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Enzymatic Approach to Bifunctional Chelating Agents

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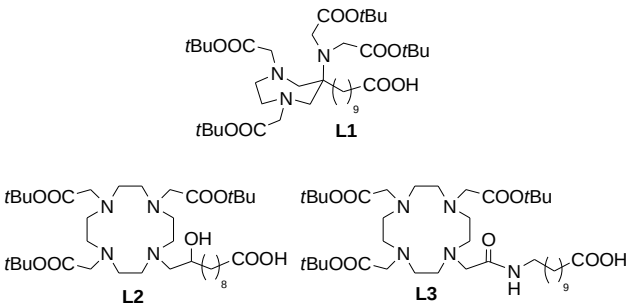
Bifunctional chelating agents (BFCAs) combine the complexing properties of a multidentate ligand with the presence of a free reactive functional group, mainly devoted to conjugation purposes. Indeed, products obtained by conjugation of a BFCA to a biomolecule and coordination of a suitable metal ion, are widely applied in nowadays medicine as diagnostic and therapeutic agents. BFCAs are generally prepared through multi-step syntheses and with extensive application of protection-deprotection strategies, due to the large number of functional groups involved. Hydrolytic enzymes, with their unique chemoselectivity, provided the best results in the preparation of three different BFCAs based on very useful and well known ligand platforms.

Introduction

Metal complexes are widely used in diagnostic and therapeutic applications.[1] Paramagnetic complexes of Gd(III) are routinely used in clinical MRI,[2] while chelates of radioactive metal ions represent the active imaging agents in PET and SPECT,[3] or radiopharmaceuticals in radiotherapy.[4] Moreover, different luminescent complexes are employed in Optical Imaging.[5] The thermodynamic and kinetic stabilities of metal complexes in biological conditions are achieved through a careful design/choice of the ligand structure, satisfying the metal requirements for a stable coordination. In addition, the structure of a metal complex needs sometimes to be modified for different application-related purposes such as: i) introduction of lipophilic/hydrophilic residues to modulate solubility properties and/or to promote the inclusion in different formulations; ii) selective targeting to a particular biological environment through conjugation to specific vectors (*e.g.* small molecules, proteins or peptides, antibodies).

BiFuctional Chelating Agents (BFCAs) combine the coordination properties of a multidentate ligand with the presence of a free reactive functional group, devoted to conjugation or tailoring purposes. Several BFCAs are commercially available, although their number is still limited, if the wide variety of metal ions to be used is taken into account. BFCAs are usually prepared through a multi-step synthesis and with extensive application of protection-deprotection strategies, due to the large number of fuctional groups involved.[6,7]

In this article we describe the synthesis, through a chemoenzymatic approach, of three selected BFCAs (Scheme 1) with different structures. The exquisite chemoselectivity of hydrolytic enzymes is exploited to handle the significant number of functional groups of the selected polyaminopolycarboxylic ligands. The choice of the ligand platforms has fallen on three chelating agents usually employed for lanthanide ions, mainly because those ions are extensively used in different imaging/therapeutic techniques, but also because of our experience developed in this field of research.[8] All BFCAs reported in this article share a remote carboxylic acid group, protected as a methyl ester during the synthesis and selectively removed by chemical or enzymatic hydrolysis in the final step.



Scheme 1. BFCAs prepared in this work.

The first BFCA (**L1**) is a derivative of the heptadentate ligand AAZTA (6-Amino-6-methylperhydro-1,4-diazepine-*N,N',N'',N'''*-tetraacetic acid).[9] Since the first report of the formation of a promising Gd(III)-complex for MRI application, AAZTA has rapidly gained popularity, with several derivatives reported so far and continuous efforts devoted to the development of dedicated BFCAs.[10-13]

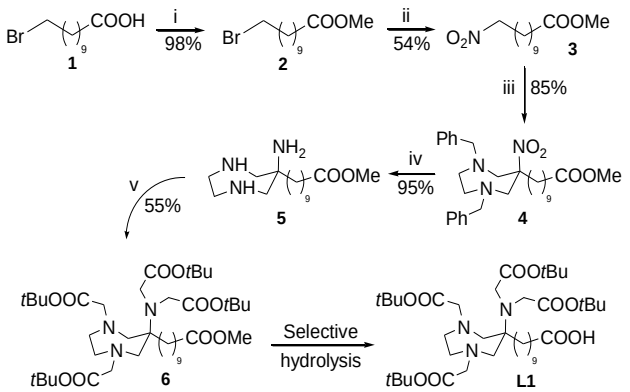
The second BFCA (**L2**) is based on the macrocyclic ligand HP-DO3A,[14] best known for its Gd-complex currently employed in clinical MRI as contrast agent under the brand name ProHance®,[15] and used with other lanthanide ions for the development of CEST-MRI imaging procedures.[16]

The third BFCA (**L3**) is built on the base structure of DOTA-monoamide (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), the latter widely represented in several of the above cited applications in clinical diagnosis and therapy.[1-4]

Results and discussion

Synthesis of protected BFCAs

Bifunctional derivatives of AAZTA usually bear the reactive functional group on the carbon atom carrying the primary amine group (position 6 of the 6-aminoperhydro-1,4-diazepine ring). This is due either to an easier synthetic access, as in the original synthesis this carbon atom derives from a suitable nitroalkane, and to stereochemical and steric considerations, as substituents placed in this position: i) retain the symmetry plane of the ligands, ii) circumvent the generation of stereocentres as would happen in any other ring or pendant arm position, iii) avoid any steric influence with the metal coordination sphere.
The first BFCA **L1** was obtained introducing on the AAZTA skeleton a C_9-alkyl spacer with a terminal carboxylic acid functional group.



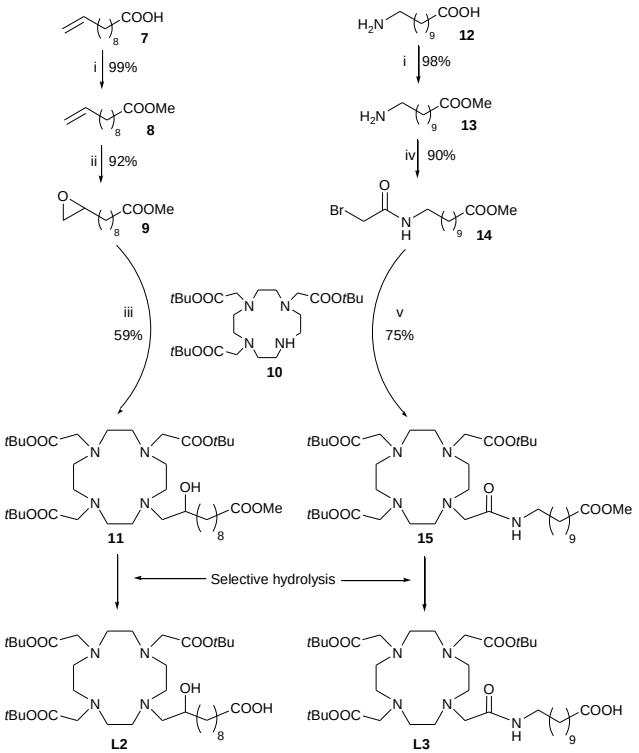
Scheme 2 Synthesis of **L1**. Reagents and conditions: (i) $SOCl_2$, MeOH, r.t.; (ii) $NaNO_2$, urea, DMAc, 50°C, 24h; (iii) *N,N'*-Dibenzylethylenediaminediacetate, paraformaldehyde, PhMe/EtOH, Rfx; (iv) $HCOONH_4$, Pd/C 10%, MeOH, reflux; (v) $BrCH_2COOtBu$, K_2CO_3, CH_3CN.

The preparation of **L1** is shown in Scheme 2 and started from 11-bromoundecanoic acid which was converted into the corresponding methyl ester **2**, then the halogen atom was substituted with a nitro group through a S_N2 reaction with sodium nitrite in *N,N*-dimethylacetamide in the presence of urea. The nitroester **3** was employed in the diazepine ring formation step, taking place through a double nitro-Mannich reaction with *N,N'*-dibenzylethylenediamine and paraformaldehyde. Catalytic transfer hydrogenation (CTH) with ammonium formate[17] and Pd/C allowed the combined removal of the benzyl groups located on the endocyclic nitrogen atoms and the reduction of the nitro group, leading to the triaminoester **5**. The amine groups were exhaustively alkylated with *tert*-butyl bromoacetate in acetonitrile in the presence of finely powdered potassium carbonate giving, after chromatographic purification, the mixed ester **6**, the latter representing the direct precursor of the BFCA **L1**.

The syntheses of the macrocyclic BFCAs are reported in Scheme 3 and both rely on the key intermediate DO3A-tBu$_3$ (1,4,7,10-tetraazacyclododecane-1,4,7-triacetate tri-*t*-butyl ester) **10**, which is commercially available or conveniently prepared following the literature procedure.[18]

The preparation of the HP-DO3A-like BFCA **L2** required the alkylation of the secondary amine group of DO3A-tBu$_3$ **10** with the epoxyester **9**, obtained in two steps from 10-undecenoic acid, to give the mixed ester precursor **11**.

The bromoacetamide **14** was needed for the synthesis of the DOTAMA (DOTA MonoAmide) BFCA **L3**. This intermediate was easily prepared by esterification of 11-aminoundecanoic acid with methanol and thionyl chloride followed by *N*-bromoacetylation in Schotten-Baumann conditions. Alkylation of DO3A-tBu$_3$ **10** with bromoacetamide **14** in refluxing acetonitrile and in the presence of triethylamine provided the mixed ester precursor **15** in good yield.



Scheme 3 Synthesis of **L2** and **L3**. Reagents and condictions; (i) $SOCl_2$, MeOH; (ii) MCPBA, CH_2Cl_2; (iii)DMAc, TEA; (iv) $BrCH_2COBr$, K_2CO_3, CH_2Cl_2/H_2O;(v) CH_3CN, Et_3N, reflux.

Selective deprotection

The protected BFCAs **6**, **11** and **15** described in the previous section share the presence of carboxylic groups protected with different ester moieties, namely *t*-butyl ester on the carboxylic groups involved in metal coordination and methyl ester on the carboxylic acid intended for conjugation purposes.
In principle, methyl ester can be selectively cleaved in the presence of *t*-butyl esters by a judicious choice of hydrolytic conditions. Nevertheless, a similar example of a mixed ester of a DOTA[19] highlighted the difficulties in finding conditions that avoid side-reactions such as hydrolysis of *t*-butyl esters and transesterification, when alcohols are used as (co)solvents.
For these reasons, compounds **6**, **11** and **15** were subjected to an extensive screening to assess and find out optimal experimental conditions leading to the BFCAs **L1-L3** in preparatively useful yields. In this work "classical" chemical hydrolysis was complemented and compared with enzymatic methods. The

exquisite chemoselectivity of hydrolytic enzymes can be useful to obtain the desired selectivity in the removal of the methyl ester groups.[20] To the best of our knowledge, only one report of the use of hydrolytic enzymes on ethyl esters of chelating agents can be found in the literature,[21] but the extreme similarity of the homogenously esterified carboxyl groups led either to incomplete reactions or to poor yields with macrocyclic substrates. Our substrates are designed to offer better structural conditions for the enzyme selectivity, presenting a single methyl ester located at the end of an aliphatic chain, strongly resembling the chemical environment of fatty acid esters.[22] The long aliphatic chain should provide a suitable spacing between the bulky coordination cages and the ester groups, allowing a proper positioning of the latter in the enzymatic active site, while minimising the steric hindrance to the reactive functional group.

The screening of "chemical" hydrolysis included two different hydroxides (LiOH, NaOH), an alkaline carbonate and an alkaline bicarbonate, sharing the same mixed solvent system 2-propanol/water 1:1. The screening of enzymes involved three lipases, *i.e.:* Porcine Pancreatic Lipase (PPL), Candida Cylindracea Lipase (CCL) and an immobilized CAL (Lipase B Candida Antarctica on Immobead 150, recombinant from *Aspergillus oryzae*), all of them run in a mixed solvent system MTBE/phosphate buffer (KH_2PO_4/K_2HPO_4, 1M, pH 7.5). The corresponding results are summarized in Table 1.

Table 1: Reaction times, yields and conversion of different ligands.(* Na^+ complexes)

	6 → L1		**11 → L2**		**15 → L3**	
	Time (h)	**Yield (%)[a]**	**Time (h)**	**Yield (%)[a]**	**Time (h)**	**Yield (%)[a]**
NaOH	4	91	4	92*	6	89*
LiOH	4	90	8	90*	6	88*
K_2CO_3	>48	0	>48	0	>48	0
$NaHCO_3$	>48	0	>48	0	>48	0
PPL	36	97	36	98	48	94
CCL	>48	0	48	87	48	63
CAL	4	99	4	99	5	98

[a]Yields determined by ^{1}H-NMRwith internal standard (diphenylmethane) on the crude isolated product.

The results of Table 1 show that potassium carbonate and sodium bicarbonate are completely ineffective with all substrates, even after 48h. Better results were obtained with alkaline hydroxides, with nearly complete conversion after 4-8h, depending on the substrate, with the AAZTA precursor reacting generally faster than the macrocyclic derivatives. It is noteworthy that in these runs, the products of the hydrolysis are unavoidably plagued by the presence of variable amounts of the alkaline cation, strongly retained in the macrocyclic cavity, a phenomenon well known for DO3A and DOTA derivatives.[23] Small amount of by-products arising from the unwanted hydrolysis of *t*-butyl groups can be detected in the crude reaction mixture, too.

The use of enzyme lead to different results. PPL provided clean reactions with all three substrates, reaching completion after 36-48h and with nearly quantitative yields. Lower performances were shown with CCL, where good results were obtained with substrate **11** after 48 h, while an incomplete conversion was obtained with ester **15** and no reaction at all was observed with the AAZTA derivative **6**.

Gratifyingly and quite unexpectedly, the best results were obtained with supported CAL: conversion was complete in 4-5 h with all the three BFCA precursors, and nearly quantitative yields were obtained from a simpler workup. No evidence of the hydrolysis by-products observed with NaOH and LiOH can be found in the reaction mixtures, leading to a simpler workup, even more facilitated by the heterogeneous nature of the catalyst, and finally to higher isolated yields.

According to all these considerations, supported CAL in phosphate buffer/MTBE proved to be the best combination for the selective removal of methyl esters of mixed ester precursors of AAZTA-, HP-DO3A and DOTAMA-based BFCAs. The latter are currently in use for the preparation of conjugates, to be reported elsewhere.

Conclusions

The preparation of BFCAs usually requires multi-step synthetic approaches and complex protection-deprotection strategies. In this work, the synthesis of three different BFCAs, based on the AAZTA, HP-DO3A and DOTAMA ligands is reported. The efficiency and selectivity of enzymes (lipases) are successfully employed for the multigram preparation of these BFCAs, with the key step represented by the selective removal of a methyl ester group in the presence of multiple *t*-butyl ester groups. The enzymatic protocol is preferred to chemical hydrolysis, where alkaline hydroxides can reach comparable hydrolytic activities but with the drawback represented by the formation of unwanted by-products. Supported CAL (Immobead 150) proved to be the most active enzyme with respect to these specific substrates. Work is in progress in order to exploit the conjugation potential of these novel BFCAs.

Experimental section

General remarks

All reagents, enzymes and solvents were obtained from Sigma-Aldrich or Alfa-Aesar and used without further purification. DO3A-tBu_3 **10** was prepared as hydrobromide following the procedure reported in literature.[18] The corresponding free base was obtained by elution of a 1% solution in MeOH/H_2O 1:1 through an ion exchange Amberlite® IRA-400 (OH^- form) column. ^{1}H-NMR and ^{13}C-NMR spectra were registered on a Jeol Eclipse ECP300 spectrometer at 300 MHz and 75.4 MHz, respectively. Mass spectra were performed on a ThermoFinningan LCQ-Deca XP-PLUS, operating in ESI-MS mode.

Synthetic procedures

Methyl 11-Bromoundecanoate (2). 11-Bromoundecanoic acid (**1**, 38.0 g, 0.14 mol) was dissolved in 200 mL of methanol and thionyl chloride (16 mL, 0.21 mol) was slowly dropped into the reaction mixture, kept in an ice bath. After the addition, the resulting solution was refluxed for 2 h, then cooled and a solution of potassium carbonate 20% was added until pH>9. The methanol

was removed under vacuum and the aqueous suspension extracted twice with dichloromethane. The organic extracts were dried over Na_2SO_4, filtered and evaporated yielding **2** as a light brown oil (38.20 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.26 (s, 10H, CH_2), 1.39 (bquint, J = 6.9 Hz, 2H, CH_2), 1.58 (bquint, J = 7.0 Hz, 2H, CH_2), 1.82 (quint, J = 6.9 Hz, 2H, CH_2), 2.27 (t, J = 7.5 Hz, 2H, CH_2), 3.37 (t, J = 6.9 Hz, 2H, CH_2), 3.63 (s, 3H, CH_3). ^{13}C {1H} NMR (75.4 MHz,$CDCl_3$) δ (ppm): 24.9 (*C*H_2), 28.1 (*C*H_2), 28.7 (*C*H_2), 29.1 (*C*H_2), 29.2 (*C*H_2), 29.3 (*C*H_2), 29.4 (*C*H_2), 32.8, (*C*H_2), 33.9 (*C*H_2), 34.1 (*C*H_2), 51.4 (*C*H_3), 174.2 (*C*). MS (ESI+) calculated for: $[C_{12}H_{23}BrO_2+H]^+$ 279.09/281.09, found 279.16/281.15.

Methyl 11-Nitroundecanoate (3). Methyl ester **2** (38.17 g, 0,14 mol) was dissolved in 50 mL of *N*,*N*-dimethylacetamide and to this solution pholoroglucinol (22.05 g, 0.14 mol) and sodium nitrite (18.88 g, 0.27 mol) were sequentially added. The reaction mixture was heated at 50 °C for 24 h. The mixture was then cooled and extracted 3 times with petroleum ether and the upper phases collected and evaporated under vacuum.The crude product was purified by silica gel column chromatography (95/5 petroleum ether/ethyl acetate) to obtain **3** as a light yellow oil (18.1 g, 54%).^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.27-1.32 (m, 12H, CH_2), 1.60 (bquint, J = 7.2 Hz, 2H, CH_2), 1.99 (quint, J = 7.2 Hz, 2H, CH_2), 2.29 (t, J = 7.5 Hz, 2H, CH_2), 3.68 (s, 3H, CH_3), 4.36 (t, 2H, CH_2). ^{13}C {1H} NMR (75 MHz,$CDCl_3$) δ (ppm): 24.9 (*C*H_2), 26.2 (*C*H_2), 27.4 (*C*H_2), 28.8 (*C*H_2), 29.1 (*C*H_2), 29.2 (*C*H_2), 29.3 (*C*H_2), 34,1 (*C*H_2), 51.4 (*C*H_3), 75.8 (*C*H_2), 51.4 (*C*H_2), 174.3 (*C*). MS (ESI+) calculated for: $[C_{12}H_{23}NO_4+H]^+$246.32, found 246.12.

Compound 4. A mixture of the nitroester **3** (18.0 g, 0.07 mol) and *N,N'*-dibenzylethylendiamine diacetate (31.7g, 0.09 mol) in 1/1 toluene/ethanol (300 mL) was brought to reflux with magnetic stirring until a clear solution was obtained. Paraformaldehyde (8.4 g, 0.28 mol) was then added in a single portion and heating was continued for additional 5 h. The solvent was removed under vacuum and the residue purified by silica gel column chromatography (95/5 petroleum ether/ethyl acetate) to obtain compound **4** as a yellow light oil (30.2 g, 85%).^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.69-1.32 (m, 14H, CH_2), 1.60 (bquint, J = 7.2 Hz, 2H, CH_2), 1.59 (bquint, 2H, CH_2), 2.29 (t, J = 7.5 Hz, 2H, CH_2), 2.51-2.65 (m, 4H, (CH_2), 2.95 (d, J = 14.1 Hz, 2H, CH_2), 3.50 (d, J = 14.1 Hz, 2H, CH_2), 3.57 (d, J = 12.9 Hz, 2H, CH_2), 3.66 (s, 3H, CH_3) 3.72 (d, J = 13.1 Hz, 2H, CH_2), 7.19-7.37 (m, 10H, Ar*H*). ^{13}C {1H} NMR (75 MHz,$CDCl_3$) δ (ppm): 23.1, 25.0 (*C*H_2), 29.2 (2x*C*H_2), 29.3, 29.33, 29.34 (*C*H_2), 34.17 (*C*H_2), 37.1 (*C*H_2), 51.4 (*C*H_3), 58.8 (*C*H_2), 61.9 (*C*H_2), 64.1 (*C*H_2), 95.2 (*C*), 127.3, 128.3, 129.2, 139.3 (*C*H), 174.4 (*C*). MS (ESI+) calculated for: $[C_{30}H_{43}N_3O_4+H]^+$ 510.33, found 510.15.

Compound 5. Compound **4** (30.0 g, 0.059 mol) was dissolved in methanol (150 mL), and 10% Pd/C (3.0 g) was added under N_2 atmosphere. Ammonium formate (36.1 g, 0.60 mol) was added in small portions, and the reaction heated to 60°C for 2h.The catalyst was removed by filtration through Celite® and the solvent removed under reduced pressure. The crude product was re-dissolved in dichloromethane and repeatedly washed with a 1M NaOH solution (25 mL x 4). The organic phase was dried over Na_2SO_4, and evaporated to obtain the triaminoester **5** as a light yellow oil (17.05 g, 95%).^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.23 (bs, 14H, CH_2), 1.57 (bt, J = 6.7 Hz, 2H, CH_2), 2.56 (t, J = 7.5 Hz, 2H, CH_2), 2.70 (m, 4H, CH_2), 2.75-3.01 (m, 4H, CH_2), 3.62 (s, 3H, CH_3). ^{13}C {1H} NMR (75 MHz, $CDCl_3$) δ (ppm): 24.9, 29.1(*C*H_2), 29.2 (2x*C*H_2) , 29.35, 29.36, 30.3 (*C*H_2), 34.0 (*C*H_2), 39.4 (*C*H_2), 51.0 (*C*H_2), 51.4 (*C*H_3), 56.4 (*C*), 59.6(*C*H_2), 174.3 (*C*). MS(ESI+) calculated for : **[**$C_{16}H_{33}N_3O_2$+H]$^+$300.17, found 300.5.

Compound 6. In a mixture of triamine **5** (17.0 g, 0.056 mol) and potassium carbonate (46.45 g, 0.34 mol) in acetonitrile, *t*-butyl bromoacetate (41 mL, 0.28 mol) was added dropwise. The reaction was stirred at room temperature overnight. The inorganic salts were removed by filtration on a Buchner funnel and the solvent removed under reduced pressure. The crude product was purified by silica gel column chromatography to obtain the mixed ester **6** as a light yellow oil (23.4 g, 55%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.22-1.30 (m, 14H, CH_2), 1.426 (s, 18H, (CH_3), 1.432 (s, 18H, (CH_3) 1.60 (m, 2H, CH_2), 2.28 (t, J = 7.5 Hz, 2H, CH_2), 2.62 (d, 2H, J = 14.4 Hz, (CH_2), 2.62-2.68 (m, 2H, CH_2), 2.72-2.80 (m, 2H, CH_2), 2.98 (d, 2H,J = 14.1 Hz, (CH_2), 3.22 (bs, 4H, CH_2), 3.62 (bs, 4H, CH_2), 3.65 (s, 3H, CH_3).^{13}C {1H} NMR (75 MHz, $CDCl_3$) δ (ppm): 22.1 (*C*H_2), 25.0, (*C*H_2), 28.2 (*C*H_3) 28.3 (*C*H_3), 29.2 (*C*H_2), 29.3 (2x*C*H_2), 29.6, 30.5 (*C*H_2), 34.14 (*C*H_2), 37.6 (*C*H_2), 51.4 (*C*H_3), 51.9 (*C*H_2), 59.4 (*C*H_2),62.6 (*C*H_2), 63.1 (*C*), (*C*H_2), 65.4 (*C*H_2), 80.2 (*C*), 80.7 (*C*), 170.9 (*C*), 172.9 (*C*), 174.3 (*C*). MS(ESI+) calculated for: **[**$C_{40}H_{73}N_3O_{10}$+H]$^+$ 756.32, found 756.5.

Methyl 10-Undecenoate (8). 10-Undecenoic acid (16.0 g, 0.087 mol) was suspended in methanol (50 mL) and thionyl chloride (8.9 mL, 0.12 mol) was added dropwise (external ice bath for cooling was required to maintain T<10°C). The resulting solution was stirred at room temperature for 12 h. A solution of aq. 20% potassium carbonate was added until pH>9, then methanol was evaporated under vacuum and the crude product extracted three times with ethyl acetate (3x25mL). The combined organic extracts were dried over Na_2SO_4, filtered and evaporated yielding compound **8** as a light brown oil (17.2 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.22-1.41 (m, 10H, CH_2), 1.61 (quint, J = 7.0 Hz, 2H, CH_2), 2.05 (quart, J = 6.7 Hz, 2H, CH_2), 2.29 (t, J = 7.5 Hz, 2H, CH_2), 3.65 (s, 3H, CH_3), 4.90-5.01 (m, 2H, CH_2), 5.79 (ddt, J_1 = 6.9 Hz, , J_2 = 9.9 Hz, , J_3 = 17.1 Hz, 1H, C*H*). ^{13}C {1H} NMR (75 MHz,$CDCl_3$) δ (ppm): 24.9, 28.1, 29.1, 29.17, 29.2, 29.4, 33.8, 34.1 (*C*H_2), 51.4 (*C*H_3), 114.2 (*C*H_2), 139.2 (*C*H), 174.2 (*C*). MS (ESI+) calculated for: $[C_{12}H_{22}O_2+H]^+$ 199.16, found 199.30.

Compound 9. Methyl 10-undecenoate **8** (2.0 g, 0.010 mol) was dissolved in CH_2Cl_2 (20 mL) and 3-chloroperbenzoic acid (70%, 4.31 g, 0.025 mol) was added. The reaction was stirred at room temperature for 3 h. A white solid precipitated and was removed by filtration on a Buchner funnel. The filtrate was thoroughly washed with 5% aq. NaOH solution (2x25 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated to obtain **9** as a colourless oil (2.11 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.22-1.62 (m, 16H, CH_2), 2.28 (t, J = 7.5 Hz, 2H, CH_2), 2.44 (dd, J_1 = 5.1 Hz, J_2 = 2.6 Hz, 1H, CH_2), 2.44 (dd, J_1 = 5.0 Hz, J_2 = 3.9 Hz, 1H, CH_2), 2.87 (m, 1H, C*H*), 3.64 (s, 3H, CH_3).

^{13}C {1H} NMR (75 MHz,$CDCl_3$) δ (ppm): 24.97, 25.9, 29.2, 29.4, 29.5, 32.5, 34.1 (*C*H_2), 47.13 (*C*H_2) 51.5 (*C*H_3), 52.39 (*C*H) 174.3 (*C*). MS (ESI+) calculated for: $[C_{12}H_{22}O_3+H]^+$ 214.1, found 214.06.

Compound 11. DO3A-tBu_3 **10** (free base, 1.0 g, 1.9 mmol) and the epoxyester **9** (447 mg, 2.1 mmol) were dissolved in *N*,*N*-dimethylacetamide (5.0 mL) and the solution was refluxed for 36 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (4 x 20 mL) and brine (3 x20 mL), dried (Na_2SO_4), filtered and evaporated under vacuum. The crude product was purified by silica gel column chromatography (eluent CH_2Cl_2/MeOH/NH_3 95/5/0.5) to obtain the mixed ester **11** as a light brown oil (820 mg, 59%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.25-1.34 (m, 12H, C*H*$_2$), 1.43 (s, 18H, (C*H*$_3$), 1.44 (s, 9H, C*H*$_3$), 1.59 (quint, J= 7.1 Hz, 2H, CH_2), 2.04-3.49 (br, 26 H, C*H*$_2$), 2.27 (t, J = 7.5 Hz, 2H, C*H*$_2$), 3.64 (s, 3H, C*H*$_3$). ^{13}C {1H} NMR (75 MHz, $CDCl_3$) δ (ppm): 24.9 (*C*H_2) 25.8 (*C*H_2), 27.9 (*C*H_3), 28.2 (*C*H_3), 29.1 (2x*C*H_2), 29.4, 29.8 34.1, 34.8 (*C*H_2), 51.4 (*C*H_3) 51.8 (*C*H_2), 52.1 (*C*H_2), 52.3 (*C*H_2), 53.7 (*C*H_2), 55.9 (*C*H_2), 56.8 (*C*H_2), 61.5 (*C*H_2), 68.1 (*C*H), 80.7 (*C*), 80.8 (*C*), 170.9 (*C*), 171.1 (*C*), 174.1 (*C*). MS (ESI+) calculated for $[C_{38}H_{72}N_4O_9+Na]^+$: 751.52, found 751.01.

Methyl 11-Aminoundecanoate (13). 11-Aminoundecanoic acid (5.0 g, 0.025 mol) was suspended in methanol (25 mL) and then thionyl chloride (3.6 mL, 0.05 mol) was added dropwise (external ice bath for cooling was required to maintain T<10°C). The mixture was stirred at room temperature for 12 h. A solution of aq. 20% potassium carbonate was added until pH>9, then methanol was evaporated under vacuum and the crude product was extracted with ethyl acetate (3x25mL), the organic phase dried (Na_2SO_4) and filtered. Removal of the solvent yielded the ester **13** as a light brown oil (5.3 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.21-1.38 (m, 12H, C*H*$_2$), 1.55 (bquint, J = 7.0 Hz, 2H, C*H*$_2$), 1.68 (bquint, J = 7.1 Hz, 2H, C*H*$_2$), 2.26 (t, J = 7.5 Hz, 2H, C*H*$_2$), 2.90 (q, J = 6.6 Hz, 2H, C*H*$_2$), 3.63 (s, 3H, C*H*)$_3$, 7.25 (bs, 2H, N*H*$_2$). ^{13}C {1H} NMR (75 MHz, $CDCl_3$) δ (ppm): 24.9, 26.6, 28.2, 29.1, 29.2, 29.25 (*C*H_2), 29.4 (2x*C*H_2), 34.1, 40.2 (*C*H_2), 51.5 (*C*H_3), 174.4 (*C*). MS (ESI+) calculated for: $[C_{12}H_{25}NO_2+H]^+$ 216.19, found 216.10.

Compound 14. Ester **13** (5.2 g, 0.024 mol) was added to a biphasic mixture of CH_2Cl_2 (25 mL) and an aqueous solution containing potassium carbonate (13.2 g, 0.096 mol, in 25 mL). Bromoacetyl bromide (3.1 mL, 0.036 mol) was added dropwise into the vigorously stirred biphasic mixture and the reaction run for 2 h. The two phases were separated and the organic layer washed with water (3x25mL) and brine (25 mL). The solvent was evaporated under reduced pressure to obtain the bromoacetamide **14** as a light brown waxy solid (7.3 g, 90%). Mp 55-56°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.18-1.36 (m, 12H, C*H*$_2$), 1.52 (bquint, J = 7.02 Hz, 2H, C*H*$_2$), 1.60 (bquint, J = 7.3 Hz, 2H, C*H*$_2$), 2.29 (t, J = 7.4 Hz, 2H, C*H*$_2$),3.27 (q, J = 6.6 Hz, 2H, C*H*$_2$), 3.65 (s, 3H, C*H*$_3$), 3.88 (s, 2H, C*H*$_2$) 6.51 (bs, 1H, N*H*). ^{13}C {1H} NMR (75 MHz, $CDCl_3$) δ (ppm): 24.9, 26.8, 29.1, 29.2 (*C*H_2), 29.27 (2x *C*H_2), 29.3(*C*H_2), 29.4 (2x*C*H_2), 34.1, 40.3 (*C*H_2), 51.4 (*C*H_3), 165.5.2 (*C*), 174.4 (*C*). MS (ESI+) calculated for: $[C_{14}H_{26}BrNO_3+H]^+$ 336.11/338.11, found 336.02/338.03.

Compound 15. DO3A-tBu_3 **10** (free base, 2.0g, 3.8 mmol) and bromoacetamide **14** (1.43 g, 4.3 mmol) were dissolved in acetonitrile (25 mL) and to the resulting mixture triethylamine (749 µL, 5.7 mmol) was added. The mixture was refluxed for 36 h. The solvent was removed under vacuum and the oily residue taken up in dichloromethane (25 mL), washed with H_2O (4x20 mL) and brine (3x20 mL), dried (Na_2SO_4), filtered and evaporated under vacuum. The crude product was purified by silica gel column chromatography (eluent CH_2Cl_2/MeOH/NH_3 95/5/0.5) to obtain the BFCA precursor **15** as a light brown oil (2.2, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.11-1.27 (m, 16H, C*H*$_2$), 1.33 (s, 9H, (C*H*$_3$), 1.34 (s, 18H, C*H*$_3$), 1.46 (quint, J= 7.2 Hz, 2H, CH_2) 2.18 (t, J = 7.5 Hz, 2H, C*H*$_2$), 2.40-3.34 (br, 24H, C*H*$_2$), 3.54 (s, 3H, C*H*$_3$) 8.67 (m, 1H, N*H*). ^{13}C {1H} NMR (75 MHz, $CDCl_3$) δ (ppm): 25.1 (CH_2) 28.0 (CH_2), 28.07 (CH_3), 28.2 (CH_3), 28.9, 29.1, 29.2, 29.5 (2x*C*H_2), 29,63, 34.1, 39.5 (*C*H_2), 51.4 (*C*H_3), 55.6 (*C*H_2), 55.8 (*C*H_2), 56.2 (*C*H_2), 56.7 (*C*H_2), 81.8 (*C*), 81.9 (*C*), 169.8 (*C*), 170.0 (*C*), 171.4 (*C*) 172.4 (*C*). MS(ESI+) calculated for: **[**$C_{40}H_{75}N_5O_9$+Na]$^+$ 792.56, found 792.05.

General procedure for chemical hydrolyses. Methyl ester (**6**, **11**, **15**, 200 mg) was dissolved in a 2-propanol/water (1:1,10 mL) mixture. The alkaline bicarbonate/carbonate/hydroxide (1.5 eq) was added in a single portion and the reaction stirred at room temperature for the reported time (Table 4). 2-Propanol was then evaporated under reduced pressure and the aqueous phase was washed with CH_2Cl_2 (3x5 mL). The organic solvent was dried (Na_2SO_4), filtered and evaporated under vacuum to give the corresponding BFCA.

General procedure for enzymatic hydrolyses. Methyl ester (**6**, **11**, **15**, 200 mg) was added to a suspension of lipase (60 mg) in a mixture of 2.0 mL of phosphate buffer (1M, pH 7.5) and 8 mL of methyl *t*-butyl ether. The reaction mixture was stirred at room temperature for the reported time (Table 4). The lipase was removed by filtration on a Celite®, the filtrate concentrated and the aqueous phase extracted with CH_2Cl_2 (3x5 mL). The organic solvent was dried (Na_2SO_4), filtered and evaporated under vacuum to give the corresponding BFCA.

Example procedures for gram-scale enzymatic hydrolyses. Methyl ester (**6** or **11**, 10.0 g) was added to a suspension of CAL lipase (3.0 g) in a mixture of 10 mL of phosphate buffer (1M, pH 7.5) and 40 mL of methyl *t*-butyl ether. The reaction mixture was stirred at room temperature for 8 h. The lipase was removed by filtration on a Celite®, the filtrate concentrated and the aqueous phase extracted with CH_2Cl_2 (3x25 mL). The organic solvent was dried (Na_2SO_4), filtered and evaporated under vacuum to give **L1** (9.4 g, 97%) or **L2** (9.6 g, 97%).

Ligand 1. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.22-1.30 (m, 14H, C*H*$_2$), 1.41 (s, 18H, C*H*$_3$), 1.42 (s, 18H, (C*H*$_3$) 1.60 (m, 2H, C*H*$_2$), 2.31 (t, J = 7.5 Hz, 2H, C*H*$_2$), 2.62 (d, 2H, J = 14.1 Hz, (C*H*$_2$), 2.62-2.68 (m, 2H, C*H*$_2$), 2.72-2.80 (m, 2H, C*H*$_2$), 2.98 (d, 2H, J = 14.4 Hz, (C*H*$_2$), 3.22 (bs, 4H, C*H*$_2$), 3.61 (bs, 4H, C*H*$_2$). ^{13}C {1H} NMR (75 MHz, $CDCl_3$) δ (ppm): 22.0 (*C*H_2), 24.7, (*C*H_2), 28.2 (*C*H_3) 28.3 (*C*H_3), 29.0 (*C*H_2), 29.2 (2x*C*H_2), 29.4,

30.4 (CH_2), 34.1 (CH_2), 37.6 (CH_2), 51.9 (CH_2), 59.3 (CH_2), 62.5 (CH_2), 63.0 (C), (CH_2), 65.4 (CH_2), 80.3 (C), 80.8 (C), 170.9 (C), 173.0 (C), 179.0 (C). MS(ESI+) calculated for: **[**$C_{39}H_{71}N_3O_{10}$+H]$^+$ 742.51, found 742.78.

Ligand 2. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.18-1.31 (m, 14H, CH_2), 1.40 (s, 9H, (CH_3), 1.41 (s, 18H, (CH_3), 1.55 (quint, J= 7.1 Hz, 2H, CH_2) 2.21 (t, J = 7.4 Hz, 2H, CH_2), 2.67-2.80 (m, 10H, CH_2), 2.88 (m, 8H, CH_2), 3.01-3.15 (m, 1H, CH), 3.25 (s, 4H CH_2), 3.29-3.37 (m, 2H CH_2). ^{13}C {1H} NMR (75 MHz, $CDCl_3$) δ (ppm): 25.3 (CH_2) 25.7 (CH_2), 27.9 (CH_3), 28.2 (CH_3), 29.1 (2xCH_2), 29.2, 29.8 34.9, 36.4 (CH_2), 49.6 (CH_2), 51.2 (CH_2), 52.8 (CH_2), 53.1 (CH_2), 56.1 (CH_2), 56.5 (CH_2), 59.7 (CH_2), 65.8 (CH), 81.2 (C), 81.4 (C), 170.6 (C), 170.8 (C), 178.7 (C). MS(ESI+) calculated for: [$C_{37}H_{70}N_4O_9$+Na]$^+$737.53, found 737.70.

Ligand 3. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.18-1.35 (m, 16H, CH_2), 1.44 (s, 9H, (CH_3), 145 (s, 18H, CH_3), 1.6 (quint, J= 7.2 Hz, 2H, CH_2) 2.18 (t, J = 7.5 Hz, 2H, CH_2), 2.75-2.88 (br, 18H, CH_2), 3.20-3.31 (m, 6H, CH_2). ^{13}C {1H} NMR (75 MHz, $CDCl_3$) δ (ppm): 25.2 (CH_2) 26.9 (CH_2), 28.0 (CH_3), 28.2 (CH_3), 29.1 (CH_2), 29.2 (2xCH_2), 29.3, 29.6, 28.7, 34.9, 39.5 (CH_2), 50.6 (CH_2) 52.6 (CH_2), 53.28 (CH_2), 53.3 (CH_2), 55.7 (CH_2), 55.8 (CH_2), 56.6 (CH_2), 80.7 (C), 81.8 (C), 169.9 (C), 170.0 (C), 170.4 (C) 177.3 (C). MS(ESI+) calculated for: **[**$C_{39}H_{73}N_5O_9$+Na]$^+$ 778.54, found 778.60.

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‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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