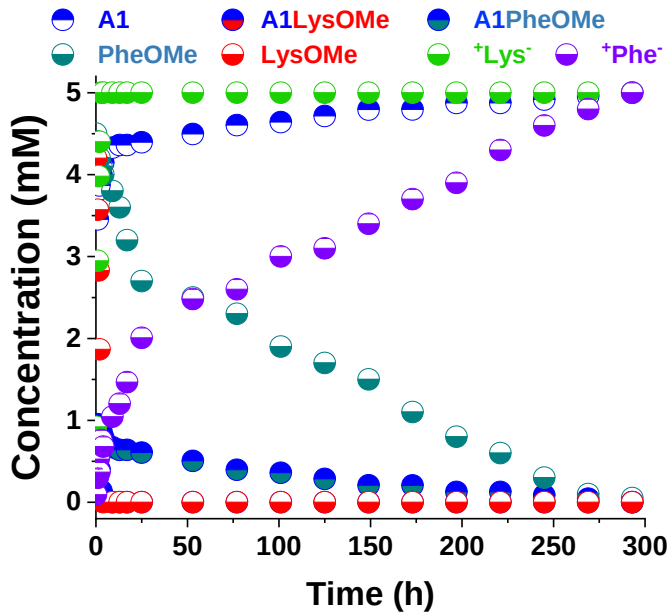

Fig. S14. Concentration profile for the different species detected in solution over 300 h for the reaction between **A1, LysOMe**, and **PheOMe** in the presence of **trypsin** (0.01 mol%). Concentrations determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K). The hydrate detected was included in the aldehyde concentration (mM). Initial concentration of reagents: 5 mM. Error bars correspond to 5% of the measurement. The (*L*) enantiomers of **AAOMe** were used in all cases.

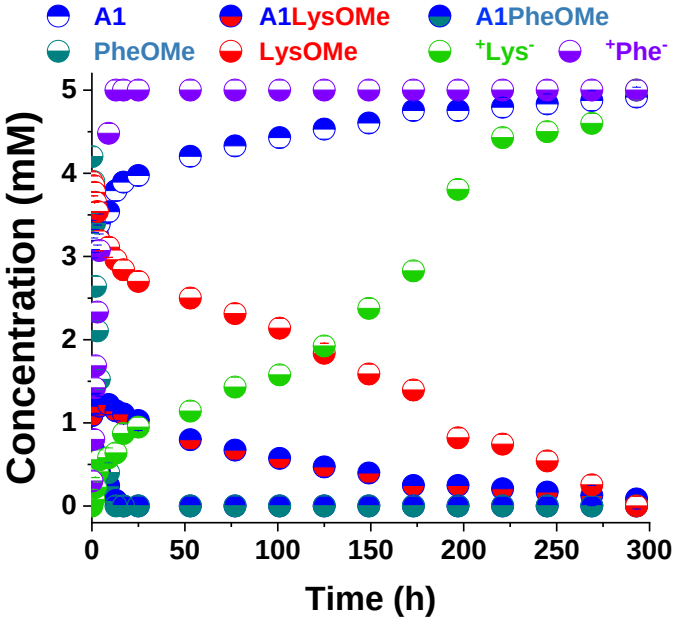

Fig. S15. Concentration profile for the different species detected in solution over 300 h for the reaction between **A1, LysOMe**, and **PheOMe** in the presence of **chymotrypsin** (0.01 mol%). Concentrations determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K). The hydrate detected was included in the aldehyde concentration (mM). Initial concentration of reagents: 5 mM. Error bars correspond to 5% of the measurement. The (*L*) enantiomers of **AAOMe** were used in all cases.

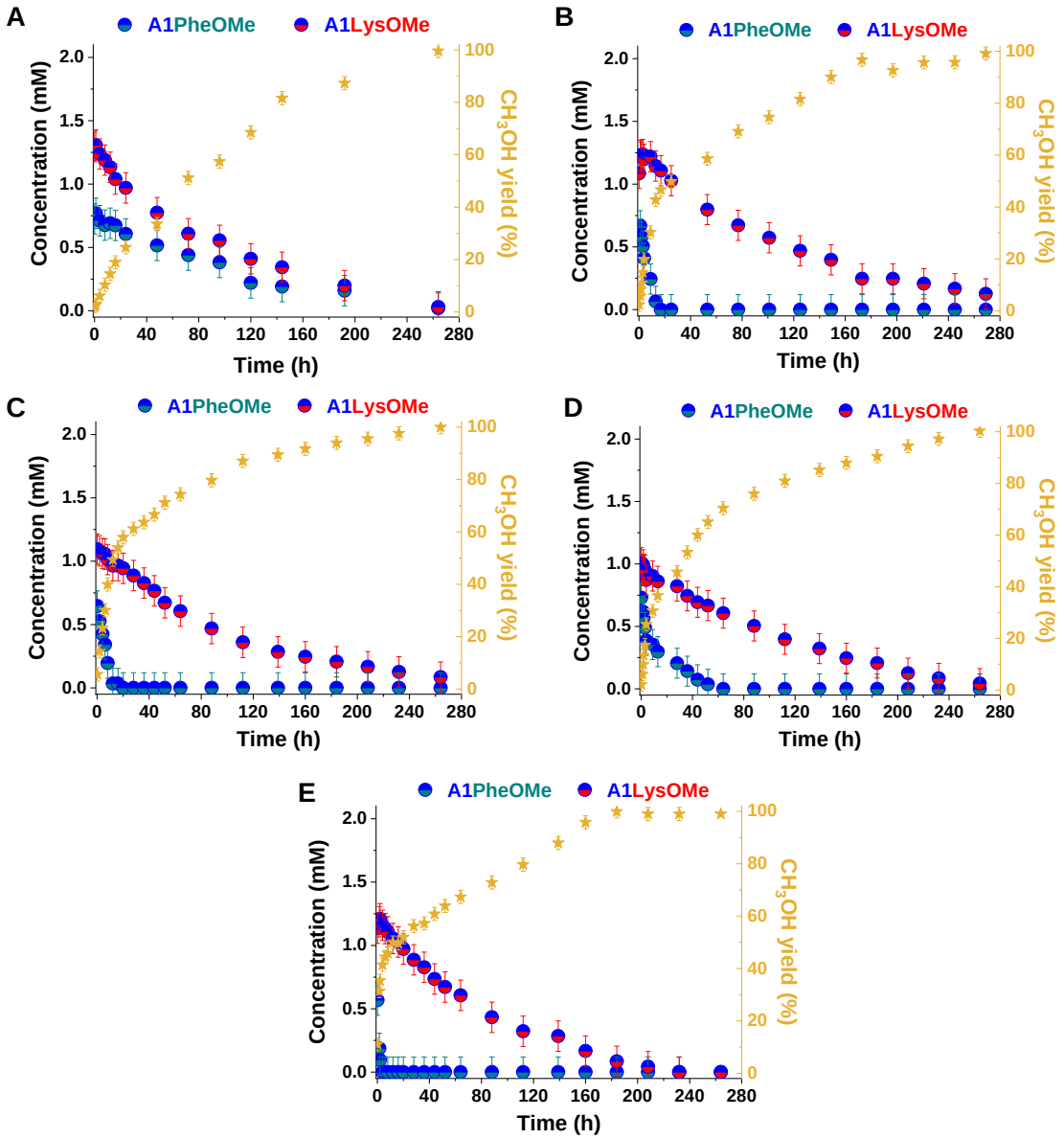

Fig. S16. Concentration profile for the different constituents detected in solution over 264 h of the **A1**, **LysOMe,** and **PheOMe** reaction in the absence (A) and presence of **chymotrypsin** (B) 0.01%, (C) 0.006%, (D) 0.003% and (E) 0.03%. Concentration and yields determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K). Initial concentration of reagents: 5 mM.

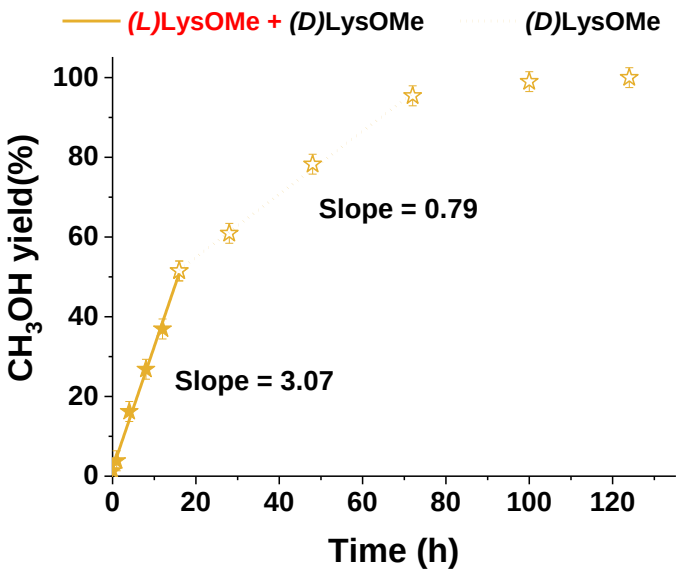

Fig. S17. CH_3OH yields calculated using ^1H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) for the reaction between **A1, (*D*)LysOMe,** and **(*L*)LysOMe** in the presence of **AChE** (0.01 mol%) over 126 h. Initial concentration of reagents: 5 mM. The two different ranges have been fitted with a linear fit and correspond to: 1) combined hydrolysis of **(*D*)LysOMe** and **(*L*)LysOMe** (continuous dark yellow line, slope = 3.07); 2) Hydrolysis of the remnant **(*D*)LysOMe** (discontinuous dark yellow line, slope = 0.79). Error bars correspond to 5% of the measurement.

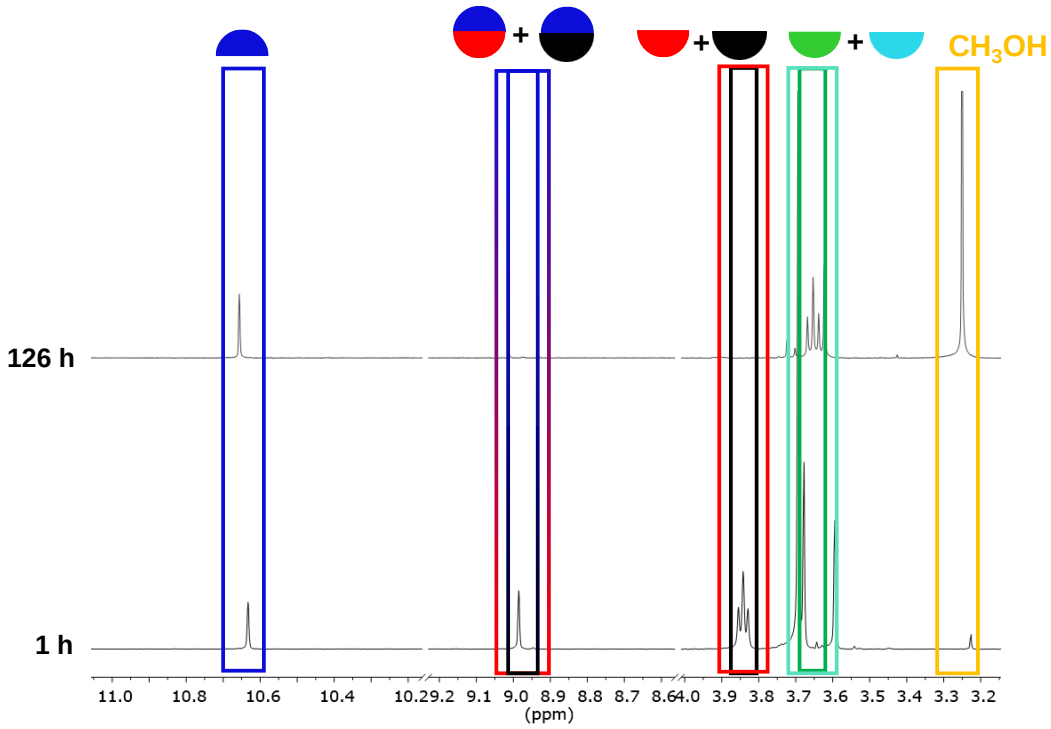

Fig. S18. Partial ^1H NMR spectra (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) monitoring the time evolution of the reaction between **A1, (*L*)LysOMe**, and **(D)LysOMe** in the presence of **AChE** (0.01 mol%)**.** Initial concentration: 5 mM. Colours correspond to the ones of Fig. 5B.

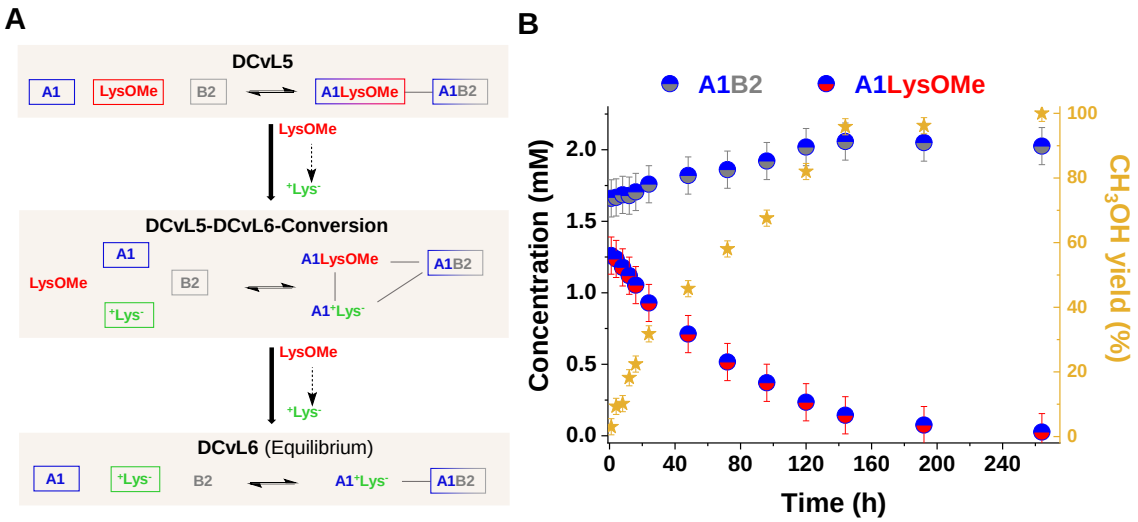

Fig. S19. (A) Representation of the reaction between **A1, LysOMe,** and **B2**. The conversion from **DCvL5** to **DCvL6** has also been represented for clarity. The predominant species (> 1 mM) are marked with a coloured rectangle.(B) Concentration profile for the different constituents detected in solution over 264 h of reaction. The (*L*) enantiomer of **LysOMe** was used. Right Y-axes correspond to the yield of methanol (dark yellow stars) calculated. Concentration and yields determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K). The hydrate detected was included in the aldehyde concentration (mM)Initial concentration of reagents: 5 mM. Error bars correspond to 5% of the measurement. Grey lines correspond to antagonistic relationships.

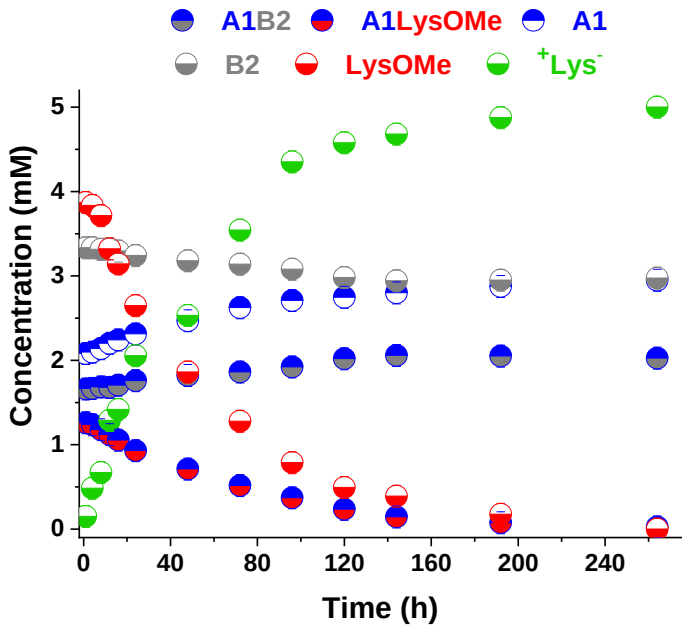

Fig. S20. Concentration profile for the different species detected in solution over 264 h for the reaction between **A1, LysOMe**, and **B2**. Concentrations determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K). The hydrate detected was included in the aldehyde concentration (mM). Initial concentration of reagents: 5 mM. Error bars correspond to 5% of the measurement. The (*L*) enantiomer of **LysOMe** was used.

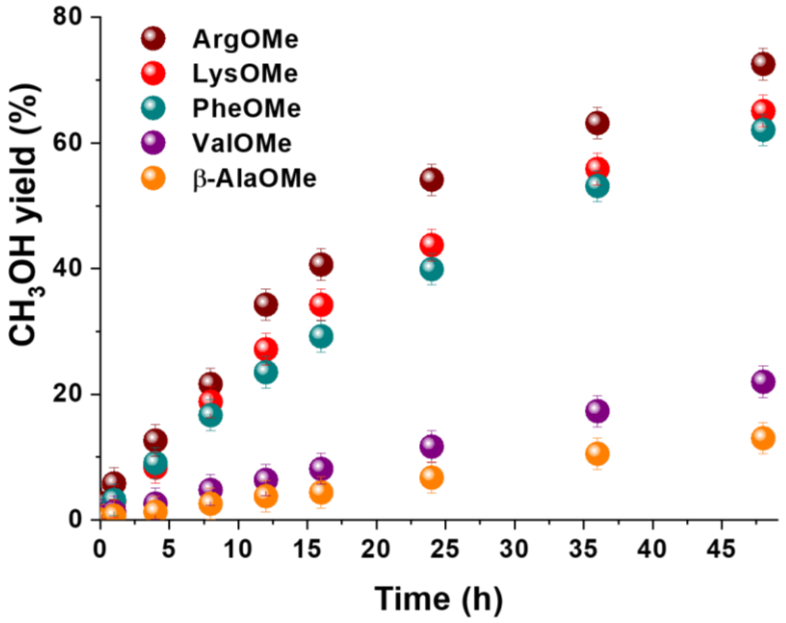

Fig. S21. Kinetic profiles for the hydrolysis of **AAOMe** (80 mM initial concentration). Yields determined by ^{1}H NMR integration (500 MHz, D_2O, pD = 7.0, PBS buffer 500 mM, 293 K) taking into account the concentration of CH_3OH produced over time. The (*L*) enantiomers of **AAOMe** were used in all cases. Error bars correspond to 5% of the measurement.

Table S10. Linear fitting parameters (linear range, 0-24 h) for the side chain effect in the AAOMe hydrolysis rates at 80 mM (Fig. S21).

AAOMe[a]	Slope[b]	Normalized slope	R^2
Arg	2.45	8.4	0.9867
Lys	1.99	6.9	0.9882
Val	0.51	1.8	0.9912
Phe	1.78	6.1	0.9921
β-Ala	0.29	1.0	0.9943

[a]**AAOMe** initial concentration: 80 Mm.
[b]Values calculated using a linear fitting with a fixed Y-intercept value of 0.

Table S11. Calculated pKa (N_α) and imine yields for the reaction between A1 and $^{+}AA^{-}$.

$^{+}AA^{-}$	pKa $^{+}AA^{-}$ [a]	**A1$^{+}AA^{-}$** yield (%) at 80 mM[b]
$^{+}Arg^{-}$	9.1	9
$^{+}Lys^{-}$	9.4	6[c]
$^{+}Phe^{-}$	9.6	4

[a] Values calculated using MarvinSketch.

[b] Values determined by ^{1}H NMR integration (500 MHz, D_2O, pD = 7.0, PBS buffer 500 mM, 293 K).

[c] A mixture of two imines was detected in the spectrum, which was assigned to the minor formation of the aldimine derivative with the Lys N_ε. The integration of both imines has been considered for the yield determination.

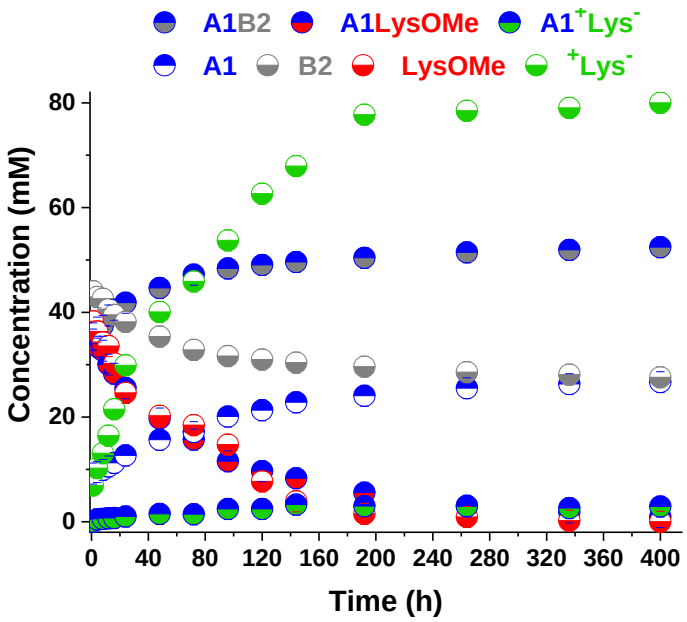

Fig. S22. Concentration profile for the different species detected in solution over 400 h of reaction between **A1, B2** and **LysOMe**. The (*L*) enantiomer of **LysOMe** was used. Concentrations determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 500 mM, 293 K). Initial concentration of reagents: 80 mM. Error bars correspond to 5% of the measurement.

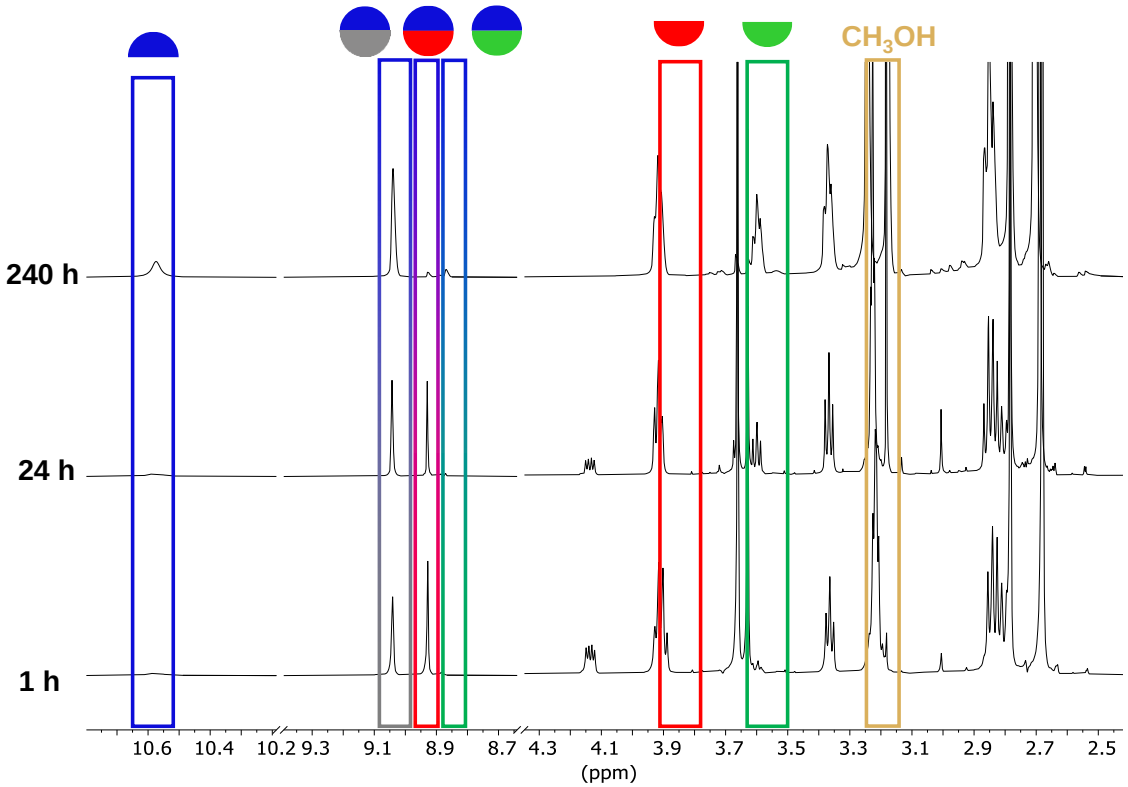

Fig. S23. Partial ^{1}H NMR spectra (500 MHz, D_2O, pD = 7.0, PBS buffer 500 mM, 293 K) monitoring the time evolution of the reaction between **A1, LysOMe**, and **B2.** Initial concentration: 80 mM. Colours correspond to the ones of Fig. S22.

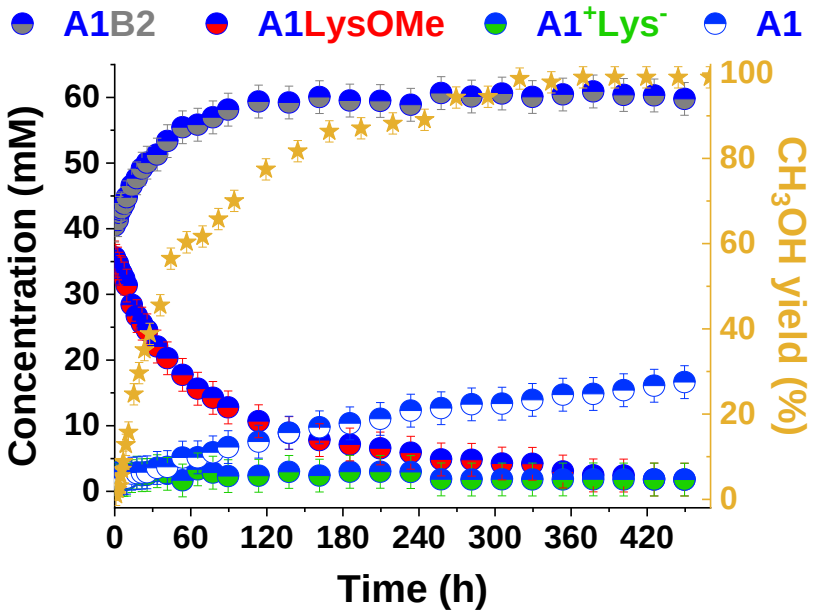

Fig. S24. Concentration profile for **A1, A1LysOMe**, **A1^{+}Lys^{-}**, and **A1B2** over 450 h for the reaction between **A1, LysOMe**, and **B2**. Concentrations determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, 293 K). The hydrate detected was included in the aldehyde concentration (mM). Initial concentration of reagents: 80 mM. Error bars correspond to 5% of the measurement. The (*L*) enantiomer of **LysOMe** was used.

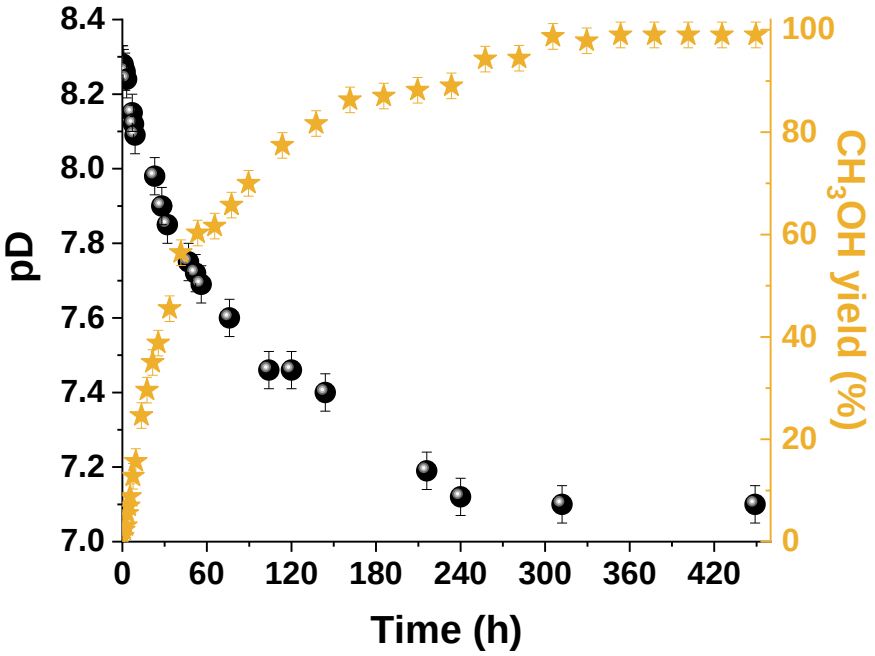

Fig. S25. pD monitoring for the reaction between **A1, LysOMe**, and **B2** over 450 h (D_2O, 293 K). Yields of CH_3OH (%) have also been added. Initial concentration of reagents: 80 mM. The (*L*) enantiomer of **LysOMe** was used.

Table S12. Calculated pKa (N_α) and imine yields for the reaction between different pseudopeptides **AlaNHCX** and **A1**.

X	n	pKa **AlaNHCX**[a]	**A1AlaNHCX yield** (%) at 80 mM[b]
5	3	8.4	48
6	4	8.4	46
7	5	8.4	52

[a] Values calculated using MarvinSketch.

[b] Values determined by ^{1}H NMR integration (500 MHz, D_2O, pD = 7.0, PBS buffer 500 mM, 293 K).

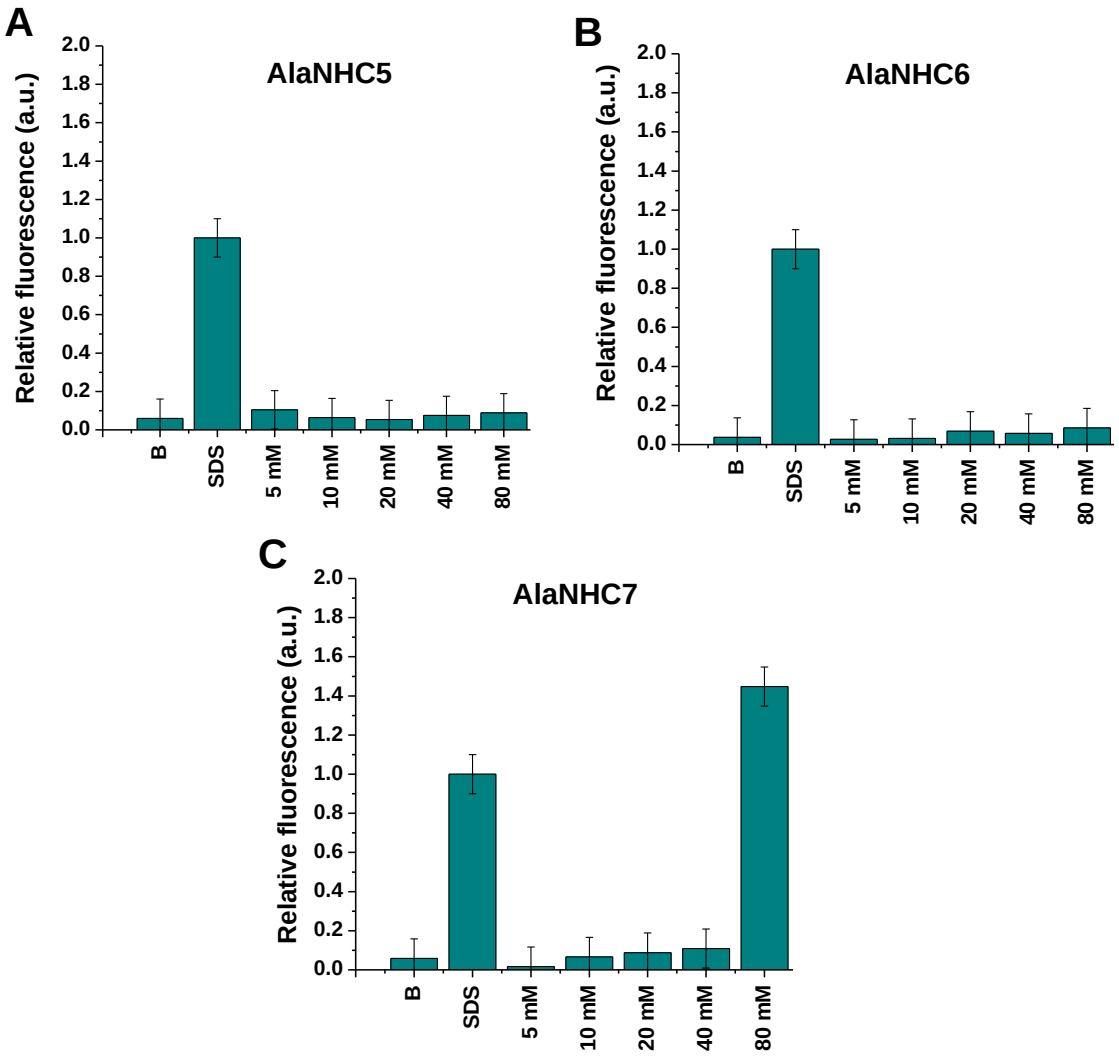

Fig. S26. Relative emission fluorescence intensities measured for the parent amines **AlaNHCX** at different concentrations. The values of the buffer solution (B) and SDS blank (SDS) have been included in each case. Excitation wavelength: 515 nm. Emission wavelength: 585 nm. The relative fluorescence values have been obtained as follows: Relative fluorescence = $F_{intensity}$sample/$F_{intensity}$SDS. We have assigned the presence of micelles to values > 0.8. Conditions: D_2O, pD = 7.0, PBS buffer 500 mM, 293 K.

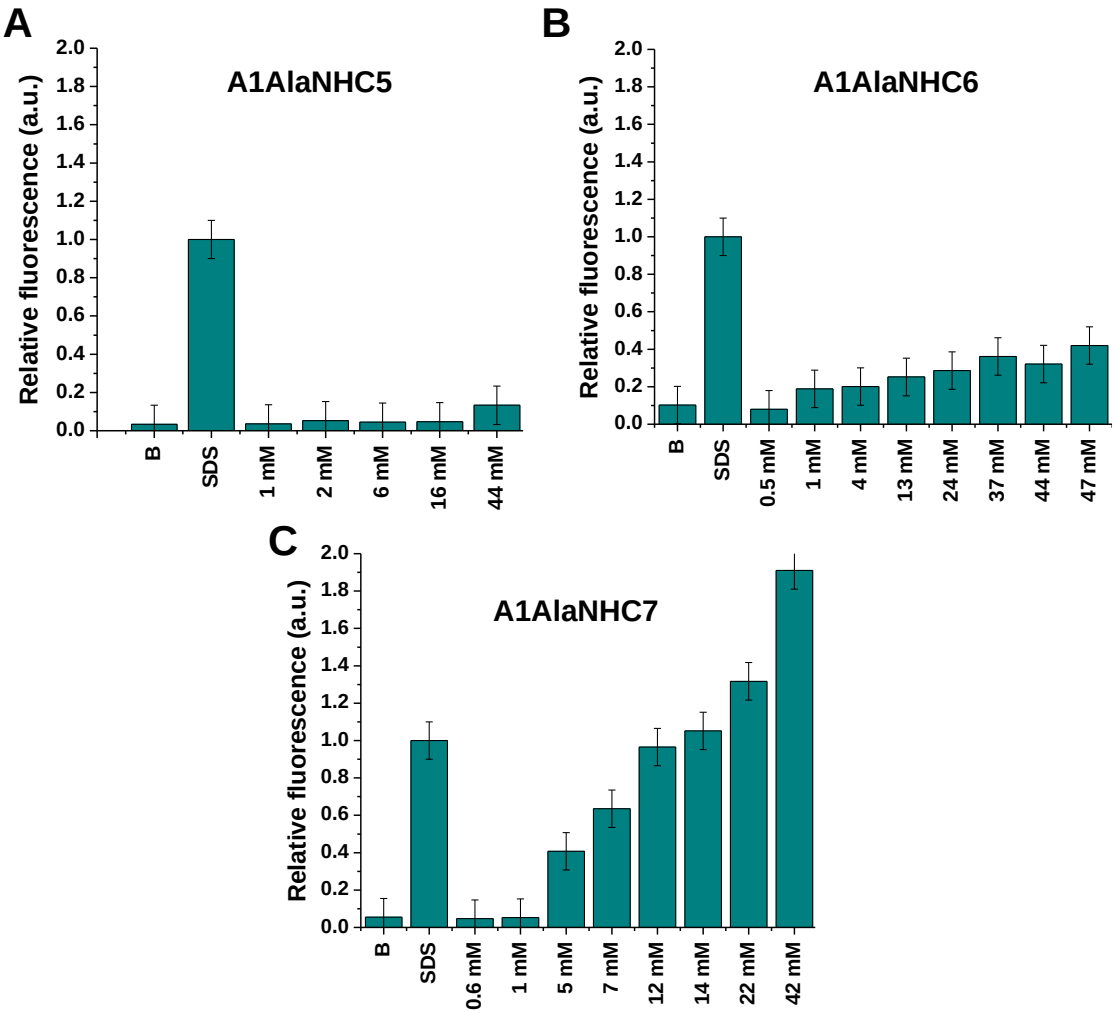

Fig. S27. Relative emission fluorescence intensities measured for the aldimines **A1AlaNHCX** at different concentrations. The values of the buffer solution (B) and SDS blank (SDS) have been included in each case. Excitation wavelength: 515 nm. Emission wavelength: 585 nm. The relative fluorescence values have been obtained as follows = $F_{intensity}$sample/$F_{intensity}$SDS. We have assigned the presence of micelles to values > 0.8. Imine concentrations determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 500 mM, 293 K). Maximum reagent concentration employed: 80 mM for **A1** and **AlaNHCX**.

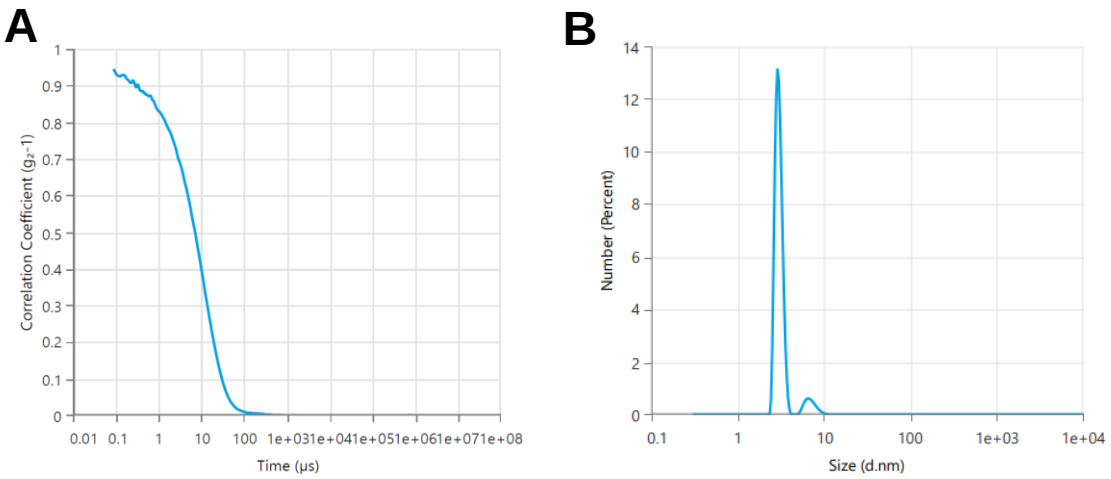

Fig. S28 DLS results showing (A) the correlogram and (B) number-size plot for the **A1AlaNHC7** sample (12 mM). Conditions: D_2O, pD = 7.0, PBS buffer 500 mM, 293 K.

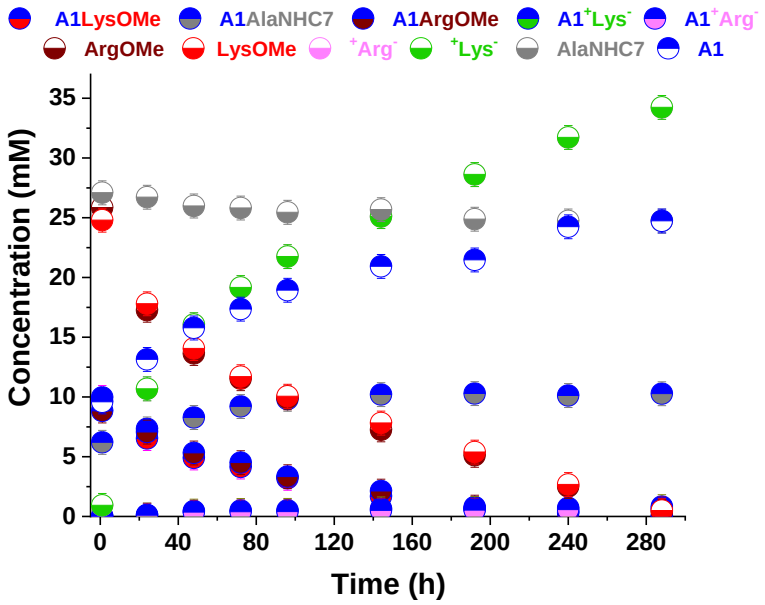

Fig. S29. Concentration profile for the different species detected in solution over 290 h of the reaction between **A1, LysOMe, ArgOMe**, and **AlaNHC7**. The (*L*) enantiomers of **AAOMe** were used in all cases. Concentrations determined by ^1H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 500 mM, 293 K). Initial concentration of reagents: 35 mM. Error bars correspond to 5% of the measurement. The data points of $^+$**Arg**$^-$ overlap with the ones of $^+$**Lys**$^-$.

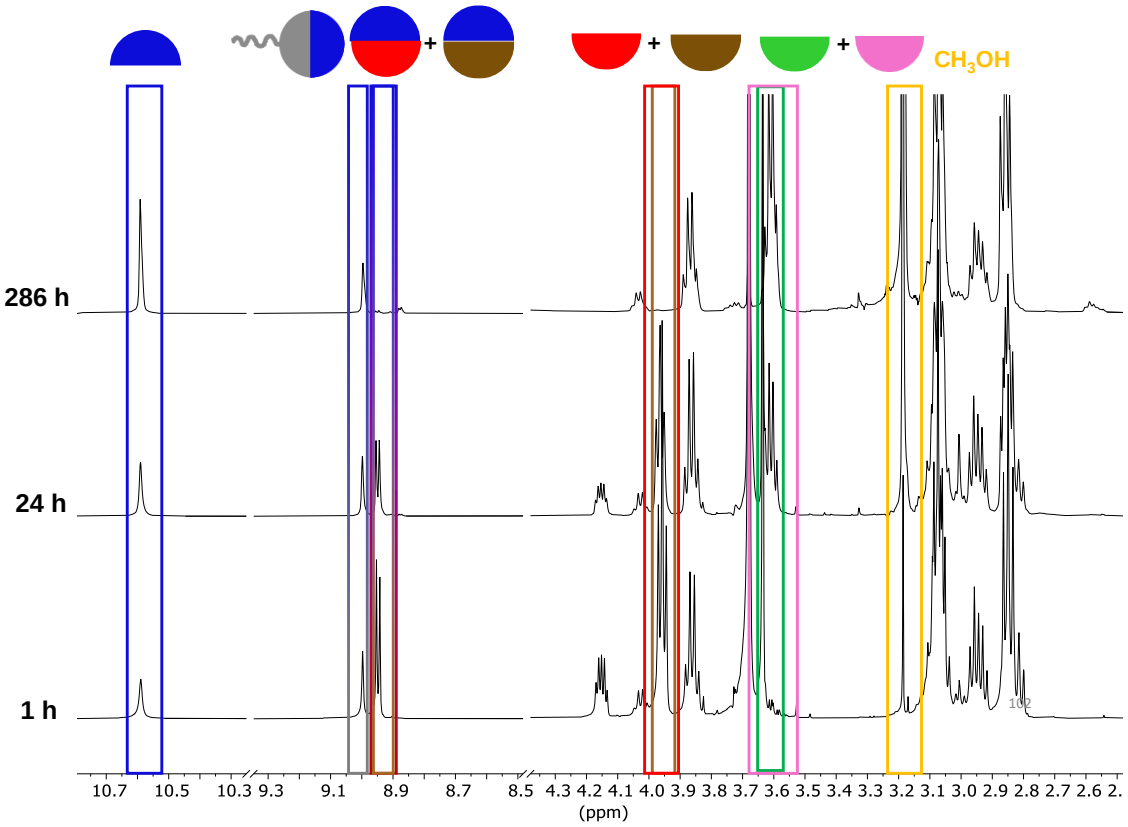

Fig. S30. Partial ^{1}H NMR spectra (500 MHz, D_2O, pD = 7.0, PBS buffer 500 mM, 293 K) monitoring the time evolution of the reaction between **A1, LysOMe**, **ArgOMe**, and **AlaNHC7.** Initial concentration: 35 mM. Colours correspond to the ones of Fig. S29.

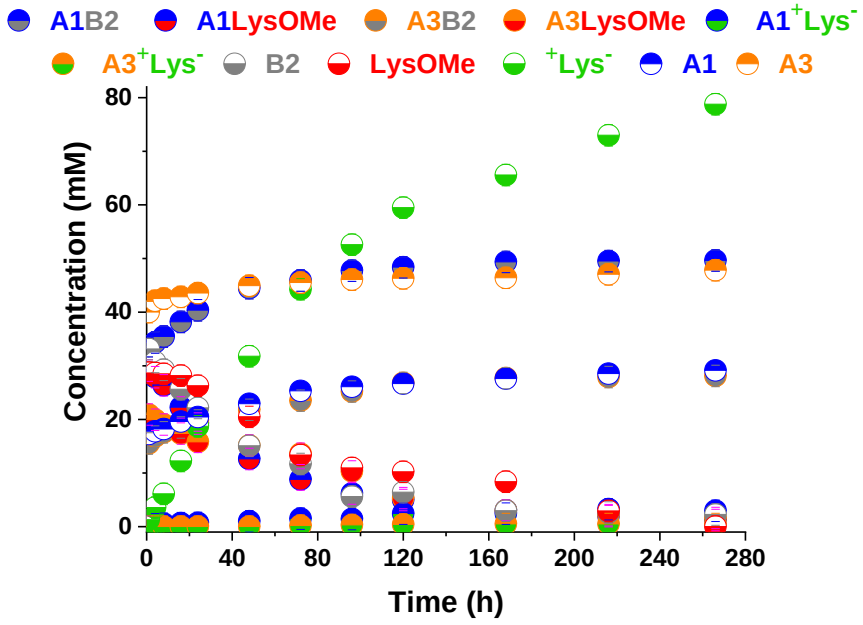

Fig. S31. Concentration profile for the different species detected in solution over 290 h of the reaction between **A1, LysOMe, A3**, and **B2**. The (*L*) enantiomer of **LysOMe** was used. Concentrations determined by ^{1}H NMR spectroscopy (500 MHz, D_2O, pD = 7.0, PBS buffer 500 mM, 293 K). Initial concentration of reagents: 80 mM. Error bars correspond to 5% of the measurement.

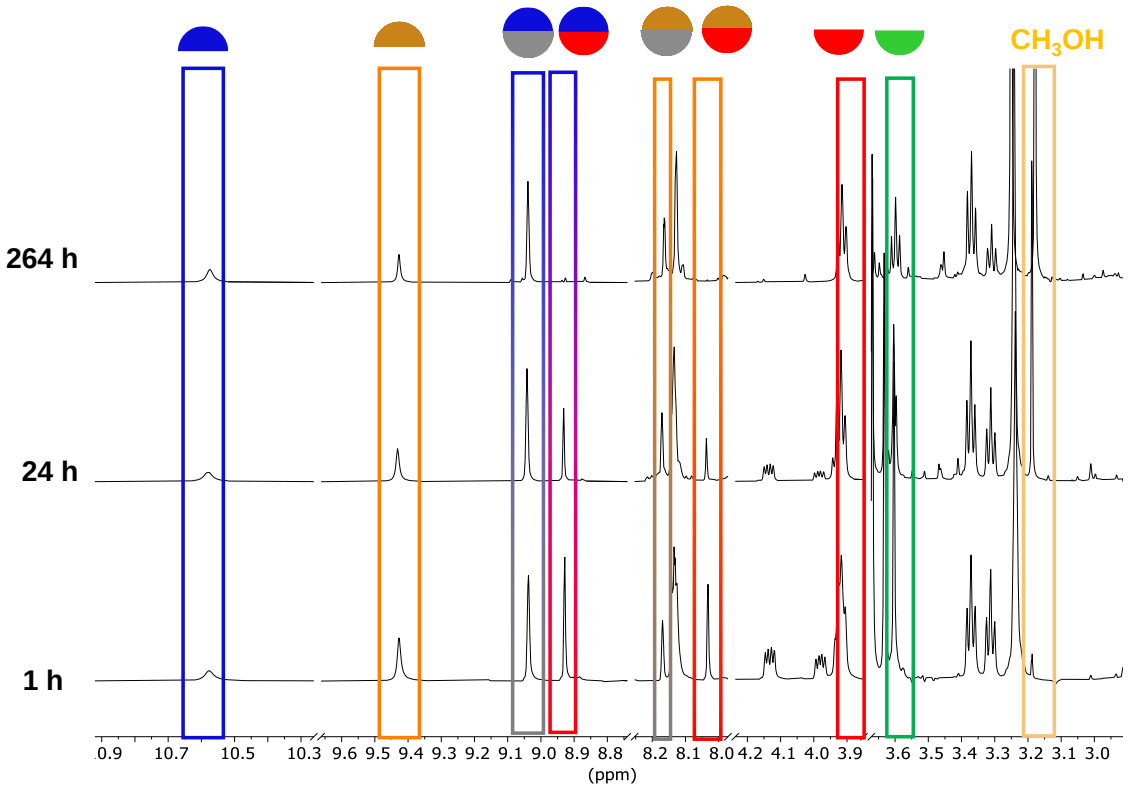

Fig. S32. Partial ^{1}H NMR spectra (500 MHz, D_2O, pD = 7.0, PBS buffer 500 mM, 293 K) monitoring the time evolution of the reaction between **A1, A3**, **LysOMe**, and **B2.** Initial concentration: 80 mM. Colours correspond to the ones of Fig. S31.

3. Characterization

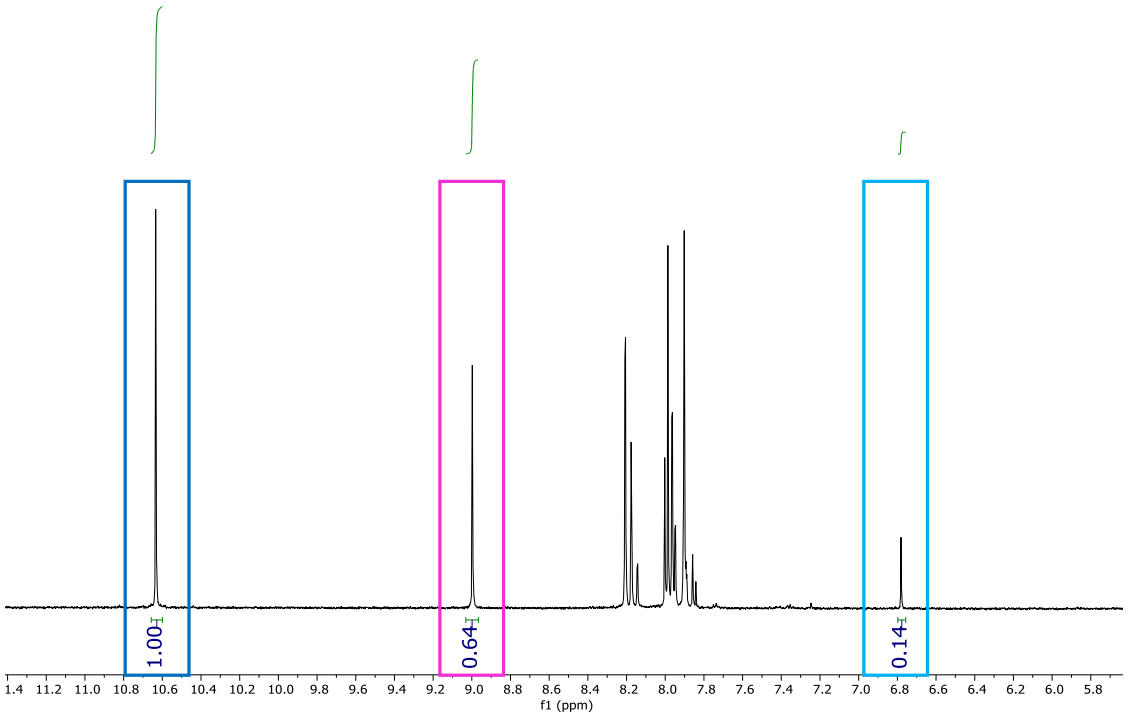

Fig. S33. Product abundance quantification using ^{1}H-NMR spectrum (500 MHz, D_2O + phosphate buffer 50mM, pD 7.0, 293 K). The crude corresponds to the condensation reaction between **ArgOMe** and **A1** (5 mM each) after 1 h of equilibration. The signal highlighted in dark blue (10.6 ppm) corresponds to **A1**, the signal in pink (9.0 ppm) to imine **A1ArgOMe,** and the signal in light blue (6.8 ppm) to the **A1**-hydrate. The abundance of **A1ArgOMe** was calculated to be 36%.

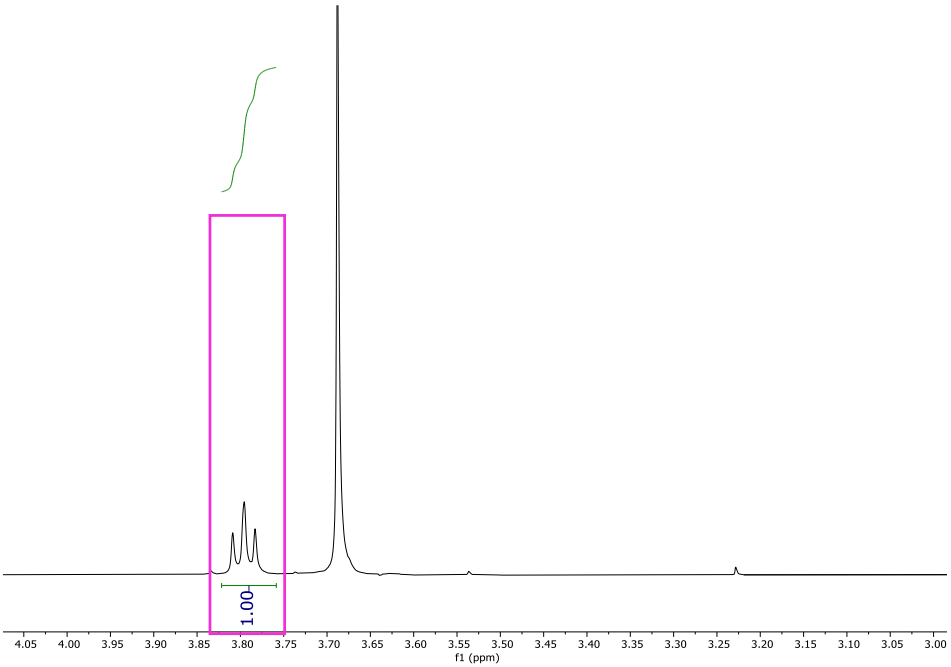

Fig. S34. Hydrolysis product abundance quantification using ^{1}H-NMR spectrum (500 MHz, D_2O + phosphate buffer 50mM, pD 7.0, 293 K). The spectrum corresponds to the hydrolysis of **LysOMe** (5 mM) after 5 min of reaction. The signal highlighted in pink (3.8 ppm) corresponds to **LysOMe.**

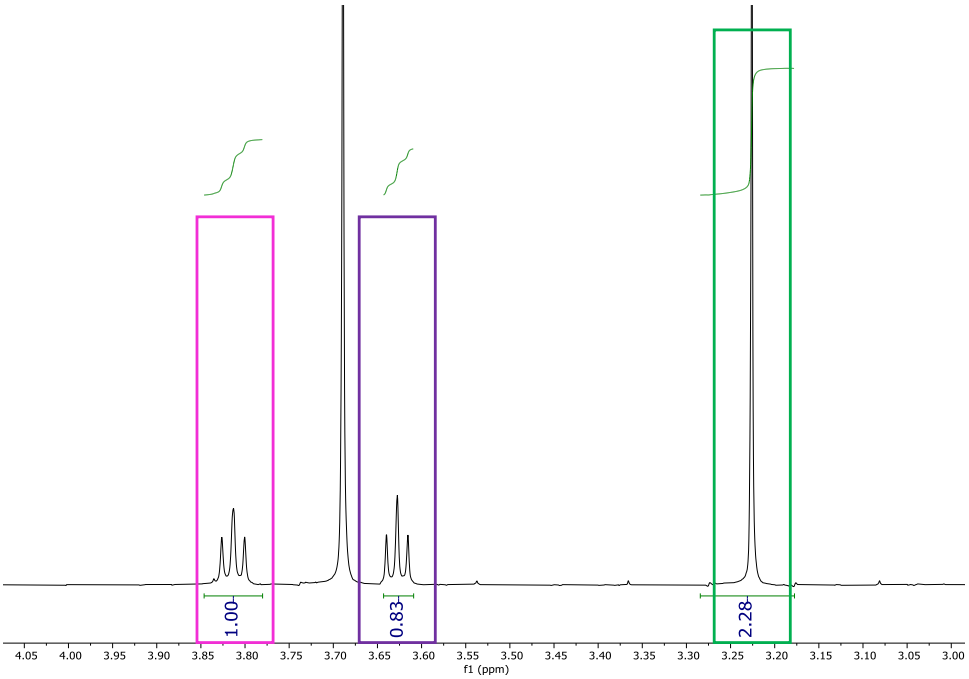

Fig. S35. Hydrolysis product abundance quantification using ^{1}H-NMR spectrum (500 MHz, D_2O + phosphate buffer 50mM, pD 7.0, 293 K). The spectrum corresponds to the hydrolysis of **LysOMe** (5 mM) after 48 h of reaction. The signal highlighted in pink (3.8 ppm) corresponds to **LysOMe**, the one in purple (3.6 ppm) to $^{+}$**Lys**$^{-}$, and the one in green (3.2 ppm) to CH3OD. The expected 1 : 3 $^{+}$**Lys**$^{-}$: CH3OD ratio is observed.

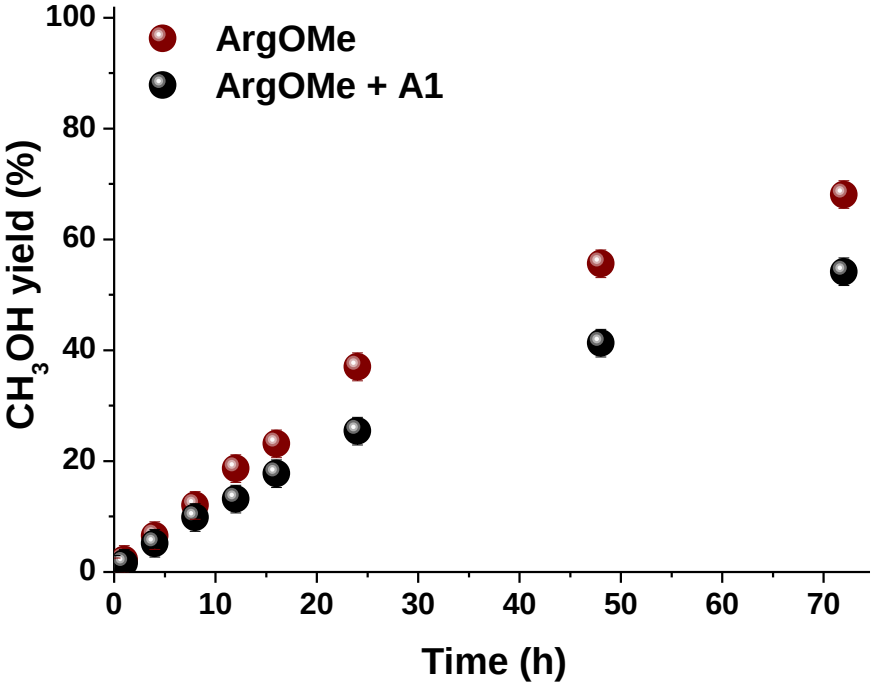

Fig. S36. Kinetic profiles for the hydrolysis of **ArgOMe** in the presence (black spheres) and absence (wine spheres) of **A1**. Yields determined by ^{1}H NMR integration (500 MHz, D_2O, pD = 7.0, PBS buffer 50 mM, 293 K) taking into account the concentration of CH_3OH produced over time. Initial concentrations: 5 mM. The (*L*) enantiomer of **ArgOMe** was used in all cases. Error bars correspond to 5% of the measurement.

Table S13. Linear fitting parameters (linear range, 0-24 h) for effect of A1 addition in the ArgOMe hydrolysis rate (Fig. S36).

A1[a]	Slope[b]	Normalized slope	R^2
-	1.52	1.0	0.9988
5 mM	1.09	0.7	0.9973

[a]**ArgOMe** initial concentration: 5 Mm.

[b]Values calculated using a linear fitting with a fixed Y-intercept value of 0.

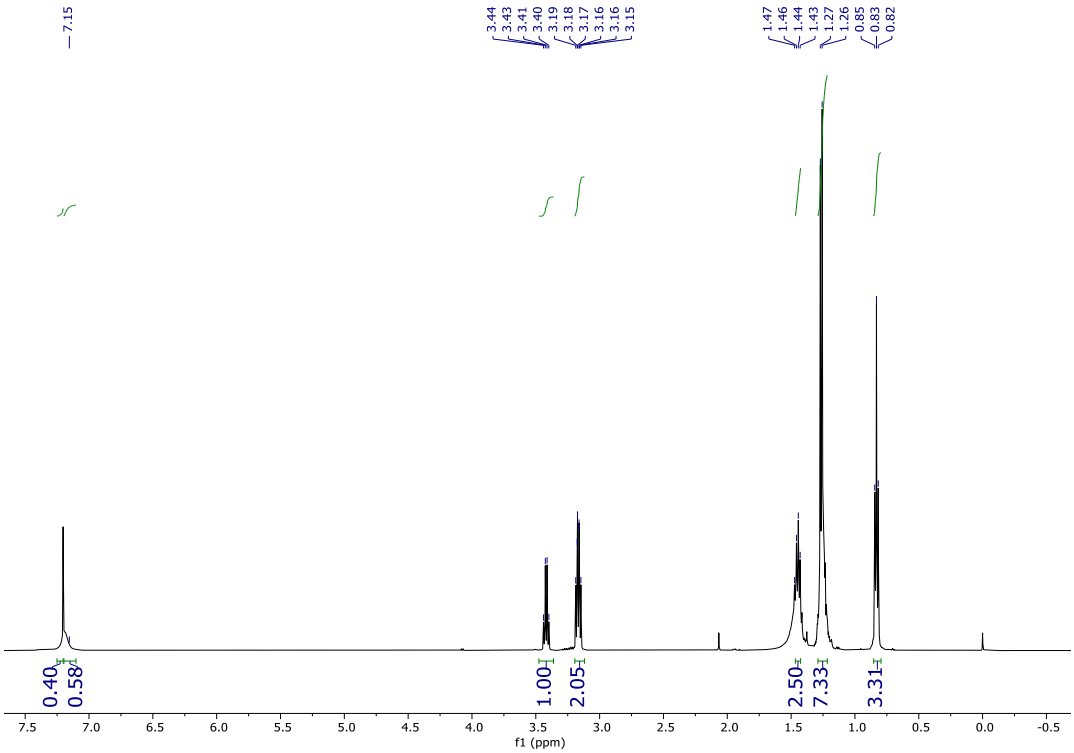

Fig. S37. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$, 293 K) of **AlaNHC5**

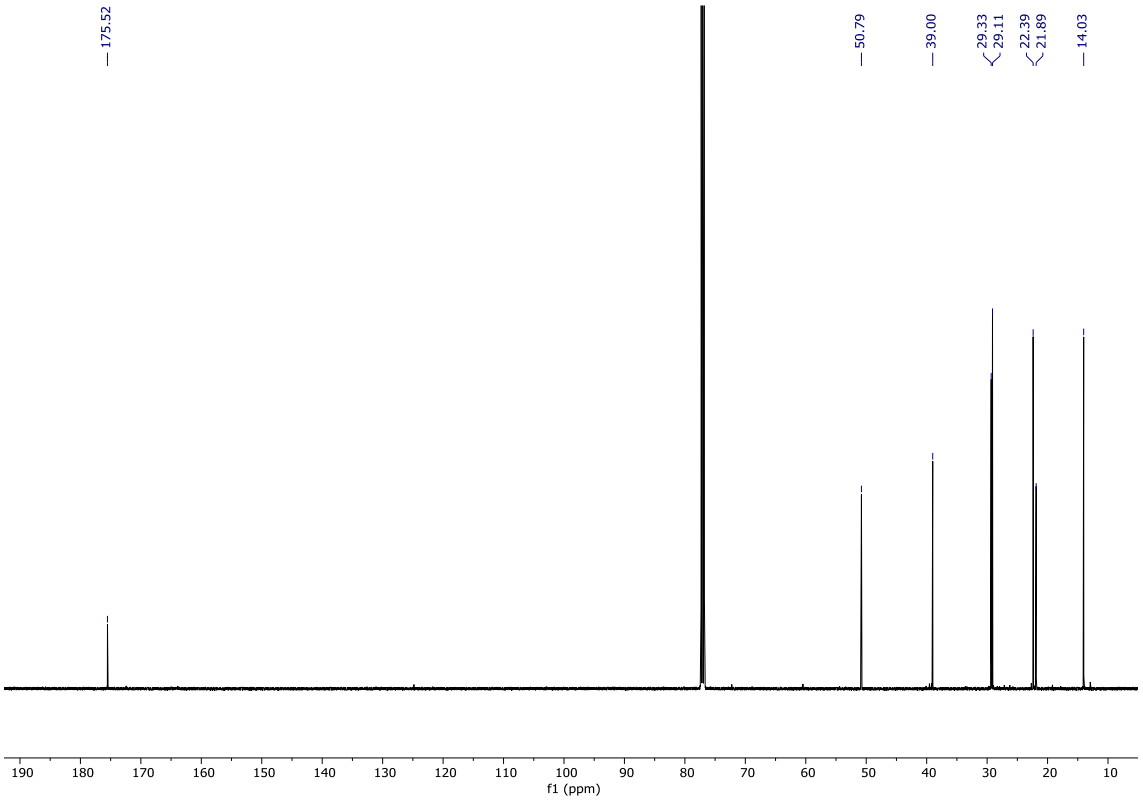

Fig. S38. $^{13}C\{^{1}H\}$-NMR spectrum (125 MHz, $CDCl_3$, 293 K) of **AlaNHC5**

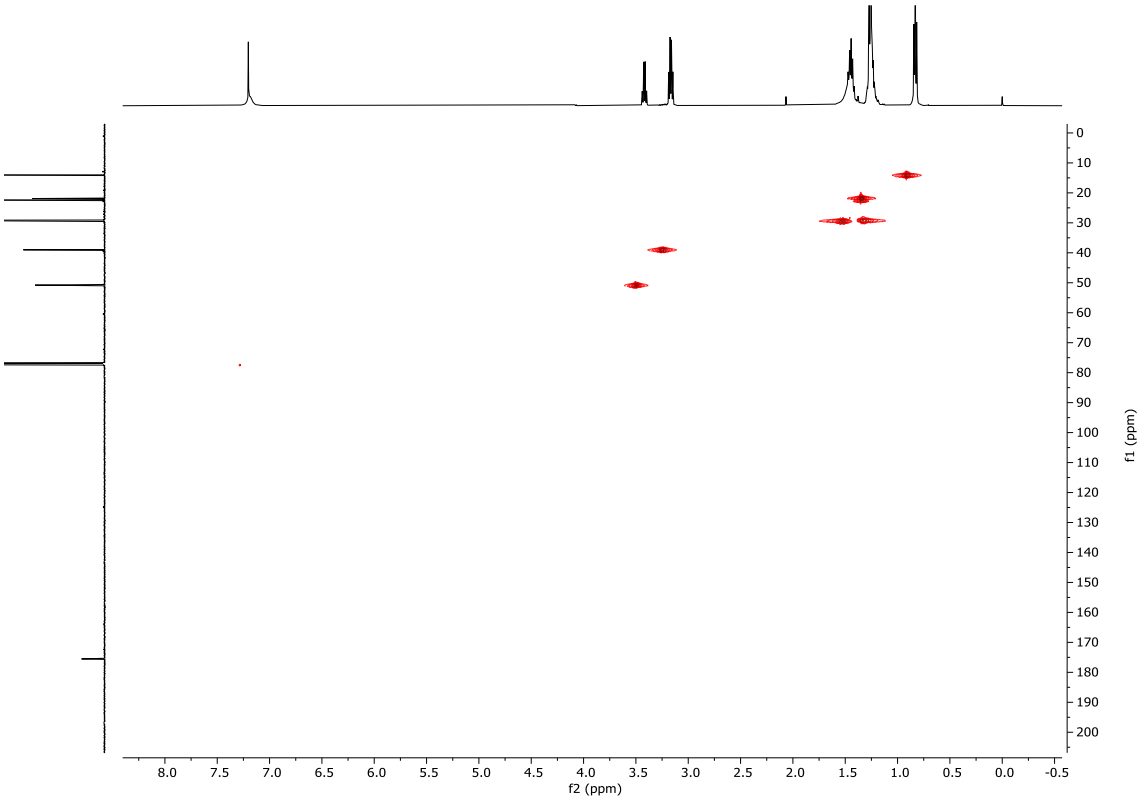

Fig. S39. 2D-HSQC spectrum (500 MHz, $CDCl_3$, 293 K) of **AlaNHC5**

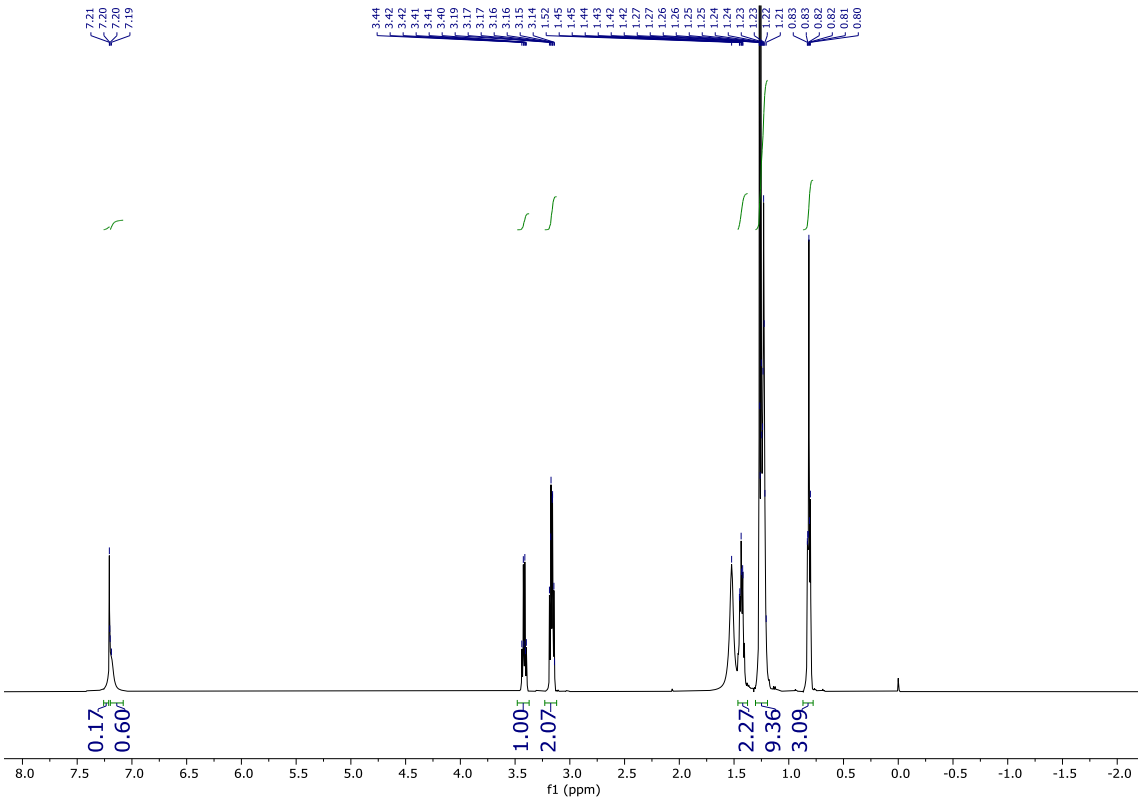

Fig. S40. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$, 293 K) of **AlaNHC6**

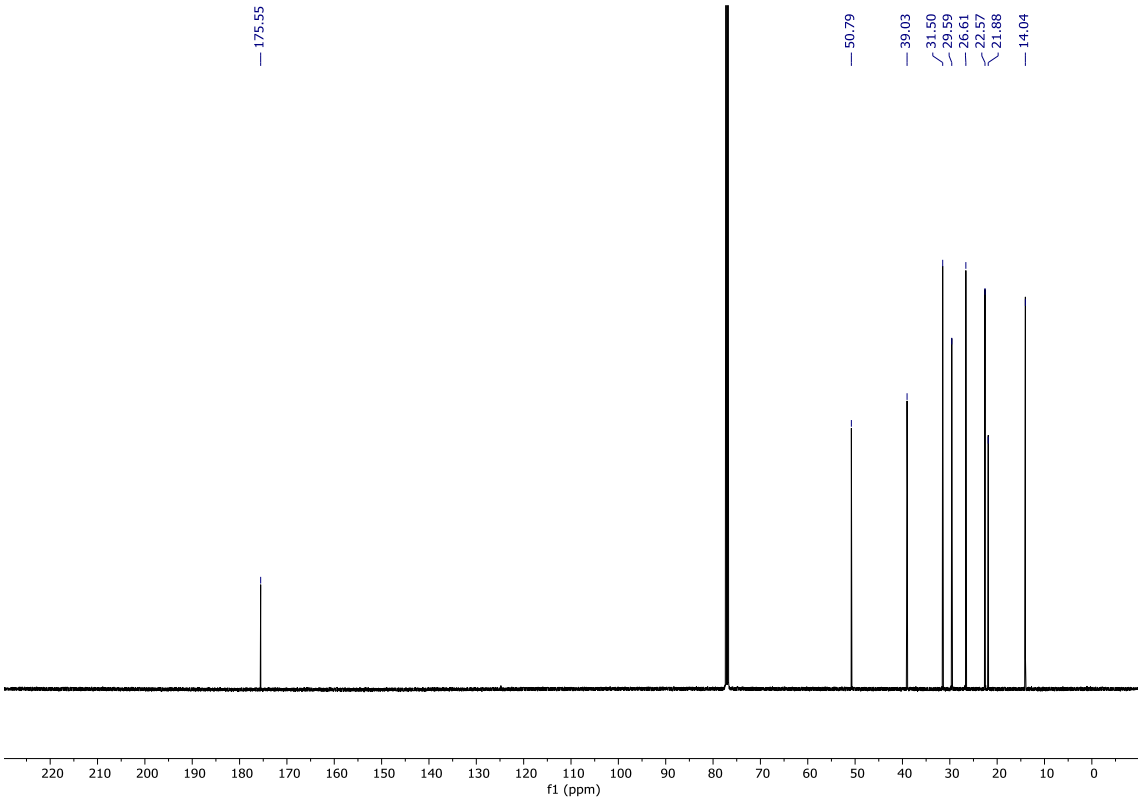

Fig. S41. $^{13}C\{^{1}H\}$-NMR spectrum (125 MHz, $CDCl_3$, 293 K) of **AlaNHC6**

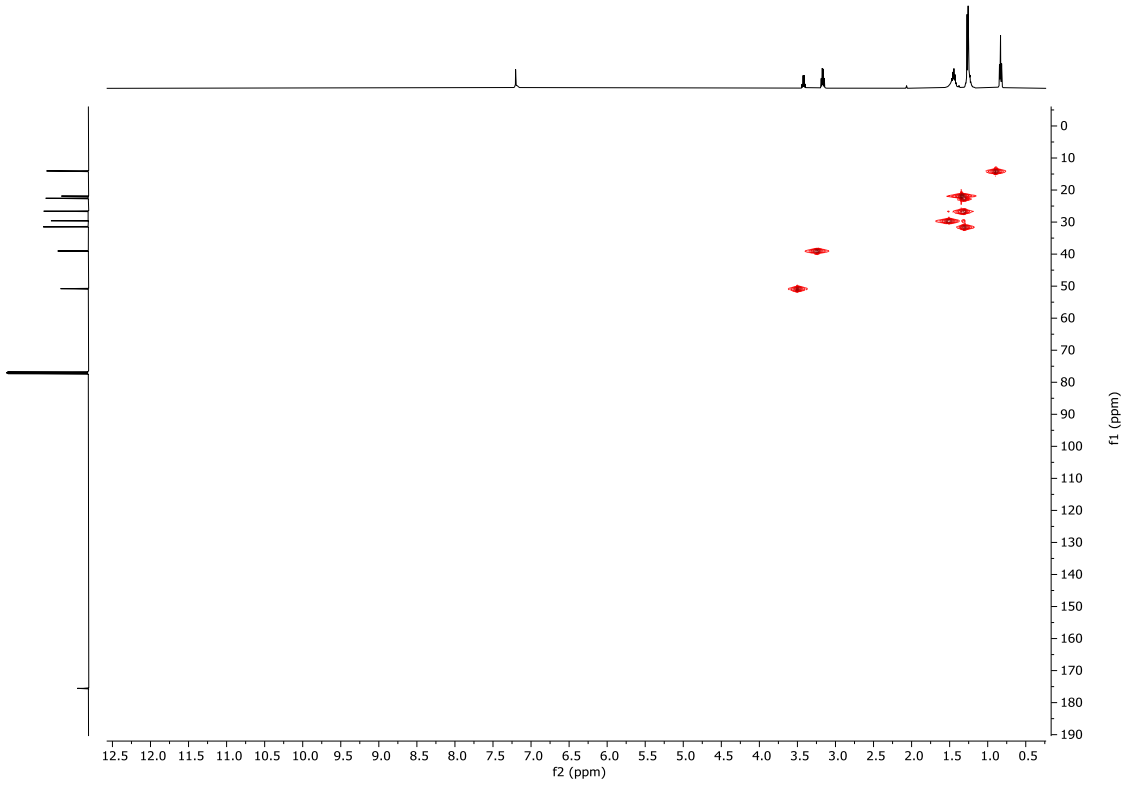

Fig. S42. 2D-HSQC spectrum (500 MHz, $CDCl_3$, 293 K) of **AlaNHC6**

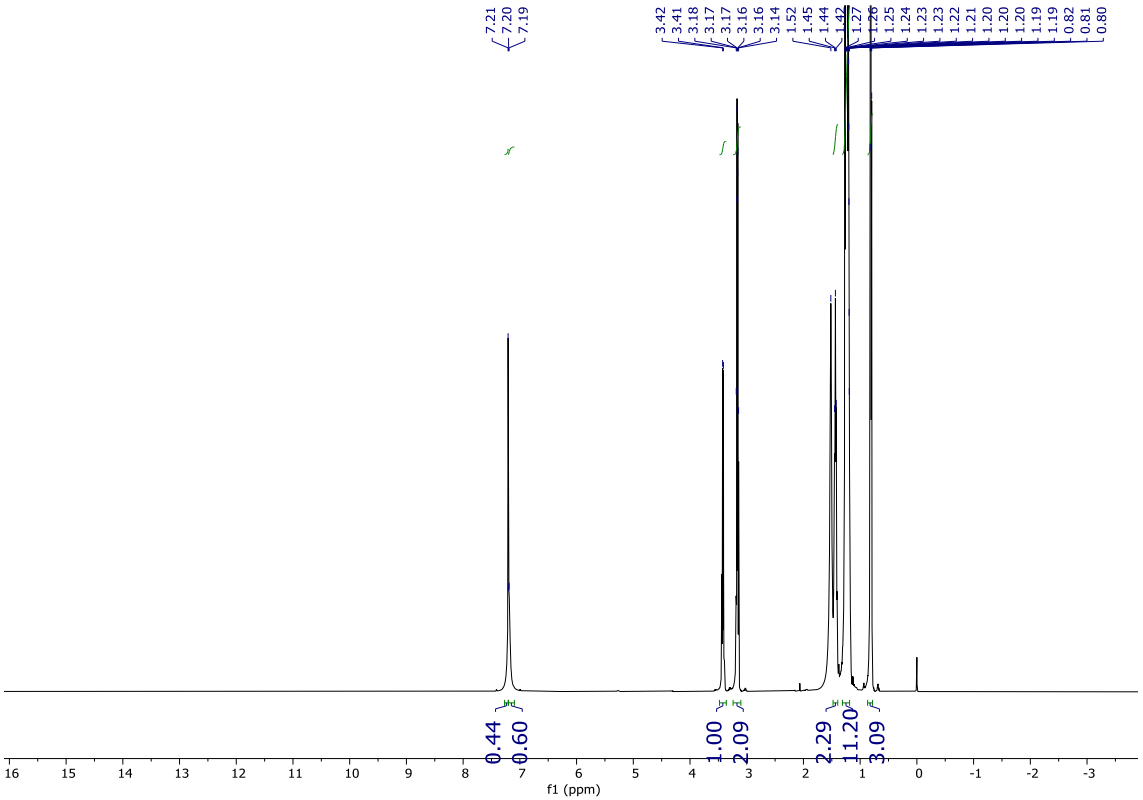

Fig. S43. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$, 293 K) of **AlaNHC7**

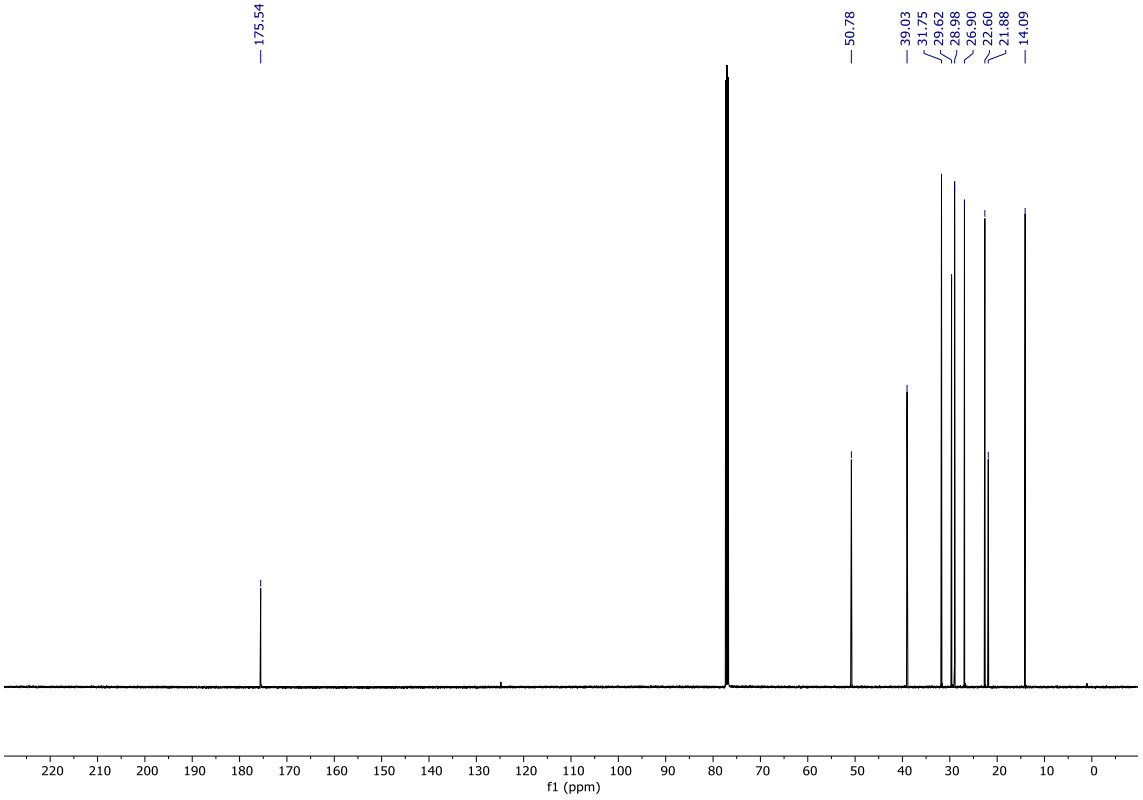

Fig. S44. $^{13}C\{^{1}H\}$-NMR spectrum (125 MHz, $CDCl_3$, 293 K) of **AlaNHC7**

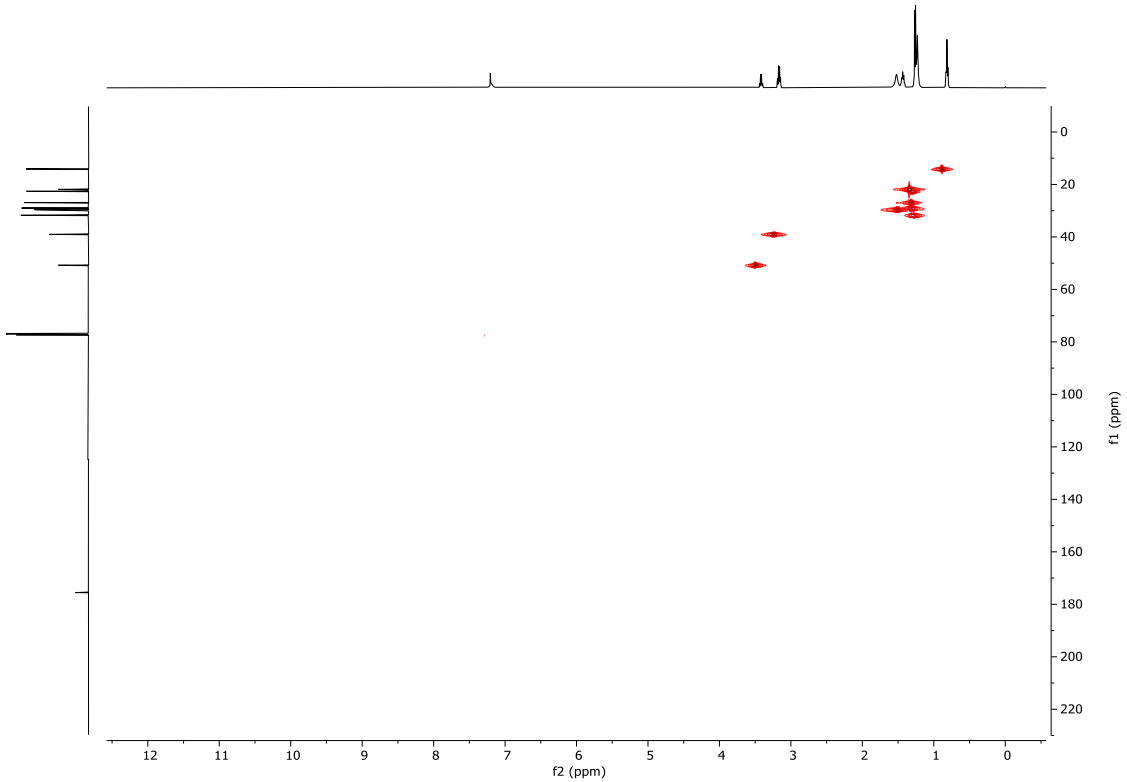

Fig. S45. 2D-HSQC spectrum (500 MHz, $CDCl_3$, 293 K) of **AlaNHC7**

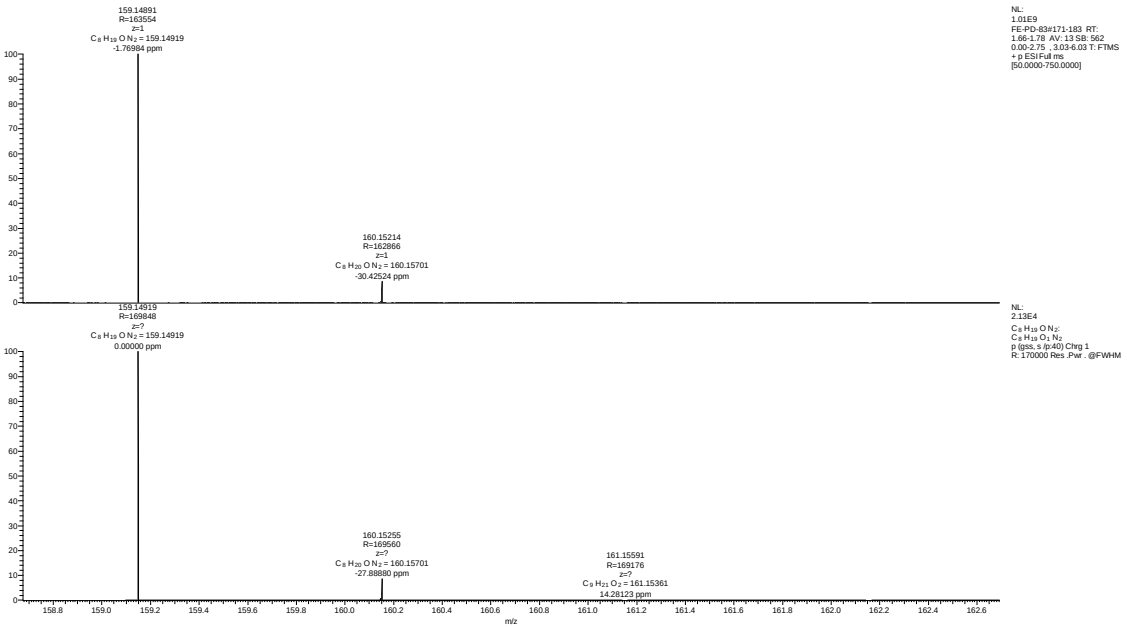

Fig. S46. HRMS-QTOF(+) spectrum (MeOH) of **AlaNHC5**: experimental (above) and calculated (below).

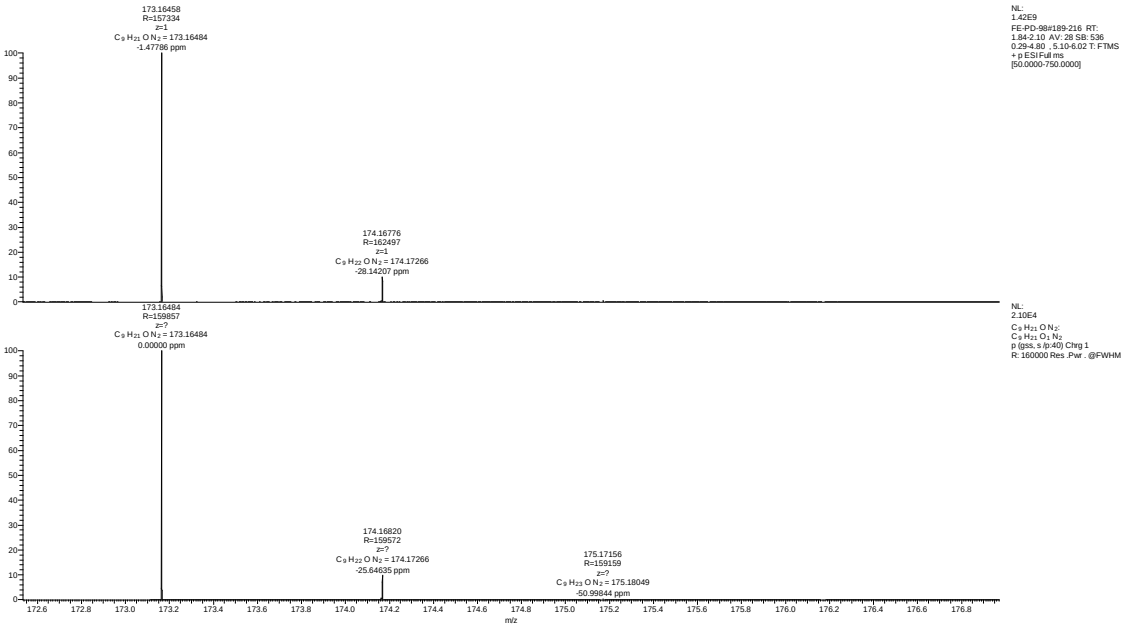

Fig. S47. HRMS-QTOF(+) spectrum (MeOH) of **AlaNHC6**: experimental (above) and calculated (below).

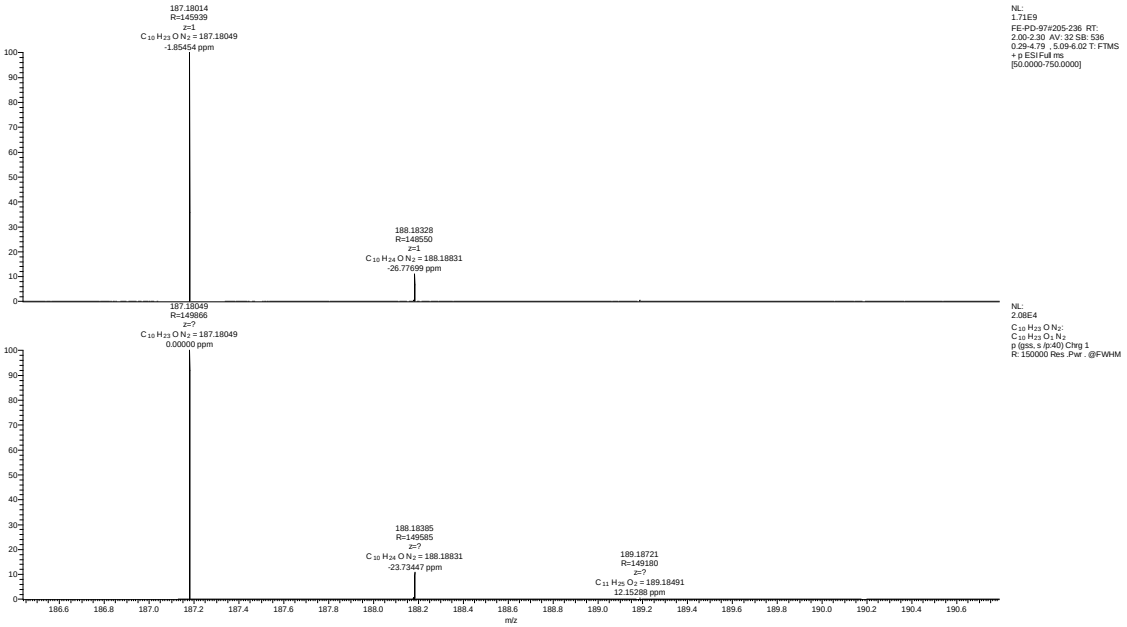

Fig. S48. HRMS-QTOF(+) spectrum (MeOH) of **AlaNHC7**: experimental (above) and calculated (below).

4. References

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