Full Paper

Novel Deoxyxylulosephosphate-Reductoisomerase Inhibitors: Fosmidomycin Derivatives with Spacious Acyl Residues

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1-Deoxy-D-xylulose-5-phosphate reductoisomerase (Dxr) represents an essential enzyme of the mevalonate-independent pathway of the isoprenoid biosynthesis. Using fosmidomycin as a specific inhibitor of Dxr, this enzyme was previously validated as target for the treatment of malaria and bacterial infections. The replacement of the formyl residue of fosmidomycin by spacious acyl residues yielded inhibitors active in the micromolar range. As predicted by flexible docking, evidence was obtained for the formation of a hydrogen bond between an appropriately placed carbonyl group in the acyl residue and the main-chain NH of Met214 located in the flexible catalytic loop of the enzyme.

Keywords: 1-Deoxy-D-xylulose-5-phosphate-reductoisomerase inhibitors / Non-mevalonate biosynthesis / Structure-activity relationships

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Introduction

Isopentenyldiphosphate is the common precursor of a wide variety of isoprenoid compounds. The biosynthesis of isopentenyldiphosphate can be achieved by two different pathways. Higher animals and some other organisms exclusively use the well-known mevalonate pathway. In contrast, the alternative pathway, the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway is unique to a variety of microorganism including such important pathogens as the malaria parasite *Plasmodium falciparum* and *Mycobacterium tuberculosis*, the causative agent of tuberculosis. Therefore, enzymes of the DOXP pathway provide attractive targets for the development of novel antimicrobial agents. One important step of the DOXP pathway is the reductive isomerisation of DOXP to 2-*C*-methyl-D-erythriol 4-phosphate, catalyzed by DOXP-reductoisomerase (Dxr) [1]. Fosmidomycin and its derivative FR900098 are well-known inhibitors of Dxr [2]. The efficacy of fosmidomycin for the treatment of acute uncomplicated malaria was recently proven in several clinical trials [3], in addition to some earlier studies on the treatment of urinary tract infections. However, relatively high doses of fosmidomycin were required to achieve the desired results. This seems in part to be due to the highly polar nature of this molecule.

Therefore, we addressed the question whether it might be possible to obtain more lipophilic Dxr inhibitors by replacing the formyl or acetyl residue of fosmidomycin or FR900098, respectively, by more spacious residues [4].

Chemistry

Synthesis of the target compounds **7a – j** started with the substitution of the bromine of the acetal **1** by dibenzylphosphite **2** to yield the intermediate **3** (Scheme 1). Acidic hydrolysis of the acetalic substructure of intermediate **3** yielded the free aldehyde **4** which underwent a reductive amination with *O*-benzylhydroxylamine to obtain the protected hydroxylamine **5**. This was acylated by appro-

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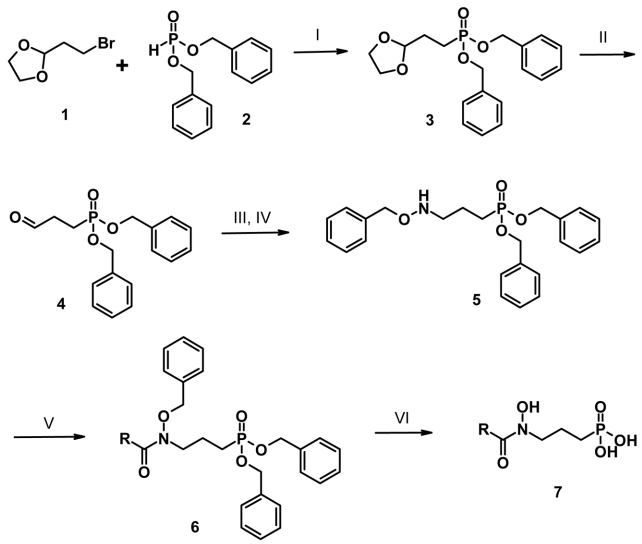


(I) NaH, DMF; (II) HCl/H_2O; (III) *O*-benzylhydroxylamine; (IV) $Na[B(CN)H_3]$, HCl, MeOH; (V) R-CO-Cl (glutaric anhydride/pyridine in case of **6d**); (VI) H_2, Pd/C, MeOH.

Scheme 1. Synthesis route of compounds**1**–**7**.

priate acyl chlorides to yield the fully benzyl protected intermediate **6**. The yields of the acylation were different and in most cases not satisfying. The preparation was not optimized because it was our main intention to get the compounds for biological testing. The final step in the synthesis was the hydrogenolytic removal of the benzyl protective groups providing the target compounds **7**. Under these conditions, the keto groups of **7b** and **7c** are stable.

DOXP reductoisomerase inhibition assay

Recombinant Dxr of *E. coli* was prepared as described previously [5]. In brief, the *dxr* gene was PCR-amplified from genomic DNA and inserted into the pQE9 expression vector (Qiagen, Hilden, Germany). The resulting protein with an amino-terminal His_6-tag was purified by immobilized metal affinity chromatography on Talon superflow resin (Clontech, Heidelberg, Germany) followed by anion exchange chromatography on source 15Q (GE Healthcare, Munich, Germany). Final purification was achieved by gel permeation chromatography using an XK 16/60 Superdex 75 column (GE Healthcare). The protein was concentrated to 15 mg/mL by ultrafiltration and stored in aliquots at −20°C until use. The assay was performed in a reaction mixture containing 100 mM Tris HCl (pH 7.5), 0.2% bovine serum albumin, 1 mM $MnCl_2$, 1 mM DOXP, 0.3 mM NADPH, and 1 μg/mL recombinant DOXP reductoisomerase of *E. coli* in a final volume of 0.25 mL. The mixture without substrate was pre-incubated with a serial dilution of the test compounds on a 96-well plate at 30°C for 5 min. The reaction was started by the addition of DOXP. The decrease of absorption was monitored at 340 nm using a SpectraMax 340PC microplate reader (Molecular Devices, Ismaning, Germany).

It is well known that fosmidomycin and its analogues are slow binding inhibitors of DXR. With respect to the slow, tight-binding properties of fosmidomycin, the inhibitors were pre-incubated with the enzyme and NADPH before the reaction was started by addition of DOXP.

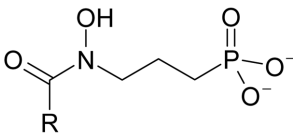
Flexible docking

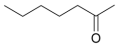
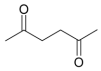
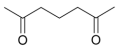
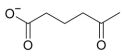
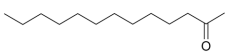
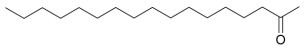
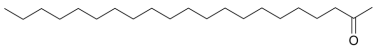
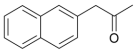
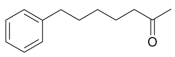
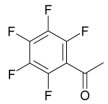
The protein structure was taken from pdb-entry 1ONP [6]. Ligands and solvent molecules were removed, but the metal ion was included as part of the protein. To suggest a reasonable cofactor binding mode NADPH coordinates were transferred from pdb ID: 1JVS [7] after both protein structures were superimposed, based on C_a coordinates. A subsequent minimization of the transferred cofactor with the MAB force field, as implemented in MOLOC [8], revealed no significant movements. For the use within AutoDock 3.0 [9], polar hydrogens were added with the PROTONATE utility from AMBER [10], AMBER united atom force field charges were assigned [11], and solvation parameters were added using the ADDSOL utility from AutoDock 3.0. Ligand structures were built in mol2-format, partial atomic charges were assigned (AM1 Hamiltonian [12]) within the semiempirical package MOPAC 6.0 [13] and all bonds except the one in the C(=O)–NO-fragment, were kept rotatable. Docking runs were performed using the Lamarckian genetic algorithm as implemented in AutoDock 3.0, using an initial population of 50 randomly placed individuals, a maximum number of 1.5×10^6 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism value of 1. Generated ligand docking solutions, mutually differing by rmsd ≤1 Å were clustered together and the lowest docking energy found for one entry of a cluster was used as representative. The results of the highest ranked clusters were chosen to compare structure-activity relationships. This protocol was initially validated by docking fosmidomycin back into its original protein crystal structure (1ONP) [14].

Results and discussion

Aiming at the development of more lipophilic inhibitors, a series of fosmidomycin derivatives with variable acyl residue was synthesized. As a general finding, the introduction of all alternative acyl residues tested led to a significant loss of Dxr inhibitory activity compared to the lead compounds fosmidomycin and FR900098 (Table 1).

Table 1. Structure and activity of compounds **7a – e.**[a)]



Compd.	R-CO-	IC_{50}(μM)
fosmidomycin		0.035
FR900098		0.035
7a		10
7b		20
7c		5.4
7d		>30
7e		9.0
7f		5.6
7g		> 30
7h		15
7i		5.1
7j		1.3

[a)] IC_{50} values were determined for the inhibition of recombinant Dxr from *E. coli*.

As observed via flexible docking of all compounds, the acyl substituents prevent the formation of the desired hydroxamate-metal interaction geometry. This results in a loss of binding affinity. However, it seems possible that some activity could be regained again by an appropriately placed hydrogen-bond acceptor forming a hydrogen bond with the backbone NH of Met214 (Figure 1). It was predicted by crystallographic studies (pdb entry 1ONP [6]) that Met214 is part of a flexible loop which covers the active-site cleft after substrate binding. Indeed, the 5-oxohexanoic acid derivative **7c** displayed a twofold enhanced activity in comparison to the hexanoic acid derivative **7a** (IC_{50} = 5.4 μM vs. IC_{50} = 10 μM). This effect proved to be dependent on the length of the substitution as the docking mode of **7b** shows a less favorable interaction to Met214 as well as on the chemical nature of the modification. The derivative **7d** showed decreased activity although the interaction to Met214 is comparable to **7c**. Considering the desolvation needed to get the ligand inside the protein cavity, it is obvious that binding of the 5-oxohexanoyl derivative **7c** is more favorable than the diacid derivate **7d**.

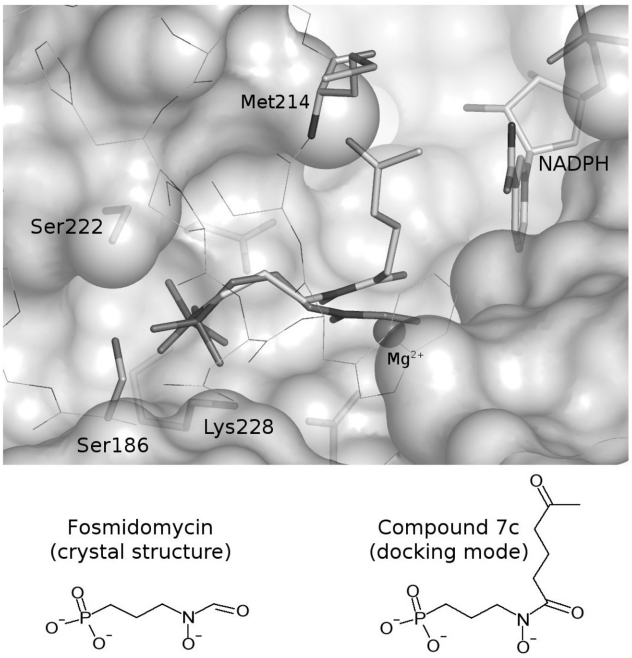


Figure 1. Docking solution of compound **7c** (light grey) showing a possible hydrogen bond between the carbonyl oxygen and the main-chain NH of Met214 (heavy atom distance 2.8 Å) superimposed with the fosmidomycin crystal structure as observed in 1ONP (dark grey). The picture shows the cofactor in sticks at the right side. The sphere represents the metal. The protein is covered by a surface but the flexible loop is shown in thin sticks. Residues interacting to the phosphonate group are labeled.

A comparable increase in activity as with the introduction of the 5-carbonyl group into the acyl chain could also be obtained by the elongation of the acyl chain by 10 methylene units (hexadecanoic acid derivative **7f**: IC_{50} = 5.6 μM), while the dodecanoyl derivative **7e** displayed similar activity as the hexanoyl derivative **7a**. Further elongation of the acyl chain, however, led to a virtually complete loss of activity (**7g**: IC_{50} > 30 μM). Inhibitor **7i** (IC_{50} = 5.1 μM) demonstrated that a part of the alkyl chain can be replaced by a phenyl residue, provided, the whole molecule has a critical length since the shorter naphthylacetyl derivative **7h** was threefold less active. In contrast, the pentafluoro benzoyl derivative was the best compound in this series with an IC_{50} of 1.3 μM.

In the light of the relatively low activity of the compounds against *E.coli* Dxr no further assays against *P. falci-*

parum Dxr or cultured parasites will be performed. Our results are in accordance to other work published in which no improvement on inhibitory activity could be achieved by modifying the acyl residue [14, 15].

In summary, several Dxr inhibitors active in the micromolar range were obtained by replacing the formyl group of fosmidomycin by more lipophilic and spacious acyl residues. Introduction of a carbonyl group into the acyl residue resulted in a slight increase in activity in comparison to the compound lacking this moiety, presumably by forming a hydrogen bond to a main-chain NH of a flexible loop of the target enzyme located over the active site. However, with respect to the reduced activity compared to the parent compound alternative modifications of the fosmidomycin lead structure need to be investigated in order to develop new inhibitors with improved therapeutic potential.

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Experimental

^{1}H- and ^{13}C-NMR (COM: Complete decoupling, DEPT: Distortionless Enhancemant by Polarisation Transfer) spectra were recorded on a Jeol Eclipse 400 and a Jeol Eclipse 500 spectrometer (JEOL, Tokyo, Japan). Mass spectra were obtained with a PE Biosystems API 2000 (Applied Biosystems, Foster City, CA, USA) or with a Jeol MStation JMS 700 (in case of FAB spectra). IR spectra were recorded on a JASCO FT/IR – 410 Fourier Transform Infrared Spectrometer (JASCO Germany, Gross-Umstadt, Germany). Microanalyses were obtained from an elementar vario el. Melting points were obtained with a Reichert Austria microscope (C. Reichert, Vienna, Austria) and are uncorrected. Liquid chromatography was carried out using silica gel 60 from ICM Silitech.

(2-[1,3]-Dioxolan-2-yl-ethyl)phosphonic acid dibenzyl ester 3

Sodium hydride (528 mg; 22 mmol) was suspended in DMF under argon atmosphere and the mixture was cooled with an ice bath. Then, 4.4 mL (20 mmol) dibenzylphosphite were added dropwise. After 1 h, 3.0 mL (25 mmol) 2-(2-bromoethyl)-1,3-dioxolan were added with further cooling. The mixture was stirred overnight at room temperature. The mixture was diluted with dichloromethane and washed with water and brine, and dried (Na_2SO_4). After evaporation of the solvent, the residue was purified by column chromatography eluting with dichloromethane methanol 98 : 2. Yield 90%. IR (film), cm^{-1}: 3090, 3064, 3033, 2953, 2887. ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 1.85–1.96 (m, 4 H, 2 × CH_2); 3.81–3.93 (m, 4 H, 2 × O-CH_2); 4.88–4.90 (m, 1 H, O-CH); 4.95–5.07 (m, 4 H, 2 × P-O-CH_2); 7.29–7.37 (m, 10 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 125 MHz, COM), δ (ppm): 19.93 (d, $^1J_{C,P}$ = 144 Hz, P-CH_2); 26.78 (d, $^2J_{C,P}$ = 3.9 Hz, P-CH_2–*CH_2*); 65.05 (2 × O-CH_2); 67.11 (d, $^2J_{C,P}$ = 6.6 Hz, 2 × P-O-CH_2); 103.20 (d, $^3J_{C,P}$ = 19.1 Hz, O-CH); 127.84, 128.32, 128.55 (10 Ar-C); 136.41 (d, $^3J_{C,P}$ = 5.6 Hz, 2 q Ar-C). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 33.81. CI-MS, m/z: 363 ([M+1]$^+$).

(3-Oxopropyl)phosphonic acid dibenzyl ester 4

An amount of 5.5 g (15 mmol) of (2-[1,3]-dioxolan-2-yl-ethyl)phosphonic acid dibenzyl ester **3** was dissolved in a few mL acetone and 30 mL 2 N HCl were added. After stirring at room temperature for three days, the solution was extracted three times with dichloromethane. The organic extracts were combined, dried (Na_2SO_4), and then the solvent was removed by rotary evaporation. Yield 71%. IR (film), cm^{-1}: 3090, 3065, 3033, 2953, 2894, 2831, 2732, 1724 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 2.01–2.07 (m, 2 H, CH_2); 2.66–2.72 (m, 2 H, CH_2); 4.94–5.08 (m, 4 H, 2 × P-O-CH_2); 7.32–7.38 (m, 10 H, Ar-H); 9.69 (s, 1 H, H–C=O). ^{13}C-NMR ($CDCl_3$, 125 MHz, COM), δ (ppm): 18.47 (d, $^1J_{C,P}$ = 144 Hz, P-CH_2); 36.80 (d, $^2J_{C,P}$ = 3.9 Hz, P-CH_2-*CH_2*); 67.51 (d, $^2J_{C,P}$ = 5.6 Hz, 2 × P-O-CH_2); 128.10, 128.65, 128.75 (10 Ar-C); 136.24 (d, $^3J_{C,P}$ = 5.8 Hz, 2 q Ar-C), 199.03 (d, $^3J_{C,P}$ = 16.5 Hz (C=O). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 32.58. CI-MS, m/z: 319 ([M+1]$^+$).

3-[(Benzyloxyamino)propyl]phosphonic acid dibenzyl ester 5

To a stirred solution of 3.2 g (10 mmol) **4** in 10 mL methanol was added dropwise a solution of 1.6 g (10 mmol) *O*-benzylhydroxylammonium chloride and 1 g (10 mmol) triethylamine in 10 mL methanol. The reaction mixture was refluxed for 1 h, cooled to room temperature, and 20 mL methanol were added. After addition of 1.9 g (30 mmol) sodium cyanoborohydride, 20 mL conc. HCl were added dropwise and the mixture was stirred overnight. Half of the methanol was removed by rotary evaporation. The remaining residue was treated with ice-water, adjusted to pH 10 with aqueous KOH solution and extracted with dichloromethane. The combined organic layers were dried over Na_2SO_4 and concentrated. The remaining oil was purified by column chromatography with ethylacetate : methanol 95 : 5 as eluent. Yield 71%. IR (film), cm^{-1}: 3247 (NH), 3088, 3963, 3031. ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 1.77–1.85 (m, 4 H, 2 × CH_2); 2.91 (m, 2 H, N-CH_2); 4.64 (s, 2 H, N-O-CH_2); 4.94–5.07 (m, 4 H, 2 × P-O-CH_2); 5.52 (bs, 1 H, NH); 7.27–7.40 (m, 15 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 100 MHz, COM), δ (ppm): 20.50 (d, $^2J_{C,P}$ = 4.6 Hz, P-CH_2-*CH_2*); 23.56 (d, $^1J_{C,P}$ = 141.4 Hz, P-CH_2); 52.01 (d, $^3J_{C,P}$ = 16.9 Hz, N-CH_2); 67.07 (d, $^2J_{C,P}$ = 6.5 Hz, 2 P-O-CH_2); 76.36 (N-O-CH_2); 127.93, 127.90, 128.38, 128.48 128.58 (15 Ar-C); 136.42 (d, $^3J_{C,P}$ = 6.1 Hz, 2 P-O-CH_2-*C*); 137.80 (1 q Ar-C). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 34.00. CI-MS, m/z (%): 426 (46, [M+H]$^+$).

General procedure for the preparation of benzyl protected derivatives 6

Method A (Synthesis of acid chlorides):

The appropriate carboxylic acid was dissolved or suspended in a small amount of dichloromethane. After addition of oxalyl chloride (0.2 mL per mmol acid) and DMF (two drops per mmol acid) the mixture was stirred at room temperature for 2 h. Volatiles were evaporated *in vacuo* and the resulting residue was used without further purification.

Method B:

1 mmol 3-[(Phenylmethoxyamino)propyl]phosphonic acid dibenzyl ester **5** and 1.1 mmol triethylamine were dissolved in

dichloromethane. The appropriate acid chloride (1.1 mmol) was added dropwise and the reaction mixture was stirred overnight. The solution was washed with aqueous $NaHCO_3$ and water and was dried (Na_2SO_4). The solvent was removed and the residue was purified by column chromatography on silica gel, eluting with dichloromethane methanol mixtures.

Method C:

1 mmol 3-[(Phenylmethoxyamino)propyl]phosphonic acid dibenzyl ester **5** and 2 mmol of the appropriate acid chloride were dissolved in toluol and refluxed for 2 h. The solution was washed with aqueous $NaHCO_3$ and water and was dried (Na_2SO_4). The solvent was removed and the residue was purified by column chromatography on silica gel, eluting with dichloromethane methanol mixtures.

*{3-[Hexanoyl(benzyloxy)amino]propyl}phosphonic acid dibenzyl ester **6a***

Prepared according to general method B from commercially available hexanoylchloride. Yield 18%. IR (film), cm^{-1}: 1661 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), d (ppm): 0.88 (t, ^3J = 7 Hz, 3 H, CH_3); 1.22 – 1.32 (m, 4 H, 2 × CH_2); 1.54 – 1.61 (m, 2 H, CH_2); 1.71-1.94 (m, 4 H, 2 × CH_2); 2.35 (t, ^3J = 7.5 Hz, 2 H, CH_2); 3.67-3.62 (m, 2 H, N-CH_2); 4.75 (s, 2 H, N-O-CH_2); 4.92 – 5.07 (m, 4 H, 2 × P-O-CH_2); 7.30 – 7.37 (m, 15 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 125 MHz, COM), δ (ppm): 13.93 (CH_3); 20.13 (P-CH_2-*CH_2*); 22.40 (CH_2); 23.36 (d, $^1J_{C,P}$ = 143 Hz, P-CH_2); 24.23 (CH_2); 31.54 (CH_2); 32.28 (CH_2); 67.14 (d, $^2J_{C,P}$ = 6.7 Hz, 2 P-O-CH_2); 76.34 (N-O-CH_2); 127.89, 128.36, 128.38, 128.47, 128.55, 128.57, 128.67, 128.90, 129.09 (15 Ar-C); 136.27, 136.32, 136.35 (3 q Ar-C); 172.01 (N-C=O). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 33.17. HRFAB-MS *m/z* 524.2564 $[M+H]^+$ (calcd. 524.2566 for $C_{30}H_{38}NO_5PH$).

*{3-[(4-Oxopentanoyl)-(benzyloxy)amino]propyl}-phosphonic acid dibenzyl ester **6b***

Prepared acording to the general methods A and B from levulinic acid. Yield 12%. IR (film), cm^{-1}: 1716 (C=O), 1657 (N-C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 1.71 – 1.78 (m, 2 H, CH_2); 1.87 – 1.97 (m, 2 H, CH_2); 2.17 (s, 3 H, CH_3); 2.66 – 2.71 (m, 4 H, C=O-CH_2-CH_2); 3.62 – 3.66 (m, 2 H, N-CH_2); 4.82 (s, 2 H, N-O-CH_2); 4.92-5.06 (m, 4 H, 2 × P-O-CH_2); 7.30 – 7.35 (m, 15 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 100 MHz, COM, DEPT), δ (ppm): 20.13 (d, $^2J_{C,P}$ = 4.5 Hz, P-CH_2-*CH_2*); 23.25 (d, $^1J_{C,P}$ = 141 Hz, P-CH_2); 26.29 (N-C=O-*CH_2*); 29.89 (CH_3); 37.43 (H_3C-C=O-*CH_2*); 45.58 (N-CH_2); 67.12 (d, $^2J_{C,P}$ = 6.7 Hz, 2 × P-O-CH_2); 76.38 (N-O-CH_2); 127.86, 128.30, 128.51, 128.63, 128.84, 129.13 (15 Ar-C); 134.39 (1 q Ar-C); 136.32 (d, $^3J_{C,P}$ = 5.6 Hz, 2 P-O-CH_2-*C*); 173.57 (N-C=O); 207.26 (H_3C-*C*=*O*). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 33.16. HRFAB-MS *m/z* 524.2199 $[M+H]^+$ (calcd. 524.2202 for $C_{29}H_{34}NO_6PH$).

*{3-[(5-Oxohexanoyl)-(benzyloxy)amino]propyl}-phosphonic acid dibenzyl ester **6c***

Prepared acording to the general methods A and B from 4-acetyl butyric acid. Yield 5% [16]. IR (film), cm^{-1}: 1713 (C=O), 1658 (N-C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 1.70 – 1.93 (m, 6 H, 3 × CH_2); 2.08 (s, 3 H, CH_3); 2.37 (t, ^3J = 7.0 Hz, 2 H, CH_2); 2.43 (t, ^3J = 7.1 Hz, 2 H, CH_2); 3.64 (t, ^3J = 6.4 Hz, 2 H, N-CH_2); 4.73 (s, 2 H, N-O-CH_2); 4.92-5.06 (m, 4 H, 2 × P-O-CH_2); 7.30 – 7.36 (m, 15 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 100 MHz, COM, DEPT), δ (ppm): 16.42 (d, $^2J_{C,P}$ = 4.1 Hz, P-CH_2-*CH_2*); 18.87 (H_3C-C=O-CH_2-*CH_2*); 23.25 (d, $^1J_{C,P}$ = 141 Hz, P-CH_2); 26.04 (N-C=O-*CH_2*); 29.90 (CH_3); 42.39 (H_3C-C=O-*CH_2*); 46.23 (N-CH_2); 61.52 (d, $^2J_{C,P}$ = 6.7 Hz, 2 × P-O-CH_2); 76.63 (N-O-CH_2); 127.91, 128.22, 128.48, 128.54, 128.56, 129.48 (15 Ar-C); 138.00 (d, $^3J_{C,P}$ = 5.4 Hz, 2 x P-O-CH_2-*C*); 139.95 (1 q Ar-C); 173.59 (N-C=O); 207.82 (H_3C-*C*=*O*). ^{31}P-NMR ($CDCl_3$, 161 MHz), δ (ppm): 33.96. HRFAB-MS *m/z* 538.2352 $[M+H]^+$ (calcd. 538.2359 for $C_{30}H_{36}NO_6PH$).

*{3-[Glutaryl(benzyloxy)amino]propyl}phosphonic acid dibenzyl ester **6d***

1 mmol 3-[(Phenylmethoxyamino)propyl]phosphonic acid dibenzyl ester **5** and 1.5 mmol glutaric anhydride were dissolved in pyridine and heated at 80°C under argon atmosphere for 2 h. The pyridine was removed under vacuum. The residue was dissolved in ethylacetate/water, the pH was adjusted to 1 – 2 with 2 N HCl and the solution was extracted with ethyl acetate. The combined organic layers were dried (Na_2SO_4), the solvent was removed, and the remaining residue was purified by column chromatography on silica gel, eluting with dichloromethane methanol 95 : 5. Yield 63%. IR (film), cm^{-1}: 3064 (OH), 1726 (C=O), 1659 (N-C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 1.73 – 1.80 (m, 2 H, CH_2); 1.85 – 1.93 (m, 4 H, 2 × CH_2); 2.35 (t, ^3J = 7.1 Hz, 2 H, CH_2); 2.44 (t, ^3J = 6.9 Hz, 2 H, CH_2); 3.64 (m, 2 H, N-CH_2); 4.73 (s, 2 H, N-O-CH_2); 4.92 – 5.05 (m, 4 H, 2 × P-O-CH_2); 7.28 – 7.36 (m, 15 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 125 MHz, COM), δ (ppm): 19.58 (CH_2); 20.13 (CH_2); 23.28 (d, $^1J_{C,P}$ = 141 Hz, P-CH_2); 31.15 (CH_2); 33.18 (CH_2); 45.68 (N-CH_2); 67.33 (d, $^2J_{C,P}$ = 6.7 Hz, 2 × P-O-CH_2); 76.37 N-O-CH_2); 127.94, 128.39, 128.55, 128.68, 128.95, 129.19 (15 Ar-C); 134.20 (1 q Ar-C); 136.18 (d, $^3J_{C,P}$ = 5.6 Hz, 2 × P-O-CH_2-*C*); 174.22 (N-C=O); 177.06 (COOH). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 33.16. HRFAB-MS *m/z* 540.2136 $[M+H]^+$ (calcd. 540.2151 for $C_{29}H_{34}NO_7PH$).

*{3-[Dodecanoyl(benzyloxy)amino]propyl}phosphonic acid dibenzyl ester **6e***

Prepared according to general method B from commercially available lauroyl chloride. Yield 30%. IR (film), cm^{-1}: 1662 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 0.88 (t, ^3J = 7.0 Hz, 3 H, CH_3); 1.25 (m, 16 H, 8 × CH_2); 1.56 (t, ^3J = 7.4 Hz, 2 H, CH_2); 1.72 – 1.78 (m, 2 H, CH_2); 1.87-1.96 (m, 2 H, CH_2); 2.35 (t, ^3J = 7.4 Hz, 2 H, CH_2); 3.65 (m, 2 H, N-CH_2); 4.74 (s, 2 H, N-O-CH_2); 4.92 – 5.05 (m, 4 H, 2 × P-O-CH_2); 7.30 – 7.37 (m, 15 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 125 MHz, COM), δ (ppm): 14.07 (CH_3); 20.20 (d, $^2J_{C,P}$ = 5.6 Hz, P-CH_2-*CH_2*); 22.64 (*CH_2*-CH_3); 23.37 (d, $^1J_{C,P}$ = 142 Hz, P-CH_2); 25.55, 29.29, 29.36, 29.39, 29.46, 29.59, 31.87, 32.35 (9 × CH_2); 45.42 (N-CH_2); 67.12 (d, $^2J_{C,P}$ = 5.8 Hz, 2 × P-O-CH_2); 76.36 (N-O-CH_2); 127.87, 128.33, 128.54, 128.65, 128.87, 129.06 (15 Ar-C); 134.42 (1 q Ar-C); 136.31 (d, $^3J_{C,P}$ = 6.6 Hz, 2 × P-O-CH_2-*C*); 175.16 (C=O). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 33.18. CI-MS *m/z* 608 $[M+H]^+$. Anal. calcd. for $C_{36}H_{50}NO_5P$ (%): C 71.14, H 8.29, N 2.30. Found (%): C 71.06, H 8.40, N 2.31.

*{3-[Hexadecanoyl(benzyloxy)amino]propyl}phosphonic acid dibenzyl ester **6f***

Prepared according to general method B from commercially available palmitoyl chloride. Yield 41%. IR (film), cm^{-1}: 1661 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 0.86 (t, ^3J = 7 Hz, 3 H, CH_3); 1.24 – 1.28 (m, 2 H) (CH_2); 1.53 – 1.55 (m, 24 H, CH_2); 1.72 – 1.77 (m, 2 H, CH_2); 1.89 – 1.90 (m, 2 H, CH_2); 2.32 – 2.35 (m, 2 H, CH_2); 3.60 – 3.64 (m, 2 H, N-CH_2); 4.72 (s, 2 H, N-O-CH_2); 4.92 – 5.06 (m, 4 H, 2 × P-O-CH_2); 7.30 – 7.35 (m, 15 H, Ar-H). ^{13}C-NMR ($CDCl_3$,

125 MHz, COM, DEPT), δ (ppm): 14.05 (CH_3); 20.15 (d, $^2J_{C,P}$ = 4.5 Hz, P-CH_2-*CH_2*); 22.61 (*CH_2*-CH_3); 23.32 (d, $^1J_{C,P}$ = 142 Hz, P-CH_2); 24.52, 29.27, 29.32, 29.35, 29.43, 29.56, 29.58, 29.61, 31.84, 32.30 (13 × CH_2); 45.38 (N-CH_2); 67.09 (d, $^2J_{C,P}$ = 5.9 Hz, 2 × P-O-CH_2); 76.31 (N-O-CH_2); 127.83, 128.29, 128.49, 128.61, 128.82, 129.03 (15 Ar-C); 134.36 (1 q Ar-C); 136.26 (d, $^3J_{C,P}$ = 5.7 Hz, 2 × P-O-CH_2-*C*); 175.06 (C=O). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 33.20. CI-MS *m/z* 664 $[M+H]^+$. Anal. calcd. for $C_{40}H_{58}NO_5P$ (%): C 72.37, H 8.81, N 2.11. Found (%): C 72.63, H 8.72, N 2.06.

{3-[Icosanoyl(benzyloxy)amino]propyl}phosphonic acid dibenzyl ester ***6g***

Prepared according to the general methods A and C from arachidic acid. Yield 8% [16]. IR (KBr), cm^{-1}: 1652 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 0.88 (t, ^3J = 6.8 Hz, 3 H, CH_3); 1.19–1.30 (m, 32 H, 16 × CH_2); 1.54–1.65 (m, 2 H, CH_2); 1.72–1.79 (m, 2 H, CH_2); 1.86–1.95 (m, 2 H, CH_2); 2.28–2.36 (m, 2 H, CH_2); 3.65 (m, 2 H, N-CH_2); 4.74 (s, 2 H, N-O-CH_2); 4.92–5.08 (m, 4 H, 2 × P-O-CH_2); 7.28–7.36 (m,15 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 125 MHz, COM, DEPT), δ (ppm): 14.08 (CH_3); 22.65, 24.55, 24.87, 25.17, 29.32, 29.38, 29.41, 29.43, 29.50, 29.62, 29.68, 31.86, 33.44, 35.33, 37.27 (20 × CH_2); 67.16 (d, $^2J_{C,P}$ = 6.5 Hz, 2 × P-O-CH_2); 76.37 (N-O-CH_2); 127.88, 127.92, 128.35, 128.44, 128.55, 128.58, 128.60, 128.65, 128.78, 128.87, 129.07 (15 Ar-C); 134.32, 136.28, 136.36 (3 q Ar-C); 173.31 (C=O). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 33.18. HRFAB-MS *m/z* 720.4761 $[M+H]^+$ (calcd. 720.4757 for $C_{44}H_{66}NO_5PH$).

{3-[(2-Naphthalen-2-yl-acetyl)(benzyloxy)amino]propyl}-phosphonic acid dibenzyl ester ***6h***

Prepared according to general method C from commercially available 2-(2-naphthyl)acetyl chloride. Yield 42%. IR (film), cm^{-1}: 1659 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 1.69–1.76 (m, 2 H, CH_2); 1.90–1.97 (m, 2 H, CH_2); 3.74 (m, 2 H, N-CH_2); 3.87 (s, 2 H, C=O-CH_2); 4.74 (s, 2 H, N-O-CH_2); 4.87–4.98 (m, 4 H, 2 × P-O-CH_2); 7.29–7.45 (m, 18 H, Ar-H); 7.66 (s, 1 H, Ar-H); 7.74–7.80 (m, 3 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 125 MHz, COM), δ (ppm): 19.95 (d, $^2J_{C,P}$ = 4.2 Hz, P-CH_2-*CH_2*); 22.93 (d, $^1J_{C,P}$ = 142 Hz, P-CH_2); 39.63 (C=O-CH_2); 45.02 (N-O-CH_2); 66.94 (d, $^2J_{C,P}$ = 7.2 Hz, 2 × P-O-CH_2); 76.17 (N-O-CH_2); 125.46, 125.85, 127.29, 127.43, 127.45, 127.72, 127.95, 128.19, 128.38, 128.57, 128.84, 129.03 (23 Ar-C); 132.10, 132.18, 133.25, 134.05, 136.07, 136.13 (7 q Ar-C); 172.64 (C=O). ^{31}P-NMR ($CDCl_3$, 162 MHz), δ (ppm): 33.04. HRFAB-MS *m/z* 594.2411 $[M+H]^+$ (calcd. 594.2409 for $C_{36}H_{36}NO_5PH$).

{3-[(Benzyloxy)(8-phenyl-octanoyl)amino]propyl}-phosphonic acid dibenzyl ester ***6i***

Prepared according to the general methods A and B from 8-phenyl octanoic acid. Yield 30%. IR (film), cm^{-1}: 1661 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 1.29–1.33 (m, 6 H, 3 × CH_2); 1.54–1.60 (m, 4 H, 2 × CH_2); 1.72–1.79 (m, 2 H, CH_2); 1.86–1.93 (m, 2 H, CH_2); 2.31-2.34 (m, 2 H, CH_2); 2.56–2.61 (m, 2 H, CH_2); 3.65 (m, 2 H, N-CH_2); 4.74 (s, 2 H, N-O-CH_2); 4.92–5.05 (m, 4 H, 2 × P-O-CH_2); 7.15–7.36 (m, 15 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 125 MHz, COM), δ (ppm): 20.18 (d, $^2J_{C,P}$ = 5.6 Hz, P-CH_2-*CH_2*); 23.35 (d, $^1J_{C,P}$ = 142 Hz, P-CH_2), 24.52, 28.99, 29.12, 29.22, 29.31, 31.44, 35.90 (7 x CH_2); 67.09 (d, $^2J_{C,P}$ = 6.8 Hz, 2 × P-O-CH_2); 76.36 (N-O-CH_2); 125.52, 127.90, 128.19, 128.37, 128.56, 128.68, 128.91, 129.09 (20 Ar-C); 136.25, 136.30 (4 q Ar-C). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 33.33. HRFAB-MS *m/z* 628.3190 $[M+H]^+$ (calcd. 628.3192 for $C_{38}H_{46}NO_5PH$).

{3-[(Benzyloxy)(pentafluorophenyl)amino]propyl}-phosphonic acid dibenzyl ester ***6j***

Prepared according to general method B from commercially available pentafluorobenzoic acid chloride. Yield 68%. IR (film), cm^{-1}: 1667 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 1.81–1.88, (m, 2 H, CH_2); 2.00–2.07 (m, 2 H, CH_2); 3.84 (t, ^3J = 6.7 Hz, 2 H, N-CH_2); 4.59 (s, 2 H, N-O-CH_2); 4.95–5.08 (m, 4 H, 2 × P-O-CH_2); 7.24–7.35 (m, 15 H, Ar-H). ^{13}C-NMR ($CDCl_3$, 125 MHz, COM, DEPT), δ (ppm): 19.99 (d, $^2J_{C,P}$ = 5.0 Hz, P-CH_2-*CH_2*); 23.15 (d, $^1J_{C,P}$ = 143 Hz, P-CH_2); 45.71 (d, $^3J_{C,P}$ = 17.1 Hz, N-CH_2); 67.26 (d, $^2J_{C,P}$ = 5.8 Hz, 2 × P-O-CH_2); 77.20 (N-O-CH_2); 110.73 (m, N-(C=O)-*C*); 127.93, 128.43, 128.59, 128.66, 129.03, 129.21 (15 Ar-C); 133.28 (N-O-CH_2-*C*); 136.27 (d, $^3J_{C,P}$ = 5.8 Hz, 2 × P-O-CH_2-*C*); 138.18 (m, 1 q CF); 142.12 (m, 2 q CF); 144.18 (m, 2 q CF) 175.06 (C=O). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 32.57. HRFAB-MS *m/z* 620.1624 $[M+H]^+$ (calcd. 620.1626 for $C_{31}H_{27}F_5NO_5PH$).

General procedure for the preparation of phosphonic acids 7

An amount of 1 mmol of the appropriate benzyl-protected compound was dissolved in methanol (10 mL) and hydrogenated at atmospheric pressure with 10% Pd/C (20 mg) as a catalyst. The mixture was filtered, and the solvent was removed by rotary evaporation.

[3-(Hexanoylhydroxyamino)propyl]phosphonic acid ***7a***

Yield 93%. IR (film), cm^{-1}: 3131 (OH), 1615 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 0.86 (t, ^3J = 7 Hz, 3 H, CH_3); 1.22–1.31 (m, 4 H, 2 × CH_2); 1.40–1.53 (m, 4 H, 2 × CH_2); 1.67–1.82 (m, 2 H, CH_2); 2.33 (t, ^3J = 7.4 Hz, 2 H, C=O-CH_2); 3.50 (t, ^3J = 6.9 Hz, 2 H, N-CH_2). ^{13}C-NMR (DMSO-d_6, 100 MHz, COM), δ (ppm): 13.79 (CH_3); 20.49 (P-CH_2-*CH_2*); 21.87 (CH_2); 23.82 (CH_2); 25.10 (d, $^1J_{C,P}$ = 134 Hz, P-CH_2); 30.98 (CH_2); 31.55 (CH_2); 47.82 (d, $^3J_{C,P}$ = 16.1 Hz, N-CH_2); 172.65 (N-C=O). ^{31}P-NMR (DMSO-d_6, 161 MHz), δ (ppm): 26.26. HRFAB-MS *m/z* 254.1182 $[M+H]^+$ (calcd. 254.1180 for $C_9H_{20}NO_5PH$).

{3-[Hydroxy(4-oxopentanoyl)amino]propyl}phosphonic acid ***7b***

Yield 99%. IR (film), cm^{-1}: 1716 (C=O), 1652 (N-C=O). ^{1}H-NMR (DMSO-d_6, 500 MHz), d (ppm): 1.42–1.52 (m, 2 H, P-CH_2); 1.68–1.79 (m, 2 H, P-CH_2-*CH_2*); 2.10 (s, 3 H, CH_3); 2.57–2.64 (m, 2 H, N-C=O-CH_2); 3.41–3.64 (m, 4 H, H_3C-C=O-*CH_2* + N-CH_2). ^{13}C-NMR (Methanol-d_4, 100 MHz, COM, DEPT), δ (ppm): 18.89 (P-CH_2-*CH_2*); 21.65 (N-C=O-*CH_2*); 28.08 (d, $^1J_{C,P}$ = 134 Hz, P-CH_2); 29.85 (CH_3); 38.73 (H_3C-C=O-*CH_2*), 53.39 (d, $^3J_{C,P}$ = 7.7 Hz, N-CH_2). ^{31}P-NMR (Methanol-d_4, 202 MHz), δ (ppm): 27.15. HRFAB-MS *m/z* 254.0807 $[M+H]^+$ (calcd. 254.0794 for $C_8H_{16}NO_6PH$).

{3-[Hydroxy(5-oxohexanoyl)amino]propyl}phosphonic acid **7c**

Yield 90%. IR (film), cm^{-1}: 1713 (C=O), 1633 (N-C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 1.35–1.45 (m, 4 H, 2 × CH_2); 1.61–1.73 (m, 2 H, CH_2); 2.06 (s, 3 H, CH_3); 2.32 (t, ^3J = 7.3 Hz, 2 H, CH_2); 2.44 (t, ^3J = 7.2 Hz, 2 H, CH_2); 3.50 (t, ^3J = 6.5 Hz, 2 H, N-CH_2). ^{13}C-NMR (DMSO-d_6, 125 MHz, COM, DEPT), δ (ppm): 19.16 (d, $^2J_{C,P}$ = 3.8 Hz, P-CH_2-*CH_2*), 21.16 (C=O-CH_2-*CH_2*), 25.69 (d, $^1J_{C,P}$ = 137 Hz, P-CH_2), 30.38 (CH_3), 31.54 (N-C=O-*CH_2*), 42.84 (H_3C-C=O-*CH_2*), 48.69 (d, $^3J_{C,P}$ = 18.2 Hz, N-*CH_2*), 173.22 (N-C=O), 209.00 (H_3C-*C*=*O*). ^{31}P-NMR (DMSO-d_6, 202 MHz), δ (ppm): 27.15. HRFAB-MS *m/z* 268.0945 $[M+H]^+$ (calcd. 268.0950 for $C_9H_{18}NO_6PH$).

{3-[Glutaryl(hydoxyamino)propyl}phosphonic acid ***7d***

Yield 90%. IR (Film), cm^{-1}: 3150 (OH), 1713 (C=O), 1616 (N-C=O). ^{1}H-NMR (DMSO-d_6, 500 MHz), δ (ppm): 1.43-1.50 (m, 2 H, CH_2); 1.66 – 1.74 (m, 4 H, 2 × CH_2); 2.23 (t, ^3J = 7.3 Hz, 2 H, CH_2); 2.36 – 2.39 (m, 2 H, CH_2); 3.51 (t, ^3J = 6.7 Hz, 2 H, N-CH_2). ^{13}C-NMR (DMSO-d_6, 125 MHz, COM), δ (ppm): 19.58 (CH_2); 20.38 (CH_2); 21.24 (d, $^1J_{C,P}$ = 141 Hz, P-CH_2); 30.77 (CH_2); 32.98 (CH_2); 53.71 (N-CH_2); 170.78 (N-C=O); 174.13 (COOH) ^{31}P-NMR (DMSO-d_6, 202 MHz), δ (ppm): 26.72. HRFAB-MS *m/z* 268.0588 $[M-H]^+$ (calcd. 268.0590 for $C_8H_{15}NO_7P$).

{3-[Dodecanoyl(hydoxyamino)propyl}phosphonic acid ***7e***

Yield 97%. IR (KBr), cm^{-1}: 3127 (OH), 1589 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 0.86 (t, ^3J = 6.8 Hz, 3 H, CH_3); 1.24 (s, 16 H, 8 × CH_2); 1.42 – 1.50 (m, 4 H, 2 × CH_2); 1.68 – 1.75 (m, 2 H, CH_2); 2.32 (t, ^3J = 7.5 Hz, 2 H, CH_2); 3.51 (t, ^3J = 6.9 Hz, 2 H, N-CH_2). ^{13}C-NMR (DMSO-d_6, 125 MHz, COM, DEPT), δ (ppm): 13.87 (CH_3); 22.00, 28.62, 28.76, 28.81, 28.86, 28.95, 31.20, 31.58 (17 × CH_2); 173.57 (C=O). ^{31}P-NMR (DMSO-d_6, 161 MHz), δ (ppm): 26.26. HRFAB-MS *m/z* 338.2097 $[M+H]^+$ (calcd. 338.2096 for $C_{15}H_{32}NO_5PH$).

[3-Hexadecanoyl(hydroxyamino)propyl]phosphonic acid ***7f***

Yield 99%. IR (film), cm^{-1}: 3065 (OH), 1661 (C=O). ^{1}H-NMR (DMSO-d_6, 500 MHz), δ (ppm): 0.85 (t, ^3J = 6.8 Hz, 3 H, CH_3); 1.23 (s, 24 H, 12 × CH_2); 1.44 – 1.50 (m, 4 H, 2 × CH_2); 1.68 – 1.73 (m, 2 H, CH_2); 2.32 (t, ^3J = 7.0 Hz, 2 H, CH_2); 3.51 (t, ^3J = 6.7 Hz, 2 H, N-CH_2). ^{13}C-NMR (DMSO-d_6, 125 MHz, COM, DEPT), δ (ppm): 13.82 (CH_3); 20.28, 20.34, 21.98, 24.12, 24.29, 25.36, 28.58, 28.75, 28.79, 28.85, 28.89, 28.94, 31.18, 31.57 (16 × CH_2); 45.58 (d, ^3J = 14.4 Hz, N-CH_2); 172.73 (C=O). ^{31}P-NMR ($CDCl_3$, 202 MHz), δ (ppm): 27.35. HRFAB-MS *m/z* 394.2697 $[M + H]^+$ (calcd. 394.2722 for $C_{19}H_{40}NO_5PH$).

{3-[Hydroxy(icosanoyl)amino]propyl}phosphonic acid ***7g***

Yield 99%. IR (KBr), cm^{-1}: 1652 (C=O). ^{1}H-NMR (DMSO-d_6, 500 MHz), δ (ppm): 0.85 (t, ^3J = 7.0 Hz, 3 H, CH_3); 1.18 – 1.27 (m, 36 H, 18 × CH_2); 1.47 – 1.54 (m, 2 H, CH_2); 1.60 – 1.67 (m, 2 H, CH_2); 1.73 – 1.85 (m, 2 H, CH_2); 2.63 – 2.64 (m, 2 H, CH_2); 3.57 (m, 2 H, N-CH_2). ^{13}C-NMR (DMSO-d_6, 125 MHz, COM), d (ppm): 12.29 (CH_3); 17.66, 21.30, 25.53, 25.86, 28.86, 28.88, 28.90, 28.95, 30.77, 32.37 (16 × CH_2); 174.80 (C=O). ^{31}P-NMR (DMSO-d_6, 202 MHz), δ (ppm): 26.85.

{3-[Hydroxy(2-naphthalen-2-yl-acetyl)amino]propyl}-phosphonic acid ***7h***

Yield 62%, Mp. 161°C. IR (KBr), cm^{-1}: 3049 (OH), 1591 (C=O). ^{1}H-NMR (DMSO-d_6, 500 Hz), δ (ppm): 1.48 – 1.55 (m, 2 H, CH_2); 1.71 – 1.79 (m, 2 H, CH_2); 3.57 – 3.60 (m, 2 H, N-CH_2); 3.89 (s, 2 H, C=O-CH_2); 7.39 – 7.50 (m, 3 H, Ar-H); 7.72 (s, 1 H, Ar-H); 7.83 – 7.88 (m, 3 H, Ar-H). ^{13}C-NMR (DMSO-d_6, 125 MHz, COM, DEPT), δ (ppm): 22.70 (d, $^2J_{C,P}$ = 6.7 Hz, P-CH_2-*CH_2*); 24.91 (d, $^1J_{C,P}$ = 138 Hz, P-CH_2); 38.56 (C=O-CH_2); 48.11 (d, $^3J_{C,P}$ = 19.3 Hz, N-CH_2); 125.34, 125.86, 127.26, 127.32, 127.51, 128.00 (7 Ar-C); 131.64, 132.89, 133.60 (3 q Ar-C); 170.60 (C=O). ^{31}P-NMR (DMSO-d_6, 202 MHz), δ (ppm): 26.97. HRFAB-MS *m/z* 324.0966 $[M+H]^+$ (calcd. 324.1000 for $C_{15}H_{19}NO_5PH$).

{3-[Hydroxy(8-phenyl-octanoyl)amino]propyl}-phosphonic acid ***7i***

Yield 66%, Mp. 102°C. IR (KBr), cm^{-1}: 3118 (OH), 1710 (C=O). ^{1}H-NMR (DMSO-d_6, 500 Hz), δ (ppm): 1.24 – 1.28 (m, 6 H, 3 × CH_2); 1.44 – 1.50 (m, 6 H, 3 × CH_2); 1.68 – 1.76 (m, 2 H, CH_2); 2.33 (t, ^3J = 7.3 Hz, 2 H, CH_2); 2.56 (t, ^3J = 7.7 Hz, 2 H, CH_2); 3.51 (t, ^3J = 6.8 Hz, 2 H, N-CH_2); 7.15 – 7.19 (m, 3 H, Ar-H); 7.25 – 7.28 (m, 2 H, Ar-H). ^{13}C-NMR (DMSO-d_6, 125 MHz, COM), δ (ppm): 21.06, 22.29, 24.36, 24.52, 28.47, 28.60, 28.69, 30.89, 31.57, 35.04 (9 x CH_2); 125.45, 128.08, 128.13 (5 Ar-C); 142.22 (q Ar-C). ^{31}P-NMR (DMSO-d_6, 202 MHz), δ (ppm): 26.86. HRFAB-MS *m/z* 358.1801 $[M+H]^+$ (calcd. 358.1783 for $C_{17}H_{28}NO_5PH$).

{3-[Hydroxy(pentafluorophenyl)amino]propyl}-phosphonic acid ***7j***

Yield 96%, Mp. 102°C. IR (KBr), cm^{-1}: 3118 (OH), 1710 (C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 1.53 – 1.60 (m, 2 H, CH_2); 1.81 – 1.89 (m, 2 H, CH_2); 3.75 (t, ^3J = 6.7 Hz, 2 H, N-CH_2). ^{13}C-NMR (DMSO-d_6, 100 MHz, COM), δ (ppm): 20.17 (d, $^2J_{C,P}$ = 3.9 Hz, P-CH_2-*CH_2*); 24.69 (d, $^1J_{C,P}$ = 138 Hz, P-CH_2); 48.50 (d, $^3J_{C,P}$ = 18.4 Hz, N-CH_2); 111.46 (m, N-(C=O)-*C*); 135.62-143.42 (m, 5 q CF); 156.99 (C=O). ^{31}P-NMR (DMSO-d_6, 202 MHz), δ (ppm): 26.86. HRFAB-MS *m/z* 350.0217 $[M+H]^+$ (calcd. 350.0210 for $C_{10}H_{10}F_5NO_5PH$).

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[16] The reason for the low yields of the acylation is unknown. No further investigations of the reaction have been performed.

