Supporting Information

One-Step Biocatalytic Synthesis of Sustainable Surfactants by Selective Amide Bond Formation

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Methods and materials

Materials and instrumentation
All chemicals and buffers were bought from Sigma Aldrich, Fluorochem or Fischer Scientific. Medium for cell growth was bought from Formedium. All materials relating to molecular biology work were purchased from New England Biolabs (NEB). All NMR spectra were recorded using a Bruker Avance 400 instrument.

HPLC analyses were performed using an Agilent 1260 Infinity II system.

LC/MS analyses were performed using an Agilent 1200 series LC system equipped with a G1379A degasser, a G1312A binary pump, a G1329 autosampler unit, a G1316A temperature-controlled column compartment and a G1315B diode array detector. Compounds were ionized using API-electrospray technique and detected in positive mode on the LCMS System. Drying gas temperature 250 °C at 12 L min⁻¹, and nebulizer pressure at 25 psig.

On both LC/MS and HPLC systems an ACE5 C18 column was used (Dimensions: 250 x 4.6 mm).

For HRMS analyses an Agilent 1200 series LC system was used, coupled to an Agilent 6520 QTOF mass spectrometer, ESI positive mode. The data was analysed using Agilent MassHunter software.

Protein expression and purification
CARmm-A and CHU genes, plasmids and expression strains (E. coli BL21 (DE3)) were prepared using previously described methods.[1]

For protein expression, autoclaved baffled flasks containing 700 ml auto-induction medium containing the appropriate antibiotic, were inoculated with E. coli BL21 (DE3) cells and were grown at 30°C for 72 hours. Cells were harvested by centrifugation and the cell pellet was stored in zip-lock bags at -80°C.

To lyse the cells for purification, the cell pellet was resuspended in Equilibration buffer (50 mM Tris.HCl pH 8, 200 mM NaCl). The cells were then sonicated 20s on/20s off 25 times. The lysis mixture was subsequently centrifuged, the supernatant collected and the pellet discarded. The supernatant was then mixed with Ni-NTA agarose and left shaking at 4°C for 30 minutes. This mixture was then poured into a gravity column and was washed with Wash Buffer (10 mM imidazole, 50 mM Tris.HCl pH 8, 200 mM NaCl). The protein was then eluted using Elution Buffer (200 mM imidazole, 50 mM Tris.HCl pH 8, 200 mM NaCl). The eluted protein was concentrated using Vivaspin centrifugal concentrators (30.000 MWCO, Sartorius) and then desalted using PD-10 columns (GE Healthcare) following the respective protocols. Purity was checked using SDS-PAGE (staining with Instant Blue (Expedeon)) and concentration was determined by measuring absorbance at 280 nm using Nanodrop (Thermo Fisher).

For the production of cell-free lysates, the frozen cell pellets were resuspended in reaction buffer (100 mM HEPBS pH 8.5), then sonicated and subsequently centrifuged as described above. The supernatant protein concentration was measured, aliquoted and stored at -20°C.

Biotransformation procedure
In an example CARmm-A biotransformation, 5 mM of the carboxylic acid substrate (from a 0.5M stock in DMSO), 50 mM of amine (from a 0.25 M stock in buffer, adjusted to pH 8.5), 17.1 mM ATP (from a 0.1 M stock in buffer, adjusted to pH 8.5), 66.5 mM MgCl₂ and CARmm-A (1 mg/mL) were added to HEPBS buffer (100 mM, pH 8.5) to a total volume of 0.5 mL in a 1.5 mL Eppendorf tube. The reaction was placed in a 37°C incubator for 16 hours shaking at 250 rpm.

The reaction was stopped by adding an equal volume of MeOH and shaking the mixture. This mixture was centrifuged and the supernatant was filtered and added to an HPLC vial for analysis.
Acylation of $^{13}$C-decanoic acid

To investigate the reaction selectivity of amidation versus esterification, $^{13}$C-labelled decanoic acid was reacted with amino sugar 1 (Figure S1).

**Figure S1:** Reaction scheme for the CARmm-A catalysed reaction between $^{13}$C-labelled 9 and 1 to synthesise $^{13}$C-labelled 13. Reaction conditions: Carboxylic acid (10 mM), 1 (175 mM), ATP (50 mM), MgCl2 (50 mM), CARmm-A (14 μM), HEPBS buffer (100 mM), 5% DMSO, 0.5 mL scale, pH 8.5, 37 °C, 250 rpm, 16 h.

The crude biotransformation was mixed 1:1 with MeOD and analysed using $^{13}$C-NMR. For a negative control, a reaction without adding the enzyme catalyst was included, as well as a commercial standard of MEGA-10. The carbonyl regions of the $^{13}$C-NMR spectra of these samples are shown in Figure S2.

**Figure S2:** $^{13}$C-NMR (MeOD, 101 MHz) spectra of the CARmm-A catalysed biotransformation between $^{13}$C-labelled decanoic acid and 1 (top), a commercially bought standard of MEGA-10 (middle) and the no enzyme control (bottom), zoomed in to the carbonyl region of the spectrum (~150-190 ppm).

As the carbonyl region of the $^{13}$C-NMR spectrum does not show any peaks other than the acid and amide peaks, it suggests the reaction is selective towards a single product without any unwanted ester byproducts.
To investigate the activity of amino sugars, initial CAR<sub>mm</sub>-A catalysed biotransformations using 3-fluoro cinnamic acid and amino sugar 1 were performed and analysed by $^{19}$F-NMR (using previously described methods<sup>[1]</sup>, figure S3).

![Figure S3](image)

**Figure S3**: Reaction conditions: Carboxylic acid (5 mM), 1 (175 mM), ATP (50 mM), MgCl<sub>2</sub> (50 mM), CAR<sub>mm</sub>-A (1 mg/mL), HEPES buffer (100 mM), 5% DMSO, 0.5 mL scale, pH 8.5, 37 °C, 250 rpm, 16 h.

Figure S4 shows the crude biotransformation (top) and the same biotransformation when spiked with the 3-fluoro cinnamic acid substrate (bottom). This indicated that the substrate in the biotransformation has been completely converted to product.

![Figure S4](image)

**Figure S4**: $^{19}$F-NMR spectra of the crude biotransformation between 3-fluoro cinnamic acid and amino sugar 1 (top) and the same biotransformation spiked with 3-fluoro cinnamic acid (bottom).

To identify the reaction product in this reaction, it was additionally analysed by LC-MS (Figure S5). It was found again that the substrate had been fully converted to the product. The product showed m/z values at 344, 366 and 382 which correspond to the mass of amide 35 ([M+H], [M+Na] and [M+K] respectively).
We also investigated whether sorbitol (a poly-alcohol derivative of glucose) would lead to ester formation when used as a nucleophile using the optimized reaction conditions and 3-fluoro cinnamic acid (Figure S6). We observed that the $^{19}$F-NMR spectra for the sorbitol experiment was identical to the experiment that contained no nucleophile. A very small new peak appeared in these experiments which is the small amount of acyl adenylate that is present in solution. This peak did not show in the no enzyme control experiment as expected. Therefore we concluded that no ester formation occurs when using sorbitol as a nucleophile. Furthermore, a positive control reaction between 3-fluoro cinnamic acid and 1 was performed showing full conversion to the amide product.

Figure S5: LC chromatogram of the 3-fluoro cinnamic acid substrate at 280nm (top), LC chromatogram of the crude CARmm-A catalysed reaction between 3-fluoro cinnamic acid and 1 at 280 nm (middle), and the mass spectrum of the product peak from the middle LC chromatogram showing m/z values at 344, 366 and 382 (bottom).
Figure S6: $^{19}$F-NMR spectra of the crude biotransformation between 3-fluoro cinnamic acid and sorbitol (top), with no nucleophile (2nd row), with no enzyme (3rd row), and a positive control using N-methyl-D-glucamine as a nucleophile (bottom). Reaction conditions: Carboxylic acid (5 mM), nucleophile (50 mM), AMP (17.1 mM), MgCl₂ (66.5 mM), Polyphosphate (14.9 mg/ml), CHU (13 μM), CAR-A (28 μM), HEPBS buffer (100 mM), 1% DMSO, 0.5 mL scale, pH 8.5, 30 °C, 250 rpm, 16 h.
Optimisation

Initial reaction test for reacting 7 with 1 resulting in MEGA-8 (11) was performed using previously reported conditions using an excess of amine and ATP. The conversion was determined by RP-HPLC at a wavelength of 210 nm, using a commercial standard as a reference for a calibration curve (Figure S7 and S8). The calculated conversion of this reaction was >99%.

![MEGA-10 calibration curve](image)

**Figure S7**: Signal values for the different concentrations of the dilution series of MEGA-10 (left) and the values plotted in a calibration curve (right).

| Conc. (mM) | Signal (mAU) |
|------------|-------------|
| 0.625      | 687         |
| 1.25       | 1366        |
| 2.5        | 2708        |
| 5          | 5684        |
| 10         | 11350       |

**Figure S8**: Reaction scheme for the CARmm-A catalysed reaction between octanoic acid and 1 to synthesise 2. HPLC chromatograms showing the reaction using 1 mM octanoic acid, with the product peak integrated showing its mAU value. Reaction conditions: Octanoic acid 7 (1 mM), Amine 1 (175 mM), ATP (50 mM), MgCl2 (50 mM), CARmm-A (1 mg/mL), HEPBS buffer (100 mM), 5% DMSO, 0.5 mL scale, pH 8.5, 37 °C, 250 rpm, 16 h.

For reaction optimisation we used the reaction between 9 and 1 resulting in MEGA-10 (13), using the CHU enzyme to regenerate ATP from AMP and polyphosphate as a model reaction (figure S9).

**Figure S9**: Reaction scheme for the CARmm-A catalysed reaction between 9 and 1 to synthesise 13, using the CHU enzyme to regenerate ATP from AMP and polyphosphate.
Using previously reported reaction conditions we investigated the effect of amine concentration on the conversion of substrates to 13 (Figure S10).

**Figure S10**: Effect of amine concentration on conversion to 13. Reaction conditions: Carboxylic acid (5 mM), amine (range), AMP (10 mM), MgCl2 (50 mM), Polyphosphate (100 mg/ml), CHU (13 μM), CAR-A (28 μM), HEPBS buffer (100 mM), 5% DMSO, 0.5 mL scale, pH 8.5, 37 °C, 250 rpm, 16 h.

We performed design of experiments using the software JMP® (Version 16 Pro. SAS Institute Inc., Cary, NC, 1989–2022) to optimize and better understand the CHU system. We constructed an empirical model for the effect of polyphosphate, AMP and Mg\(^{2+}\) concentrations on conversion using data from a set of biotransformation conditions generated by the software (Figure S11). Using the maximize desirability option in the prediction profiler, it was found that the optimum reaction conditions were 17.1 mM AMP, 66.5 mM MgCl2, and 14.9 mg/ml polyphosphate.

**Figure S11**: Conversions of the set of biotransformations generated by the JMP software (table) and an empirical model of the reaction. (A) The actual conversion plotted against the model prediction \(R^2 = 0.99\). (B) Pareto plots of model factor significance. (C) Snapshot of the prediction profiler set to maximum desirability, showing the optimum concentrations of Mg\(^{2+}\), PolyP and AMP as predicted by the model.

Using these optimum conditions, we further explored the effect of amine concentration (Figure S12).
Figure S12: Effect of amine concentration on conversion to 13. Reaction conditions: Carboxylic acid (5 mM), amine (range), AMP (17.1 mM), MgCl2 (66.5 mM), Polyphosphate (14.9 mg/ml), CHU (13 μM), CAR-A (28 μM), HEPBS buffer (100 mM), 5% DMSO, 0.5 mL scale, pH 8.5, 37 °C, 250 rpm, 16 h.

We then performed another round of design of experiments to gain more insight into the effects of acid, amine and CAR concentration on the conversion and the analytical yield. (Figure S13)

**Table:**

| Run | Acid (mM) | Amine (mM) | CAR (mg/mL) | Analytical Yield (mM) | Conv. (%) |
|-----|-----------|------------|-------------|-----------------------|-----------|
| 1   | 5         | 60         | 2           | 4.49                  | 95        |
| 2   | 6          | 80         | 0.5         | 3.06                  | 39        |
| 3   | 2          | 60         | 1.25        | 1.88                  | 64        |
| 4   | 2          | 80         | 0.5         | 1.97                  | 58        |
| 5   | 5          | 60         | 0.5         | 3.83                  | 77        |
| 6   | 2          | 40         | 3           | 1.82                  | 93        |
| 7   | 5          | 80         | 1.25        | 4.55                  | 91        |
| 8   | 10         | 40         | 1.25        | 3.58                  | 71        |
| 9   | 5          | 40         | 0.5         | 1.73                  | 86        |
| 10  | 10         | 80         | 3           | 4.13                  | 52        |
| 11  | 1          | 60         | 1.25        | 3.57                  | 45        |
| 12  | 5          | 60         | 1.25        | 4.39                  | 86        |
| 13  | 5          | 60         | 1.25        | 4.33                  | 87        |
| 14  | 2          | 40         | 3           | 3.77                  | 47        |
| 15  | 2          | 80         | 3           | 2.13                  | 103       |
| 16  | 8          | 40         | 0.5         | 3.05                  | 38        |

Figure S13: Conversions of the set of biotransformations generated by the JMP software (table) and an empirical model of the reaction. (A) The actual conversion plotted against the model prediction R² = 0.98. (B) Pareto plots of model factor significance. (C) Snapshot of the prediction profiler

The effects of co-solvent concentration on the reaction conversion were investigated (Figure S14).
**Figure S14**: Effect of amine concentration on conversion to 13. Reaction conditions: Carboxylic acid (5 mM), amine (50 mM), AMP (17.1 mM), MgCl2 (66.5 mM), Polyphosphate (14.9 mg/ml), CHU (13 μM), CAR-A (28 μM), HEPBS buffer (100 mM), co-solvent, 0.5 mL scale, pH 8.5, 37 °C, 250 rpm, 16 h.
Analysis of analytical scale biotransformations

Reactions shown in Table 1 were stopped after 16 hours by adding methanol in a 1:1 ratio, the mixture was centrifuged and the supernatant was used for reversed-phase HPLC and LC/MS analysis.

Method on both LC/MS and HPLC: 10 minute isocratic method of 90:10, 80:20, or 70:30 MeOH (+0.1% TFA)/H₂O (+0.1% TFA).

To calculate conversions, a calibration curve was made using a dilution series of a commercially bought standard of MEGA-10 (13) using the concentration range 0.625 mM, 1.25 mM, 2.5 mM, 5 mM and 10 mM. These samples were run using the HPLC conditions described above, detecting the amide at 210 nm, taking the mAU value of the peak of the standard at a retention time of approximately 4.8 min.

The signal values of the dilution series are shown in the table in figure S15 and were plotted in a calibration curve shown in the same figure. The equation of the trend line was then used to calculate the conversions of the N,N-di-substituted amides in Table 1 (11-14, 19-22, and 31-34).

**Figure S15:** Signal values for the different concentrations of the dilution series of MEGA-10 (left) and the values plotted in a calibration curve (right).

A second calibration curve was made using the same dilution series of a commercially bought standard of lauramide MEA (30) (Figure S16). The equation of this trend line was used to calculate the conversions of the mono-N-substituted amides in Table 1 (15-18 and 23-30).

**Figure S16:** Signal values for the different concentrations of the dilution series of lauramide MEA (left) and the values plotted in a calibration curve (right).
Figure S1: Reaction scheme for the CAR\textsubscript{mm}-A catalysed reaction between 7 and 1 to synthesise 11 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 322, [M+Na] = 344 and [M+K] = 362.

Figure S18: Reaction scheme for the CAR\textsubscript{mm}-A catalysed reaction between 8 and 1 to synthesise 12 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 336, [M+Na] = 358 and [M+K] = 376.
Figure S19: Reaction scheme for the CARnm-A catalysed reaction between 9 and 1 to synthesise 13 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 350, [M+Na] = 372 and [M+K] = 388.

Figure S20: Reaction scheme for the CARnm-A catalysed reaction between 10 and 1 to synthesise 14 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 378, [M+Na] = 400 and [M+K] = 416.
Figure S21: Reaction scheme for the CARmm-A catalysed reaction between 7 and 2 to synthesise 15 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing $[\text{M+H}] = 308$, $[\text{M+Na}] = 330$ and $[\text{M+K}] = 346$.

Figure S22: Reaction scheme for the CARmm-A catalysed reaction between 8 and 2 to synthesise 16 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing $[\text{M+H}] = 322$ and $[\text{M+Na}] = 344$. 
Figure S23: Reaction scheme for the CARmm-A catalysed reaction between 9 and 2 to synthesise 17 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 336, [M+Na] = 358 and [M+K] = 374.

Figure S24: Reaction scheme for the CARmm-A catalysed reaction between 10 and 2 to synthesise 18 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 364, [M+Na] = 386 and [M+K] = 402.
Figure S25: Reaction scheme for the CARmm-A catalysed reaction between 7 and 3 to synthesise 19 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing \([\text{M+H}] = 232\), \([\text{M+Na}] = 254\) and \([\text{M+K}] = 270\).

Figure S26: Reaction scheme for the CARmm-A catalysed reaction between 8 and 3 to synthesise 20 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing \([\text{M+H}] = 246\), \([\text{M+Na}] = 268\) and \([\text{M+K}] = 284\).
Figure S27: Reaction scheme for the CARmm-A catalysed reaction between 9 and 3 to synthesise 21 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 260, [M+Na] = 282 and [M+K] = 298.

Figure S28: Reaction scheme for the CARmm-A catalysed reaction between 10 and 3 to synthesise 22 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 288, [M+Na] = 310 and [M+K] = 326.
Figure S29: Reaction scheme for the CARmm-A catalysed reaction between 7 and 4 to synthesise 23 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 218, [M+Na] = 240 and [M+K] = 256.

Figure S30: Reaction scheme for the CARmm-A catalysed reaction between 8 and 4 to synthesise 24 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 232, [M+Na] = 254 and [M+K] = 270.
Figure S31: Reaction scheme for the CARmm-A catalysed reaction between 9 and 4 to synthesise 25 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 246, [M+Na] = 268 and [M+K] = 284.

Figure S32: Reaction scheme for the CARmm-A catalysed reaction between 10 and 4 to synthesise 26 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 274, [M+Na] = 296 and [M+K] = 312.
Figure S33: Reaction scheme for the CAR\textit{mm}-A catalysed reaction between 7 and 5 to synthesise 27 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing \([M+H] = 188\), \([M+Na] = 210\) and \([M+K] = 226\).

Figure S34: Reaction scheme for the CAR\textit{mm}-A catalysed reaction between 8 and 5 to synthesise 28 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing \([M+H] = 202\) and \([M+Na] = 224\).
Figure S35: Reaction scheme for the CARmm-A catalysed reaction between 9 and 5 to synthesise 29 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 216, [M+Na] = 238 and [M+K] = 254.

Figure S36: Reaction scheme for the CARmm-A catalysed reaction between 10 and 5 to synthesise 30 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 244, [M+Na] = 266 and [M+K] = 282.
Figure S37: Reaction scheme for the CAR\textit{mm}-A catalysed reaction between 7 and 6 to synthesise 31 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 232, [M+Na] = 254 and [M+K] = 270.

Figure S38: Reaction scheme for the CAR\textit{mm}-A catalysed reaction between 8 and 6 to synthesise 32 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 246 and [M+Na] = 268.
Figure S39: Reaction scheme for the CARmm-A catalysed reaction between 9 and 6 to synthesise 33 (top), LC chromatogram of biotransformation with product peak integrated (middle), and mass spectrum in positive mode of the product peak showing [M+H] = 260, [M+Na] = 282 and [M+K] = 298.
Preparative scale reactions

Reaction conditions: Carboxylic acid (5 mM), amine (50 mM), AMP (17.1 mM), MgCl2 (66.5 mM), Polyphosphate (14.9 mg/ml), CHU (13 μM), CAR-A (28 μM) HEPBS buffer (100 mM), 1% DMSO, 30 mL scale, pH 8.5, 37 °C, 250 rpm, 16 h

After 16 hours, the reaction mixture was centrifuged for 5 min at 4000 rpm and the supernatant was freeze-dried. The resulting residue was purified using either normal-phase flash chromatography (DCM/MeOH, Pure C-815 Flash Advanced automated flash chromatography system with UV and ELS detection (Büchi Labortechnik)) or reverse phase flash chromatography (H2O/MeOH).

MEGA-8 (11)

Exact Mass: 321.2151

Isolated yield: 20 mg (42%)

1H NMR (400 MHz, CDCl3) δ 3.95 - 3.31 (m, 8H, CH sugar), 3.02 (s, C9H), 2.87 (s, C9H), 2.35 - 2.18 (m, 2H, C7H), 1.51 - 1.46 (m, 2H, C6H), 1.26 - 1.19 (m, 8H, C2-5H), 0.82 - 0.79 (m, 3H, C1H).

Product has a cis- and trans-isomer, as observed by the C10 proton split between two signals (3.02 and 2.87).

13C NMR (101 MHz, CDCl3) δ 175.3 (C=O), 73.0 (C sugar), 71.8 (C sugar), 70.2 (C sugar), 63.8 (C sugar), 51.7 (C sugar), 37.4 (C sugar), 33.7 (C9H), 31.8 (C aliph), 29.7 (C aliph), 29.4 (C aliph), 29.1 (C aliph), 25.0 (C aliph), 22.6 (C aliph), 14.1 (C aliph).

HRMS (ESI+, m/z): calculated for (C15H32NO6+) [M+H]+: 322.2224; found: 322.2217.

N-octanoyl glucamine (15)

Exact Mass: 307.1995

Isolated yield: 25 mg (54%)

1H NMR (400 MHz, MeOD) δ 8.45 (s, 1H, NHamide), 3.73 - 3.33 (m, 7H, CH sugar), 3.16 - 3.11 (m, 1H, CH sugar), 2.11 (t, J = 7.6 Hz, 2H, C7H), 1.57 - 1.43 (m, 2H, C6H) 1.25 - 1.19 (m, 8H, C2-5H), 0.82 - 0.79 (m, 3H, C1H).

13C NMR (101 MHz, MeOD) δ 176.3 (C=O), 73.2 (C sugar), 72.8 (C sugar), 72.4 (C sugar), 70.7 (C sugar), 64.2 (C sugar), 42.8 (C sugar), 36.5 (C aliph), 32.2 (C aliph), 29.7 (C aliph), 29.5 (C aliph), 26.4 (C aliph), 23.0 (C aliph), 13.8 (C aliph).

HRMS (ESI+, m/z): calculated for (C14H29NO6) [M+H]: 308.2068; found: 308.2061.
N-(2,3-dihydroxypropyl)decanamide (25)

Isolated yield: 29 mg (78%)

$^1$H NMR (400 MHz, MeOD) $\delta$ 8.54 (s, 1H, NHamide), 3.72 – 3.60 (m, 1H, CHglyc), 3.55 – 3.33 (m, 3H, CHglyc), 3.24 – 3.12 (m, 1H, CHglyc), 2.20 (t, J = 7.5 Hz, 2H, C9H), 1.63 – 1.52 (m, 2H, C8H), 1.40 – 1.18 (m, 12H, C2-7H), 0.96 – 0.82 (m, 3H, C1H).

$^{13}$C NMR (101 MHz, MeOD) $\delta$ 176.3 (C=O), 71.5 (Cglyc), 64.4 (Cglyc), 42.7 (Cglyc), 36.4 (Calph), 32.4 (Calph), 30.0 (Calph), 29.8 (Calph), 29.7 (Calph), 26.4 (Calph), 23.1 (Calph), 13.8 (Calph).

HRMS (ESI+, m/z): calculated for (C_{13}H_{28}NO_{3})$^+$ [M+H]: 246.2064; found: 246.2060.
Gene sequences

All genes contain an N-terminal polyhistidine tag.

**CAR**mmΔ647-1175
ATGAGCCATCATCATTATCATCATACATCATGAGACCACCATGAGGGCCGATCTGGCCGCGCACAGCATATG
GCAGCATTTTGCAGGAAACTGGAAGAATGTTGAAACCCTGCGTGTTAAACCGAAACAGAGCAT
TGATCTGAAAAAAGATTTCGACACCGACTACGATCATAAAATGCTGACCAAAGAAGAAGGCGAAG
AACTGCTGAATCTGGGTATTTCAAAACTGAGCGAGATCCAAGAAAAACTGTATGCAAGCGGCA
AAAAGCGTTCTGATTGTTTTTCAGGCAATGGATGCAGCAGGTAAAGATGGCACCGTTAAACATAT
TATGACCGGTCTGAATCCGCAGGGTGTTAAAGTTACCAGCTTTAAAGTTCCGAGCAAAATCGAAC
TGAGCCATGATTATCTGTGGCGTCATTATGTTGCACTGCCTGCAACCGGTGAAATTGGTATCTTT
AATCGTAGCCACTATGAAAATGTTCTGGTTACCCGTGTTCATCCGGAATATCTGCTGAG
GGACCAGCGGTGTTACCGCAATTGAACAGGTTAATCAGAAATTCTGGGATAAACGCTTTCAGCAGA
TCAACAACTTTGAACAGCATATTAGCGAAAACGGCACCATTGTGCTGAAATTCTTTCTGCATGTGA
GCAGAACAGAAAAACGCTTTATTGAACGCATCGAAGTTCAGCGCTTAAACCGATATG

**CHU**
ATGGGCAGCAGCCTATCATCATTATCATCATACATACAGCAGCCGCGCTGTGGCCGCGCGCGGAGGATATG
GCAAAGAATGGGAACTGGAAGAATGTTGAAACCCTGCGTGTTAAACCGAAACAGAGCAT
TGATCTGAAAAAAGATTTCGACACCGACTACGATCATAAAATGCTGACCAAAGAAGAAGGCGAAG
AACTGCTGAATCTGGGTATTTCAAAACTGAGCGAGATCCAAGAAAAACTGTATGCAAGCGGCA
AAAAGCGTTCTGATTGTTTTTCAGGCAATGGATGCAGCAGGTAAAGATGGCACCGTTAAACATAT
TATGACCGGTCTGAATCCGCAGGGTGTTAAAGTTACCAGCTTTAAAGTTCCGAGCAAAATCGAAC
TGAGCCATGATTATCTGTGGCGTCATTATGTTGCACTGCCTGCAACCGGTGAAATTGGTATCTTT
AATCGTAGCCACTATGAAAATGTTCTGGTTACCCGTGTTCATCCGGAATATCTGCTGAG
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