Short Communication

Functionalization of Fatty Acid Mimetics for Solid-Phase Coupling and Subsequent Target Identification

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Fatty acid mimetics such as pirinixic acid (PA) derivatives and 2-(phenylthio)alkanoic acid derivatives are drug-like small molecules with an interesting pharmacological profile. Previously, we have characterized PA derivatives (*e.g.*, **1**) as dual agonists of peroxisome proliferator-activated receptors (PPARs) α and γ and as inhibitors of microsomal prostaglandin E_2-synthase-1 (mPGES-1) and 5-lipoxygenase (5-LO). 2-(Phenylthio)alkanoic acids (*e.g.*, **2**) were shown to act as highly active and selective PPARα agonists. Encouraged by these results, we would like to identify other target proteins and, thereby, further explore the pharmacological profile of these molecules. An elegant method to screen for potential interaction partners is the so-called ‘‘protein-fishing’’ approach. Requirement is coupling of a functionalized small molecule to a solid phase which is used for biological experiments. Ideally, the pharmacophore of the small molecule remains intact as far as possible. Here, we describe the successful design and synthesis of functionalized fatty acid mimetics, thus providing an eligible starting point for solid-phase coupling and subsequent ‘‘protein-fishing’’ experiments.

Keywords: Fatty acid mimetics / Functional groups / Pirinixic acid / Protein fishing / Solid-phase coupling

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Introduction

Fatty acid mimetics like pirinixic acid (PA) and derivatives (Fig. 1) display an interesting pharmacophore with multiple biological activities. Previously, we have shown that pirinixic acid derivatives act as activators of a subclass of nuclear receptors *i.e.*, peroxisome proliferator-activated receptors (PPARs) α and γ [1–3], and as inhibitors of distinct enzymes of the arachidonic acid cascade *i.e.*, microsomal prostaglandin E_2-synthase-1 (mPGES-1) and 5-lipoxygenase (5-LO) [4, 5]. PPAR agonists are widely used drugs in the treatment of the metabolic diseases dyslipidemia (PPARα agonists like fenofibrate) and type-2 diabetes mellitus (PPARγ agonists like pioglitazone). Inhibitors of mPGES-1 and 5-LO have shown to exert multiple anti-inflammatory effects, hence, displaying a promising alternative to classical non-steroidal anti-inflammatory drugs (NSAIDs), which are associated with severe side effects such as gastrointestinal toxicity and increased cardiovascular morbidity [6]. Our previous studies include systematic structural variations of the PA scaffold providing detailed information about structure–activity relationships (SAR). As a result, most active compounds for PPAR and mPGES-1/5-LO such as **1** (Fig. 2) contain an *n*-hexyl chain in the α-position and a biphenyl residue coupled to the pyrimidine ring [1]. Notably, the substitution pattern of the biphenyl moiety had only minor impact on the PPAR activation and the dual mPGES-1/5-LO inhibition. PA derivative **1** is a PPARα/γ dual agonist with an EC_{50} value of 0.19 μM for PPARα and 1.5 μM for PPARγ [1]. Furthermore, this compound also shows high inhibitory activities for 5-LO ($IC_{50} = 0.41$ μM) and mPGES-1

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Abbreviations: 5-lipoxygenase (5-LO); microsomal prostaglandin E_2-synthase-1 (mPGES-1); peroxisome proliferator-activated receptors (PPARs); pirinixic acid (PA).

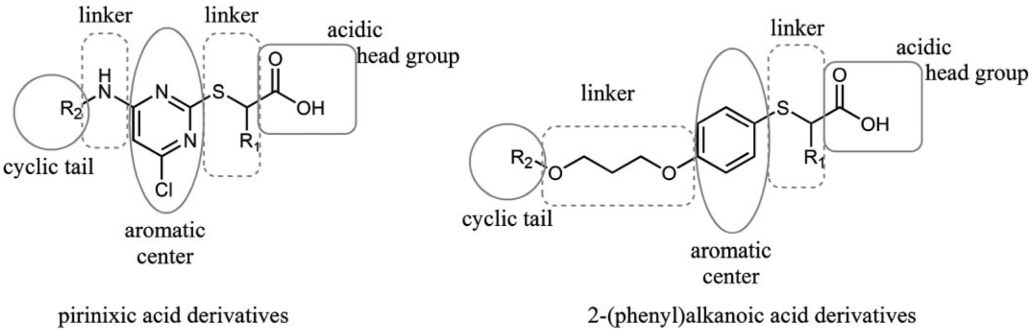


Figure 1. Scaffold of PA derivatives and 2-(phenylthio)alkanoic acid derivatives [1, 3].

($IC_{50} = 1.7$ μM). Based on these findings, we have selected the robust scaffold of **1** for the purpose of this study in order to ensure high pharmacological activity.

Structural optimization efforts of the PA lead structure yielded a novel class of 2-(phenylthio)hexanoic acid derivatives (Fig. 1) [4]. Compounds based on this novel scaffold are selective PPARα agonists with nanomolar activity. SAR revealed a high structural tolerance to modifications of the terminal phenyl residue. Encouraged by the promising PPAR activities of this series, we have selected representative **2** for this work (EC_{50} PPARα = 0.056 μM and EC_{50} PPARγ = 3.02 μM) [7].

To further investigate the pharmacological profile of the selected fatty acid mimetics (*i.e.*, compounds **1** and **2**), we were looking for a possibility to identify targets other than the known PPAR, mPGES-1, and 5-LO. An excellent screening method is the so-called ''protein-fishing'' approach, which was applied successfully in many recent research studies [8–10]. In short, the underlying strategy of this method is coupling of a functionalized small molecule to a polymer matrix as solid phase. Structural requirement for successful solid-phase coupling is the introduction of an eligible functional group (*i.e.*, amine, hydroxyl, or carboxylic acid) to the initial pharmacophore. Importantly, the position for this

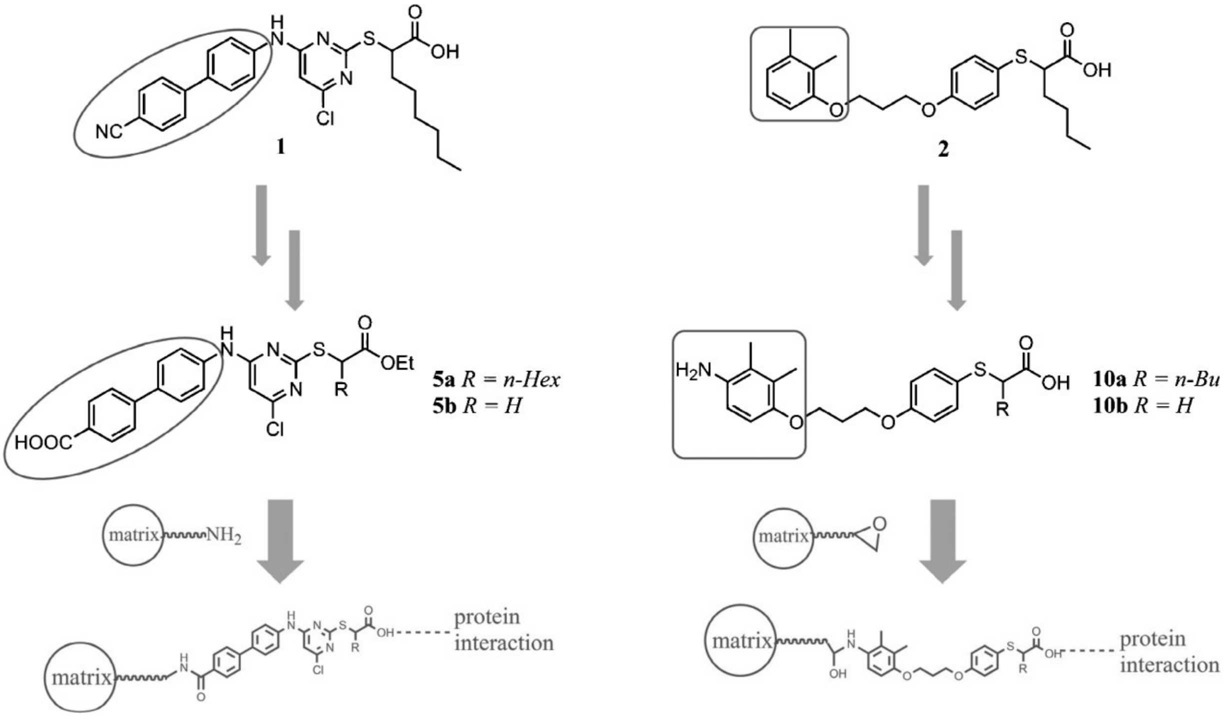


Figure 2. Functionalization of PA derivative **1** and 2-(phenylthio)alkanoic acid derivative **2**.

chemical modification has to be selected carefully to ensure that the structural entity remains untouched as far as possible.

This short communication addresses the issue of pharmacophore functionalization based on the selected fatty acid mimetics **1** and **2**. Subsequent experiments might be the incubation of the matrix-small molecule complex with cell lysates of interest in order to investigate specific ligand–protein interactions.

Results and discussion

As stated in the introduction, we have selected compounds **1** and **2** for functionalization in order to enable solid–phase coupling (Fig. 2).

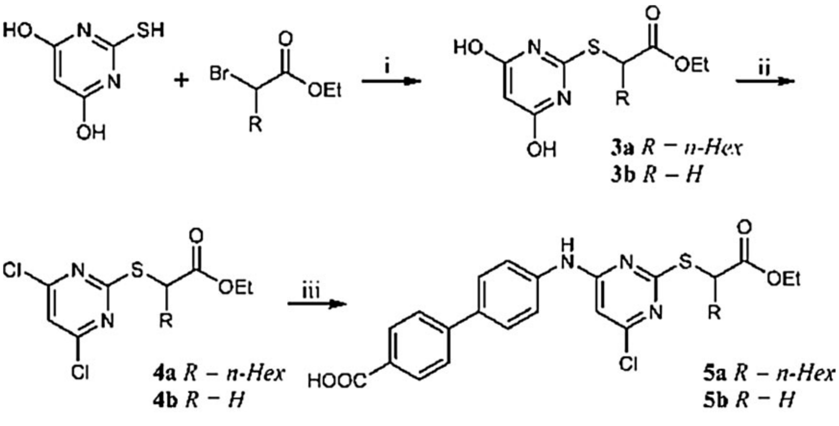
Our previous SAR studies showed clearly the importance of the carboxylic acid head group and the α-alkyl chain for pharmacological activity of **1** and **2**. In contrast, structural modifications of the lipophilic backbone were rather tolerated. Therefore, we have selected the terminal phenyl ring of both **1** and **2** for the introduction of an additional functional group (Fig. 2, **5a** and **10a**). Additionally, we have synthesized the respective α-unsubstituted derivatives (**5b** and **10b**) to provide an additional ''internal standard'' for following biological experiments. Considering the commercial availability of respective educts, these synthetic options have the following results:

1) For functionalization of the 4′-biphenyl position of compound **1**, the replacement of the cyano group by an amine (using biphenyl-4,4′-diamine) or by a carboxylic acid (using 4′-amino-biphenyl-4-carboxylic acid) was possible. To avoid a cross-linking reaction with biphenyl-4,4′-diamine in the amination step (Fig. 2; Scheme 2, step (iii)), we have decided to use 4′-amino-biphenyl-4-carboxylic acid yielding carboxylic acid derivatives **5a** and **5b**. Solid-phase coupling will be done by amidation on a Toyopearl AF-Amino-650 resin [11]. Notably, the ester moiety of **5a** and **5b** has to be cleaved after the coupling reaction in order to ensure regioselectivity.

2) For functionalization of the terminal 4-phenyl position of compound **2**, the introduction of a hydroxyl (using 3,4-dimethylhydroquinone) or an amine (using 4-amino-2,3-xylenol) were possible. Because of the susceptibility to oxidation of 2,3-dimethylhydroquinone impairing its use in a Mitsunobu reaction (Scheme 2, step (iii)), we have decided to introduce an amine by using 4-amino-2,3-xylenol. Finally, only the *boc*-protected 4-amino-2,3-xylenol reacted under Mitsunobu conditions to give **10a** and **10b**. Solid-phase coupling will be done on a Toyopearl AF-Epoxy-650 resin [12].

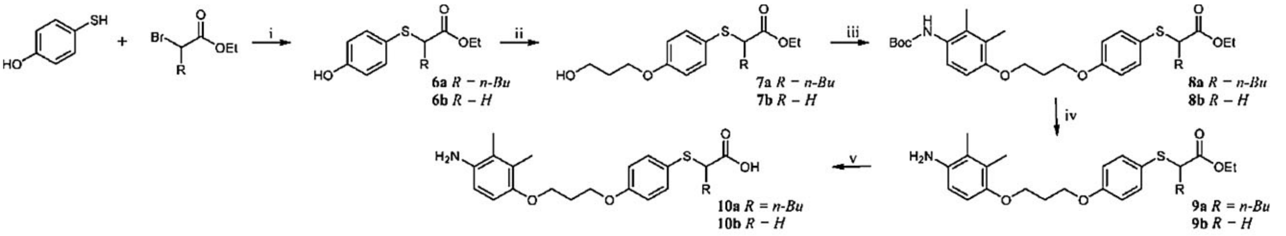
Synthesis of the pirinixic acid derivatives **5a** and **5b** is shown in Scheme 1 and was adapted from d'Atri *et al.* [4, 13]. The starting reaction was a nucleophilic substitution of 2-mercapto-pyrimidine-4,6-diol (2-thiobarbituric acid) with ethyl 2-bromooctanoate **3a** and ethyl 2-bromoacetate **3b**, respectively, in the presence of NEt_3 (Scheme 1; step (i)). Next, chlorination of the hydroxyl groups with phosphorus oxy chloride afforded the dichloropyrimidine derivatives **4a** and **4b** in quantitative yield (Scheme 1; step (ii)). We achieved the final compounds **5a** and **5b** by amination with 4′-amino(1,1′-biphenyl)-4-carboxylic acid in the presence of equimolar amounts of NEt_3 (Scheme 1; step (iii)) [13].

Preparation of the compounds **10a** and **10b** was adapted from the recently published synthesis of **2** as shown in Scheme 2 [7]. First, the esterified acidic head group was arranged by nucleophilic substitution of 4-mercaptophenol with ethyl 2-bromohexanoate **6a** and ethyl 2-bromoacetate **6b**, respectively (Scheme 2; step (i)) [15]. The propylene spacer was introduced by a Williamson-like ether synthesis with 3-bromopropan-1-ol to give **7a** and **7b** (Scheme 2; step (ii)) [16].



Reactions and conditions: (i) NEt_3, DMF, reflux, 2.5 h; (ii) $POCl_3$, *N,N*-diethylaniline, 100°C, 3 h; (iii) 4'-amino(1,1'-biphenyl)-4-carboxylic acid hydrochloride, NEt_3, EtOH, reflux, a) **5a**: 5.25 d, b) **5b**: 1 h.

Scheme 1. Synthesis of **5a** and **5b**.



Reactions and conditions: (i) NEt_3, $CHCl_3$, reflux, 1.5 h; (ii) K_2CO_3, ACN, reflux, 2.5 d; (iii) *tert*-butyl-4-hydroxy-2,3- dimethylcarbamate, PPh_3, DEAD, THF, r. t., 10 h; (iv) TFA, CH_2Cl_2, 0°C → r. t., 45 min.; (v) LiOH, H_2O/THF/MeOH, 0°C → 50°C.

Scheme 2. Synthesis of **10a** and **10b**.

To allow the subsequent Mitsunobu reaction, the amine group of 4-amino-2,3-xylenol has to be protected with di-*tert*-butyl dicarbonate without usage of any catalysts [17]. The *boc*-protected 4-amino-2,3-xylenol reacted appropriately under Mitsunobu conditions to give **8a** and **8b** (Scheme 2; step (iii)) [18]. Next, *boc*-protected amines **8a** and **8b** were deprotected by treatment with trifluoroacetic acid (Scheme 2; step (iv)) [19]. Finally, hydrolysis of the ester moiety with lithium hydroxide in a mixture of THF, water, and methanol yielded the desired products **10a** and **10b** (Scheme 2; step (v)).

In summary, we have presented the successful functionalization of selected fatty acid mimetics. A coupleable carboxylic acid group was introduced to the PA derivative **1** and a coupleable amino group to the 2-(phenylthio)alkanoic acid derivative **2** allowing regioselective solid-phase coupling. We have thus provided the chemical starting point for ''protein-fishing'' experiments, which may give valuable new insights in the pharmacology of these interesting pharmacophores.

Experimental

General

All commercially available chemicals and solvents are of reagent grade and were used without further purification unless specified otherwise. ^{1}H-NMR and ^{13}C-NMR spectra were measured in DMSO-d_6 on a Bruker AM 250, ARX 300, and AVANCE 300 spectrometer (Bruker, Rheinstetten, Germany). Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as internal standard. Mass spectra have been performed by the Institute of Organic Chemistry and Chemical Biology, Goethe University of Frankfurt, on a Fisons Instruments VG Platform II spectrometer measuring in the positive or negative ion mode (ESI–MS system). Merck silica gel 60 (Merck, Darmstadt, Germany) was used for column chromatography.

Chemistry

4′-(6-Chloro-2-(2-ethoxy-2-oxooctan)pyrimidin-4-ylamino)biphenyl-4-carboxylic acid ***5a*** *[14]*

4-Amino(biphenyl)carboxylic acid was prepared by treatment of 0.32 g of 4-amino(biphenyl)carboxylic acid hydrochloride (1.28 mmol, 1 eq) with 0.178 mL of triethylamine (1.28 mmol, 1 eq) in 40 mL EtOH. 4-Amino(biphenyl) carboxylic acid hydrochloride was suspended in ethanol. Triethylamine was added and the mixture was heated to 80°C for 1 h. The reaction mixture was filtered hot and the pure amine was obtained by concentrating the filtrate *in vacuo* (0.161 g, 59%). Next, 0.165 g of **4a** (0.47 mmol, 1 eq) and 0.1 g of 4-amino(biphenyl) carboxylic acid (0.47 mmol, 1 eq) were dissolved in 40 mL EtOH and 0.065 mL triethylamine (0.47 mmol, 1 eq) were added. The reaction mixture was heated to 80°C for 126 h. After the reaction was completed (TLC control), all volatile components were removed *in vacuo*. The purification by column chromatography (*n*-hexane/ethyl acetate, 10:1; *n*-hexane/ethyl acetate, 5:1; *n*-hexane/ethyl acetate, 3:1; ethyl acetate) gave a white solid (0.143 g, 58%). ^{1}H-NMR (300.13 MHz, DMSO-d_6) δ: 0.78 (t, 3H, $J = 6.59$ Hz, CH_3-Hex), 1.14 (t, 3H, $J = 7.14$ Hz, -CH_3), 4.06–4.13 (m, 2H, OCH_2), 4.43 (t, 1H, $J = 7.15$ Hz, SCH), 6.56 (s, 1H, Pyr-5-*H*), 7.64–7.85 (m, 6H, Ph$_1$-2,3,5,6-H, Ph$_2$-3,5-H), 8.00 (d, 2H, $J = 8.4$ Hz, Ph$_2$-2,6-H), 10.13 (s, 1H, NH), 12.95 (s, 1H, COOH). ^{13}C-NMR (62.90 MHz, DMSO-d_6) δ: 13.79 (CH_3-Hex), 13.93 (-CH_3), 21.89 (CH_2-Hex), 26.45 (CH_2-Hex), 28.01 (CH_2-Hex), 30.92 (CH_2-Hex), 31.33 (CH_2-Hex), 46.83 (S*C*H), 60.99 (OCH_2), 101.47 (Pyr-C_5), 120.87 (2C, Ph-C_2 + -C_6), 126.19 (2C, Ph′-C_3 + -C_5), 127.32 (2C, Ph-C_3 + -C_5), 129.32 (Ph-C_1), 129.95 (2C, Ph′-C_2 + -C_6), 133.83 (Ph-C_4), 138.75 (Ph′-C_4), 143.60 (Ph-C_1), 157.42 (Pyr-C_4), 160.42 (Pyr-C_2), 167.13 (Pyr-C_6), 169.63 (COOH), 171.13 (COOH). MS (EI$^-$) *m*/*e*: 526.6 [M − 1].

4′-(6-Chloro-2-(2-ethoxy-2-oxoethylthio)pyrimidin-4-ylamino)biphenyl-4-carboxylic acid ***5b*** *[14]*

0.358 g of **4b** (1.35 mmol, 1 eq) and 0.337 g of 4-amino(biphenyl) carboxylic acid hydrochloride (1.35 mmol, 1 eq) were dissolved in 10 mL ethanol. 0.38 mL triethylamine (2.7 mmol, 2 eq) were added and the reaction mixture was heated to 80°C for 1 h. All volatile components were removed *in vacuo*. The purification by column chromatography (*n*-hexane/ethylacetate, 10:1; *n*-hexane/ethyl acetate, 5:1; *n*-hexane/ethyl acetate, 3:1; ethyl acetate) yielded in a white solid (0.57 g, 95%). ^{1}H-NMR (300.13 MHz, DMSO-d_6) δ: 1.11 (t, 3H, $J = 7.14$ Hz, -CH_3), 4.01 (s, 2H, SCH_2), 4.05 (q, 2H, $J = 7.11$ Hz, OCH_2), 6.55 (s, 1H, Pyr-5-*H*), 7.65–7.83 (m, 6H, Ph$_1$-2,3,5,6-H, Ph$_2$-3,5-H), 8.00 (d, 2H, $J = 8.5$ Hz, Ph$_2$-2,6-H), 10.12 (s, 1H, NH), 12.92 (s, 1H, COOH). ^{13}C-NMR (62.90 MHz, DMSO-d_6) δ: 13.93 (-CH_3), 32.86 (SCH_2), 61.00 (OCH_2), 101.31 (Pyr-C_5), 120.75 (2C, Ph-C_2 + -C_6), 126.19 (2C, Ph′-C_3 + -C_5), 127.34 (2C, Ph-C_3 + -C_5), 129.27 (Ph-C_1), 129.97 (2C, Ph′-C_2 + -C_6), 133.71 (Ph-C_4), 138.80 (Ph′-C_4), 143.62 (Ph-C_1), 157.42 (Pyr-C_4), 160.42 (Pyr-C_2), 167.12 (Pyr-C_6), 168.60 (COOEt), 170.02 (COOH). MS (EI$^+$) *m*/*e*: 442.4 [M + 1].

*Ethyl-2-(4-(3-(4-(tert.-butoxycarbonylamino)-2,3-dimethyl-phenoxy)propoxy)-phenylylthio) hexanoate **8a** [18]*

0.76 g of **7a** (2.32 mmol, 1.1 eq) and 0.5 eq of (4-hydroxy-2,3-dimethyl-phenyl)-carbamic acid *tert*-butyl ester (2.11 mmol, 1 eq) were dissolved in 7 mL absolute THF. 0.61 g of PPh_3 (2.32 mmol, 1.1 eq) dissolved in 3 mL absolute THF were added. Next, 0.4 g DEAD (2.32 mmol, 1 eq) was added dropwise under cooling (ice bath). All steps were carried out under argon atmosphere. After stirring for 7 h at room temperature, the reaction was completed. All volatile components were removed *in vacuo*. The product was purified by column chromatography with *n*-hexane/ethyl acetate, 20:1; *n*-hexane/ethyl acetate, 15:1 and *n*-hexane/ethyl acetate) The product is a clear oil (1.0 g, 79%). ^{1}H-NMR (300.13 MHz, DMSO-d_6) δ: 0.83 (t, 3H, $J = 6.68$ Hz, CH_3-Bu), 1.06 (t, 3H, $J = 7.07$ Hz, -CH_3), 1.19–1.36 (m, 4H, CH_2-Bu), 1.41 (s, 9H, CH_3-*t*-Bu), 1.48–1.77 (m, 2H, CH_2-Bu), 2.02 (s, 3H, Ph′-3-CH_3), 2.06 (s, 3H, Ph′-2-CH_3), 2.16 (t, 2H, $J = 6.17$ Hz, -CH_2-), 3.56 (t, 1H, $J = 7.59$ Hz, SCH), 3.99 (q, 2H, $J = 7.21$ Hz, OCH_2), 4.06 (t, 2H, $J = 6.19$ Hz, Ph′-OCH_2), 4.15 (t, 2H, $J = 6.19$ Hz, Ph-OCH_2), 6.74 (d, 1H, $J = 8.89$ Hz, Ph′-6-H), 6.93 (d, 2H, Ph-3-H + -5-H + 1H, Ph′-5-H), 7.35 (d, 2H, $J = 8.69$ Hz, Ph-2-H + -6-H), 8.37 (bs, 1H, NH). ^{13}C-NMR (75.44 MHz, DMSO-d_6) δ: 11.99 (Ph′-2-CH_3), 13.68 (CH_3-Bu), 13.87 (-CH_3), 14.41 (Ph′-3-CH_3), 21.66 (CH_2-Bu), 28.15 (3C, CH_3-*t*-Bu), 28.64 (2C, -CH_2- + CH_2-Bu), 30.50 (CH_2-Bu), 50.49 (SCH), 60.37 (-OCH_2), 64.60 (2C, Ph-OCH_2 + Ph′-OCH_2), 78.08 ($C(CH_3)_3$-*t*-Bu), 109.03 (Ph′-C_6), 115.09 (2C, Ph-C_3 + -C_5), 122.52 (Ph-C_1), 124.28 (Ph′-C_5), 124.57 (Ph′-C_2), 129.50 (Ph′-C_4), 133.34 (Ph′-C_3), 135.78 (2C, Ph-C_2 + -C_6), 153.82 (Ph′-C_1), 154.16 (COO-*t*-Bu), 158.97 (Ph-C_4), 171.45 (COO). MS (EI$^+$) *m/e*: 546.9 [M + 1].

*Ethyl 2-(4-(3-(4-(tert-butoxycarbonylamino)-2,3-dimethyl-phenoxy)propoxy)-phenylylthio) acetate **8b** [18]*

^{1}H-NMR (250.13 MHz, DMSO-d_6) δ: 1.09 (t, 3H, $J = 7.1$ Hz, -CH_3), 1.41 (s, 9H, CH_3-*t*-Bu), 2.02 (s, 3H, Ph′-3-CH_3), 2.06 (s, 3H, Ph′-2-CH_3), 2.15 (t, 2H, $J = 6.09$ Hz, -CH_2-), 3.66 (s, 2H, SCH_2), 3.97–4.09 (t, 2H, Ph-OCH_2 + q, 2H, OCH_2), 4.13 (t, 2H, $J = 6.10$ Hz, Ph′-OCH_2), 6.74 (d, 1H, $J = 8.75$ Hz, Ph′-6-H), 6.92 (d, 2H, Ph-3-H + -5-H + 1H, Ph′-5-H), 7.34 (d, 2H, $J = 8.73$ Hz, Ph-2-H + -6-H), 8.36 (bs, 1H, NH). ^{13}C-NMR (75.44 MHz, DMSO-d_6) δ: 11.99 (Ph′-2-CH_3), 13.91 (-CH_3), 14.43 (Ph′-3-CH_3), 28.15 (3C, CH_3-*t*-Bu), 28.67 (-CH_2-), 36.96 (SCH_2), 60.66 (-OCH_2), 64.48 (Ph′-OCH_2), 64.56 (Ph-OCH_2), 78.09 ($C(CH_3)_3$-*t*-Bu), 108.98 (Ph′-C_6), 115.20 (2C, Ph-C_3), 124.29 (Ph′-C_5), 124.54 (Ph′-C_2), 124.74 (Ph-C_1), 129.46 (Ph′-C_4), 132.78 (2C, Ph-C_2 + -C_6), 133.35 (Ph′-C_3), 153.81 (Ph′-C_1), 154.17 (COO-*t*-Bu), 158.12 (Ph-C_4), 169.37 (COO). MS (EI$^+$) *m/e*: 490.4 [M + 1].

*Ethyl-2-(4-(3-(4-amino-2,3-dimethylphenoxy)propoxy)-phenylylthio) hexanoate **9a** [19]*

0.47 g of **8a** (0.86 mmol, 1 eq) were dissolved in CH_2Cl_2. The reaction mixture was cooled to 0°C (ice bath) and 1.92 mL TFA (25.9 mmol, 30 eq) were added. After removal of the ice bath and stirring at room temperature for 45 min, the reaction was completed. All volatile components were removed *in vacuo*. The residue was co-evaporated with toluene, then with methanol, and finally dissolved in ethyl acetate. The organic layer was extracted with 2 M HCl, saturated $NaHCO_3$ solution and brine. The remaining organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. The product is an orange oil (0.28 g, 73%). ^{1}H-NMR (300.13 MHz, DMSO-d_6) δ: 0.83 (t, 3H, $J = 6.68$ Hz, CH_3-Bu), 1.07 (t, 3H, $J = 6.99$ Hz, -CH_3), 1.15–1.45 (m, 4H, CH_2-Bu), 1.46–1.79 (m, 2H, CH_2-Bu), 1.94 (s, 3H, Ph′-3-CH_3), 2.02 (s, 3H, Ph′-2-CH_3), 2.05–2.17 (m, 2H, -CH_2-), 3.56 (t, 1H, $J = 7.54$ Hz, SCH), 3.92 (t, 2H, $J = 6.12$ Hz, Ph′-OCH_2), 4.00 (q, 2H, $J = 7.20$ Hz, OCH_2), 4.13 (t, 2H, $J = 6.21$ Hz, Ph-OCH_2), 4.33 (bs, 2H, NH_2), 6.42 (d, 1H, $J = 8.54$ Hz, Ph′-6-H), 6.55 (d, 1H, $J = 8.54$ Hz, Ph′-5-H), 6.93 (d, 2H, $J = 8.72$ Hz, Ph-3-H + -5-H), 7.35 (d, 2H, $J = 8.72$ Hz, Ph-2-H + 6-H). ^{13}C-NMR (75.45 MHz, DMSO-d_6) δ: 12.01 (Ph′-2-CH_3), 13.24 (Ph′-3-CH_3), 13.70 (CH_3-Bu), 13.88 (-CH_3), 21.68 (CH_2-Bu), 28.65 (CH_2-Bu), 28.89 (-CH_2-), 30.48 (CH_2-Bu), 50.45 (SCH), 60.38 (-OCH_2), 64.59 (Ph-OCH_2), 65.36 (Ph′-OCH_2), 111.15 (Ph′-C_6), 112.05 (Ph′-C_5), 115.05 (2C, Ph-C_3 + -C_5), 121.43 (Ph′-C_4), 122.40 (Ph-C_1), 124.91 (Ph′-C_2), 135.82 (2C, Ph-C_2 + -C_6), 140.42 (Ph′-C_3), 148.16 (Ph′-C_1), 159.02 (Ph-C_4), 171.46 (COO). MS (EI$^+$) *m/e*: 446.7 [M + 1].

*Ethyl-2-(4-(3-(4-amino-2,3-dimethylphenoxy)propoxy)-phenylylthio) acetate **9b** [19]*

^{1}H-NMR (300.13 MHz, DMSO-d_6) δ: 1.10 (t, 3H, $J = 7.09$ Hz, -CH_3), 1.94 (s, 3H, Ph′-3-CH_3), 2.02 (s, 3H, Ph′-2-CH_3), 2.04–2.15 (m, 2H, -CH_2-), 3.67 (s, 2H, SCH_2), 3.92 (t, 2H, $J = 6.11$ Hz, Ph′-OCH_2), 4.03 (q, 2H, $J = 7.14$ Hz, OCH_2), 4.12 (t, 2H, $J = 6.22$ Hz, Ph-OCH_2), 4.32 (bs, 2H, NH_2), 6.42 (d, 1H, $J = 8.64$ Hz, Ph′-6-H), 6.54 (d, 1H, $J = 8.64$ Hz, Ph′-5-H), 6.92 (dd, 2H, $J_1 = 2.22$ Hz, $J_2 = 6.78$ Hz, Ph-3-H + -5-H), 7.34 (dd, 2H, $J_1 = 2.22$ Hz, $J_2 = 6.76$ Hz, Ph-2-H + -6-H). ^{13}C-NMR (75.45 MHz, DMSO-d_6) δ: 12.02 (Ph′-2-CH_3), 13.25 (-CH_3), 13.92 (Ph′-3-CH_3), 28.91 (-CH_2-), 36.96 (SCH_2), 60.66 (-OCH_2), 64.57 (Ph-OCH_2), 65.37 (Ph′-OCH_2), 111.14 (Ph′-C_6), 112.05 (Ph′-C_5), 115.19 (2C, Ph-C_3 + -C_5), 121.43 (Ph′-C_4), 124.69 (Ph-C_1), 124.91 (Ph′-C_2), 132.78 (2C, Ph-C_2 + -C_6), 140.42 (Ph′-C_3), 148.17 (Ph′-C_1), 158.16 (Ph-C_4), 169.37 (COO). MS (EI$^+$) *m/e*: 390.3 [M + 1].

*2-(4-(3-(4-Amino-2,3-dimethylphenoxy)propoxy) phenylylthio)-hexanoic acid **10a***

0.22 g of **9a** (0.61 mmol, 1 eq) was dissolved in 12.4 mL THF and 4.1 mL methanol. 72.8 mg of LiOH (3.04 mmol, 5 eq) in 4.1 mL H_2O were added at 0°C. The reaction mixture was stirred at 40 to 50°C for 1.5 h. Subsequently, the solvent was removed *in vacuo* and the remaining residue was dissolved in H_2O. The aqueous phase was neutralized with 2 M HCl and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Finally, the crude product was recrystallized in methanol to give purified **10a** (0.135 g, 53%). ^{1}H-NMR (300.13 MHz, DMSO-d_6) δ: 0.82 (t, 3H, $J = 6.93$ Hz, CH_3-Bu), 1.15–1.45 (m, 4H, CH_2-Bu), 1.45–1.79 (m, 2H, CH_2-Bu), 1.93 (s, 3H, Ph′-3-CH_3), 2.02 (s, 3H, Ph′-2-CH_3), 2.04–2.16 (m, 2H, -CH_2-), 3.48 (t, 1H, $J = 6.26$ Hz, SCH), 3.92 (t, 2H, $J = 6.15$ Hz, Ph′-OCH_2), 4.12 (t, 2H, $J = 6.15$ Hz, Ph-OCH_2), 6.42 (d, 1H, $J = 8.65$ Hz, Ph′-6-H), 6.55 (d, 1H, $J = 8.65$ Hz, Ph′-5-H), 6.92 (d, 2H, $J = 8.63$ Hz, Ph-3-H + -5-H), 7.35 (d, 2H, $J = 8.63$ Hz, Ph-2-H + -6-H). ^{13}C-NMR (75.45 MHz, DMSO-d_6) δ: 12.03 (Ph′-2-CH_3), 13.25 (Ph′-3-CH_3), 13.72 (CH_3-Bu), 21.73 (CH_2-Bu), 28.71 (CH_2-Bu), 28.96 (-CH_2-), 30.88 (CH_2-Bu), 50.97 (SCH), 64.62 (Ph-OCH_2), 65.45 (Ph′-OCH_2), 111.19 (Ph′-C_6), 112.13 (Ph′-C_5), 115.06 (2C, Ph-C_3 + -C_5), 121.50 (Ph′-C_4), 123.38 (Ph-C_1), 124.96 (Ph′-C_2), 135.08 (2C, Ph-C_2 + -C_6), 140.38 (Ph′-C_3), 148.26 (Ph′-C_1), 158.71 (Ph-C_4), 173.00 (COO). MS (EI$^+$) *m/e*: 418.3 [M + 1].

*2-(4-(3-(4-Amino-2,3-dimethylphenoxy)propoxy) phenylylthio)-acetic acid **10b***

^{1}H-NMR (300.13 MHz, DMSO-d_6) δ: 1.94 (s, 3H, Ph′-3-CH_3), 2.02 (s, 3H, Ph′-2-CH_3), 2.06–2.15 (m, 2H, -CH_2-), 3.61 (s, 2H, SCH_2), 3.92 (t, 2H, $J = 5.65$ Hz, Ph′-OCH_2), 4.11 (t, 2H, $J = 5.65$ Hz, Ph-OCH_2), 6.42 (d, 1H, $J = 8.47$ Hz, Ph′-6-*H*), 6.55 (d, 1H, $J = 8.47$ Hz, Ph′-5-*H*), 6.91 (d, 2H, $J = 8.39$ Hz, Ph-3-*H* + -5-*H*), 7.32 (d, 2H, $J = 8.31$ Hz, Ph-2-*H* + -6-*H*). ^{13}C-NMR (75.45 MHz, DMSO-d_6) δ: 12.09 (Ph′-2-CH_3), 13.31 (Ph′-3-CH_3), 28.97 (-CH_2-), 37.19 (SCH_2), 64.59 (Ph-OCH_2), 65.40 (Ph′-OCH_2), 111.14 (Ph′-C_6), 112.14 (Ph′-C_5), 115.22 (2C, Ph-C_3 + -C_5), 121.55 (Ph′-C_4), 124.94 (Ph-C_1), 125.49 (Ph′-C_2), 131.99 (2C, Ph-C_2 + -C_6), 140.38 (Ph′-C_3), 148.24 (Ph′-C_1), 157.86 (Ph-C_4), 170.78 (*C*OO). MS (EI$^+$) *m/e*: 362.1 [M + 1].

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