This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: https://orca.cardiff.ac.uk/id/eprint/113130/

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Foley, David W., Pathak, Ravindra B., Phillips, Theresa R., Wilson, Gayle L., Bailey, Patrick D., Pieri, Myrtani, Senan, Anish and Meredith, David 2018. Thiodipeptides targeting the intestinal oligopeptide transporter as a general approach to improving oral drug delivery. European Journal of Medicinal Chemistry 156, pp. 180-189. 10.1016/j.ejmech.2018.06.064

Publishers page: http://dx.doi.org/10.1016/j.ejmech.2018.06.064

Please note:
Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher’s version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See http://orca.cf.ac.uk/policies.html for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.
Title
Thiodipeptides targeting the intestinal oligopeptide transporter as a general approach to improving oral drug delivery

Authors
David W. Foley, Ravindra B. Pathak, Theresa R. Phillips, Gayle L. Wilson, Patrick D. Bailey, Myrtani Pieri, Anish Senan and David Meredith

Affiliations
EPSAM Research Institute, Faculty of Natural Sciences, Keele University, Keele, Staffordshire, ST5 5BG, U.K.
Department of Biological & Medical Sciences, Faculty of Health & Life Sciences, Oxford Brookes University, Gipsy Lane, Headington, Oxford OX3 0BP, U.K.

Present Addresses:
DWF: Drug Discovery Unit, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK.
MP: Department of Biological Sciences, Molecular Medicine Research Center (MMRC), University Of Cyprus, Kallipoleos 75, 1678 Nicosia, Cyprus.
PDB: Vice Chancellor’s Office, London South Bank University, 103 Borough Road, London, SE1 0AA, UK.

* Corresponding Author: Dr. David Meredith. Address: Department of Biological & Medical Sciences, Faculty of Health & Life Sciences, Oxford Brookes University, Gipsy Lane, Headington, Oxford OX3 0BP, U.K. Email: dmeredith@brookes.ac.uk

Abstract
The broad substrate capacity of the intestinal oligopeptide transporter, PepT1, has made it a key target of research into drug delivery. Whilst the substrate capacity of this transporter is broad, studies have largely been limited to small peptides and peptide-like drugs. Here, we demonstrate for the first time that a diverse range of drugs can be targeted towards transport by PepT1 using a hydrolysis resistant carrier. Eleven prodrugs were synthesised by conjugating modified dipeptides containing a thioamide bond to the approved drugs ibuprofen, gabapentin, propofol, aspirin, acyclovir, nabumetone, atenolol, zanamivir, baclofen and mycophenolate. Except for the aspirin and acyclovir prodrugs, which were unstable in the assay conditions and were not further studied, the prodrugs were tested for affinity and transport by PepT1 expressed in *Xenopus laevis* oocytes: binding affinities ranged from approximately 0.1 to 2 mM. Compounds which showing robust transport in an oocyte trans stimulation assay were then tested for transcellular transport in Caco-2 cell monolayers: all five tested prodrugs showed significant PepT1-mediated transcellular uptake. Finally, the ibuprofen and propofol prodrugs were tested for absorption in rats: following oral dosing the intact prodrugs and free ibuprofen were measured in the plasma. This provides proof-of-concept for the idea of targeting poorly bioavailable drugs towards PepT1 transport as a general means of improving oral permeability.

Keywords
Prodrug; membrane transporter; intestine; PepT1; drug delivery
Introduction

The oral bioavailability of a compound is a crucial factor in its success or failure as a therapeutic agent, particularly given the convenience of this route of administration. There are two main mechanisms of absorption from the GI tract: passive diffusion [1] and carrier mediated transport [2]. The oral bioavailability of poorly absorbed drugs can be improved either by modifying their physicochemical properties to aid passive diffusion and/or by targeting of the compounds towards carrier mediated transport [3-5].

PepT1 is a proton coupled oligopeptide transporter expressed principally in the small intestine and the proximal tubule of the kidney [6]. It has a broad substrate specificity including most di- and tripeptides, β-lactam antibiotics and ACE inhibitors [7].

There are many examples of targeting PepT1 to improve the oral bioavailability of pharmacologically active compounds, usually by modifying them so that they resemble the natural di- or tripeptide substrates [8-13]. We have patented [14] a set of thiodipeptide substrates (such as A and B) that we hope can act as “carriers” for drug transport by PepT1 generally, and have previously published our work on model systems demonstrating that a variety of linkers can be employed [15, 16]. The basic premise is illustrated in Figure 1 in which drugs are conjugated directly or by a linker to our thiodipeptides, converting them into prodrugs that are PepT1 substrates.
In this paper, we apply the results of our previously reported characterisation of the structure-transport relationships for PepT1 [15] to drug delivery challenges and report proof-of-concept studies that validate our thiodipeptide carriers as a general approach for targeting a variety of drugs towards PepT1 mediated transport. We focused on two major areas that we felt could benefit from our thiodipeptide drug delivery technology:

i) **Drugs with GI side effects.** A common class of such drugs are the NSAIDs, as exemplified by aspirin and ibuprofen [17]. Whilst these drugs have high oral bioavailability, they also can cause severe gastric side effects. If a prodrug strategy could be developed so that bioavailability was retained, but active drug was not released close to the GI tract, such side effects might be significantly reduced. Prodrugs 1, 4 and 6-7 of ibuprofen, aspirin and nabumetone respectively (Figure 2) were synthesized to explore this area.

ii) **Drugs with poor oral bioavailability.** This is a major challenge in drug development. A search of ChEMBL [18] identified several marketed drugs with low, highly variable or no oral bioavailability [17]: gabapentin (an anticonvulsant and analgesic); baclofen (a GABA receptor agonist); propofol (chemotherapeutic nausea and intractable migraine); zanamivir (treatment and prophylaxis of influenza) and mycophenolic acid (an immunosuppressant). Prodrugs 2, 3, 5, and 8-11 (Figure 2) were synthesized to prove our concept in this important area.
Chemistry

The synthesis of the protected serine and aspartate carrier thiodipeptides (12 and 13), nabumetone prodrugs 6-7 and ibuprofen prodrug 1 have been reported previously [15, 16]. Our chosen drugs could readily be attached to the appropriate carrier using standard coupling reagents, except for the aspirin prodrug 4 (Table 1). This was synthesized by first using concentrated Mitsunobu conditions [19] with sonication to esterify the salicylic acid with triethylene glycol to give 22, then coupling this glycol ester to the aspartate carrier using standard coupling conditions (Scheme 1) to give 23. This indirect route was chosen because we were unable to accomplish direct esterification of aspirin with the serine carrier using a variety of coupling conditions. Deprotection was usually achieved in >85% yield using either a 33% solution of TFA in DCM or neat formic acid, except for 5, for which decomposition was avoided by using phenol as solvent [20]. Since the NMR [15] of carriers 12 and 13 show no signs of epimerisation, and the coupling reactions employ only moderate bases with the reaction centre removed from chiral centres, we believe the rotamers observed in some protected intermediates and final compounds are not due to epimerisation.

Figure 2. Rationally designed prodrugs to target PepT1 (parent drug in brackets).
Table 1. Synthesis of protected pro-drugs (non-optimized yields)

| Drug                        | Coupling Agent | Compound (Yield) |
|-----------------------------|----------------|-----------------|
| Gabapentin (Boc protected)  | HBTU           | 14 (22%)        |
| Propofol                    | DCC            | 15 (38%)        |
| Acyclovir                   | DCC            | 16 (96%)        |
| Atenolol (Boc protected)    | CDI            | 17 (36%)        |
| Zanamivir                   | HATU           | 18 (11%)        |
| Baclofen (Boc protected)    | DCC            | 19 (65%)        |
| Mycophenolic acid           | HATU           | 20 (20%)        |

Scheme 1. Synthesis of protected aspirin pro-drug. (i) PPh₃, triethylene glycol, DIAD, THF, rt, sonication, 15 min. (ii) 13 [15], HBTU, DIPEA, DMF, rt, 4 days.

Results and Discussion

The results of binding studies, trans-stimulation and Caco-2 monolayer assays are summarised in Table 2. The binding affinities of all prodrugs for PepT1 were determined by measuring the concentration at which they inhibit uptake of radiolabelled D-Phe-L-Gln in *Xenopus laevis* oocytes expressing rabbit
PepT1. Inhibition constants were calculated from standard Michaelis-Menten kinetics [21, 22]. PepT1 is a low affinity, high capacity transporter and compounds with an affinity < 1 mM are generally classed as high affinity binders of the transporter. Figure 3 shows the data for prodrugs 1 and 3, which are representative of those determined for all the prodrugs. Prodrugs 4 and 5 had limited stability in the pH 5.5 assay buffer (multiple HPLC peaks), and so no reliable affinity or transport data could be generated.

| Compound (parent drug) | K<sub>i</sub> (mM) | Trans-stimulated efflux? | Overall P<sub>app</sub> (x 10<sup>-6</sup> cm s<sup>-1</sup>) (Normalised)<sup>a</sup> | PepT1 P<sub>app</sub> (x 10<sup>-6</sup> cm s<sup>-1</sup>) Normalised<sup>a</sup> |
|-----------------------|-----------------|-------------------|-----------------------------|-----------------------------|
| FSA (control)         | 0.32 ± 0.08     | No                | 3.0 ± 0.5 (1.00)            | 1.9 ± 0.5 (1.00)            |
| 1 (ibuprofen)         | 0.26 ± 0.03     | Yes               | 3.7 ± 0.2 (1.29)            | 2.1 ± 0.3 (1.07)            |
| 2 (gabapentin)        | 1.01 ± 0.33     | Yes               | 0.6 ± 0.1 (0.46)            | 0.4 ± 0.1 (0.69)            |
| 3 (propofol)          | 0.92 ± 0.19     | Yes               | 0.5 ± 0.1 (0.41)            | 0.5 ± 0.1 (0.89)            |
| 4 (aspirin)           | nd<sup>b</sup>  |                   |                             |                             |
| 5 (acyclovir)         | nd<sup>b</sup>  |                   |                             |                             |
| 6<sup>c</sup> (nabumetone) | 0.08 ± 0.02  | Yes               | 9.7 ± 0.1 (1.94)            | 4.4 ± 0.2 (1.30)            |
| 7<sup>c</sup> (nabumetone) | 0.46 ± 0.09  | Yes               | 7.8 ± 0.2 (3.78)            | 6.3 ± 0.1 (6.52)            |
| 8 (atenolol)          | 0.44 ± 0.15     | No                | nd                          | nd                          |
| 9 (zanamivir)         | 0.13 ± 0.02     | No                | nd                          | nd                          |
| 10 (baclofen)         | 1.87 ± 0.26     | No                | nd                          | nd                          |
| 11 (mycophenolate)    | 0.21 ± 0.08     | Weak              | nd                          | nd                          |

Table 2. Summary of in vitro affinity and transport studies on rationally design prodrugs in oocytes and Caco-2 monolayers. <sup>a</sup>The PheΨ[CS-NH]-Ala (FSA) value is the mean ± RSD of six separate experiments with at least three monolayers. All other results are the mean ± RSD of one experiment with at least three monolayers. The normalized figure is to the FSA value recorded in that experiment. <sup>b</sup>Prodrug was unstable to assay buffer (pH 5.5). <sup>c</sup>Previously reported data [16].

Figure 3. The K<sub>i</sub> of prodrugs 1 and 3 for rabbit PepT1 expressed in Xenopus laevis oocytes.
As binding studies only show affinity for PepT1 and do not provide information as to whether the compound is a substrate or an inhibitor, further transport experiments were undertaken. Trans-stimulation assays were performed using radiolabelled $[^3]H$-D-Phe-L-Gln efflux from rabbit PepT1 expressing oocytes in the presence of 10 mM pro-drug. As controls, 10 mM Gly-L-Gln (a standard PepT1 substrate) or buffer lacking a substrate (negative control) were used. Figure 4 shows the efflux data for the compounds summarised in Table 2.

Prodrugs 1-3, 6-7 and 11 induced statistically significant trans-stimulation efflux in oocytes, thereby demonstrating PepT1 mediated transport [21, 22]. Most of these induced similar or greater efflux than GlyGln, however prodrug 11 only weakly triggered efflux in comparison to GlyGln.

Prodrugs that generated robust trans-stimulated efflux in oocytes were further in a Caco-2 monolayer assay to investigate further the extent and rate of trans-epithelial transport. Caco-2 cells were chosen as they are widely accepted as a good overall model for the small intestinal epithelium [23], although it has been suggested that Caco-2 cells may underestimate the in vivo trans-epithelial rate of transport [24]. Apical to basolateral transport of 2 mM pro-drug, applied to the apical side, was monitored by high performance liquid chromatography (HPLC) after one hour. The presence or absence of excess Gly-Gln allowed us to determine both the overall and PepT1 specific permeability (Table 2). The remaining prodrugs were significantly transported in both oocyte and Caco-2 monolayer assays. The PepT1 mediated Papp values are of similar magnitude to known PepT1 (pro)drug substrates [10, 16].

Based on these encouraging in vitro results, we elected to study 1 and 3 in vivo (Table 3), as simple examples of the two areas of interest to us. Administration of both 1 and 3 to rats resulted in intact prodrug being observed in the blood, with $C_{\text{max}}$ of 0.2 and 16.7 µg/mL respectively observed. Release of ibuprofen was also observed upon administration of 1, with a relative bioavailability of 2% [25]. The shift in the $T_{\text{max}}$ observed for ibuprofen following administration of 1 when compared to free ibuprofen (from 1 hour for free ibuprofen to 3.5 hours for 1) is indicative of a change in absorption mechanism. The low
bioavailability of ibuprofen can be explained by the relative stability of the prodrug 1 to metabolism, as estimated by its in vitro half-life upon incubation with rat liver homogenate of over 17 hours.

Table 3. Preliminary in vivo results. Values are mean ± standard deviation for n = 3 male Sprague-Dawley rats. IV = intra venous dosing. * As free base. ** As compared to L-Trp-L-Ala, which had a t₁/₂ of 0.15 hours under the same experimental conditions.

| Compound | Dose (mg kg⁻¹) | Assayed compound | C_{Max} (µg mL⁻¹) | AUC (µg h mL⁻¹) | t_{max} (h) | Rat liver homogenate t₁/₂ (h) | Relative F (%) |
|----------|----------------|------------------|-------------------|-----------------|-------------|-------------------------------|---------------|
| Ibuprofen | 6              | Ibuprofen        | 5.7 ± 0.6         | 14.0 ± 1.8      | 0.7 ± 0.3   | 100 [25]                      |               |
| 1        | 10             | Intact 1         | 0.2 ± 0.04        | 0.5 ± 0.1       | 1.0 ± 0.0   | 17.9                          |               |
|          |                | Ibuprofen        | 0.2 ± 0.02        | 0.7 ± 0.1       | 3.2 ± 0.2   | 2.8 ± 0.3                     |               |
| 3        | 7.6            | Intact 3         | 16.3 ± 1.8        | 3.2 ± 1.4       | 5.2 ± 0.8   | 7.3                           |               |

Regrettably, we were unable to detect free propofol released following administration of prodrug 3 because HPLC conditions to quantify free propofol could not be found. However, the relatively high C_{Max} for the intact prodrug 3 is encouraging and despite the relative metabolic stability of 3 (7-hour half-life in rat liver homogenate) it is likely some free propofol would be available systemically. This is notable given the fact that propofol itself had no oral bioavailability in either rats or humans [26].

The low bioavailability of ibuprofen following administration of prodrug 1, combined with fact that both prodrug 1 and 3 were relatively resistant to liver metabolism indicates that further work is required to design effective prodrugs suitable for therapeutic application. Nevertheless, we believe 1 and 3 serve as promising preliminary proof-of-concept to the idea that targeting PepT1 using our thiodipeptides can be used as a general strategy to overcoming oral bioavailability issues in drug discovery and development.

Conclusions

PepT1 is described in the literature as having a broad substrate capacity but, in reality, it has been limited to date to small peptides and peptide-like drugs. To better harness the capacity of this transporter as a drug delivery target, a rational and general targeting approach is required. We report here our data supporting a thiodipeptide prodrug as such a general targeting approach in vitro and in vivo.

We were excited to find that nine out of eleven of our rationally designed PepT1 targeting prodrugs displayed high affinity binding towards PepT1, and six of them triggered pro-stimulation. Additionally, prodrugs 1-3, as well as 6-7 (as previously reported) [16], were all significantly transported in Caco-2 monolayers, with prodrugs 1 and 3 showing evidence of intact absorption in vivo. This provides proof-of-concept that diverse drug types can be delivered via a PepT1 mediated pathway using thiodipeptide carriers, with implications for future drug design strategies. Preliminary in vivo data also supports the use of thiodipeptide prodrugs to confer oral bioavailability.

The drugs exemplified represent examples of several classes of drugs for which oral delivery could be therapeutically interesting. These include thiodipeptide prodrugs of NSAIDs (e.g. 1, 4, 6-7), which have high oral activity but also suffer from significant GI side effects. Examples of drugs for which oral activity is absent (e.g. 3), low (e.g. 9), or highly variable (2, 10) have also been successfully modified using our thiodipeptide approach, to target the PepT1 transporter.
There is much future work to conduct before we can confidently say that rationally targeting PepT1 is a general strategy for oral drug delivery. In particular, the complete in vivo DMPK profile of our prodrugs needs to be established and future prodrugs need to be optimised for rapid liver and/or plasma esterase metabolism and release of free drug. Optimisation of the stability of the linker is also required, as evidenced by the instability of prodrugs 4 and 5. However, our previously reported work suggests that the transporter can accommodate a wide variety of linkers [15, 16], allowing scope to tailor a specific prodrug’s DMPK properties.

The oral delivery of drugs is a major challenge in pre-clinical development and leads to significant shelving of promising lead candidates in drug discovery. Our prodrug approach may allow many such biologically active compounds to be re-evaluated by administration as PepT1 targeting thiodipeptide prodrugs. Our in vitro and preliminary in vivo data is highly encouraging and warrants further work. In particular, our recent report that large peptide drugs such as cyclosporine A [27] can be rationally targeted towards PepT1 using the same approach offers the tantalising possibility of PepT1 targeting as a solution to the delivery of both small and peptidic molecules.

Acknowledgements

Funding from The Wellcome Trust (082051/Z/07/Z) is gratefully acknowledged. The Caco-2 assays on compound 1 were carried out by colleagues at the AstraZeneca Research Laboratories, Alderley Park, Cheshire, UK, with particular thanks to Kevin Foote, Kate Harris and Venessa Zann.

Experimental Section

In Vitro Biological Studies

The Kᵢ, trans-stimulation efflux and Caco-2 assays were performed as described previously [15, 16].

Fresh rat liver homogenate was prepared by isolating liver from euthanized male rats, according to approved Home Office procedures. The liver was chopped with scissors and rinsed with medium (0.25 M sucrose, 25 mM KCl, 5 mM MgCl₂ and 50 mM Tris/HCl, pH7.5) to remove trapped blood. The liver was then homogenized in fresh medium with a loose-fitting dounce-type homogenizer and kept on ice. Liver homogenate was incubated with either 0. 5 mM compound 1 or 3, or L-Trp-L-Ala as a positive control, at 37°C. 250 μl aliquots of the homogenate were taken at 0, 0.25, 0.5, 1, 2, 6 and 24 hours. The samples were precipitated by addition an equal volume of 3% perchloric acid and centrifugation at 17000 g for 5 minutes. The perchloric acid was neutralized with 250 μl of 1 M KOH, and the sample subjected to a freeze / thaw cycle to precipitate the KClO₄ salt before again being centrifuged. The supernatant was then analysed by HPLC as for the Caco-2 permeability studies [15, 16], and the half-life of the compounds calculated according to the method of Vig et al [28].

In vivo studies

In vivo testing was carried out by Cyprotex Discovery Ltd., 15 Beech Lane, Macclesfield, Cheshire, SK10 2DR, United Kingdom or Saretius Ltd, Science & Technology Centre, Earley Gate, University of Reading, Reading, Berkshire RG6 6BZ, United Kingdom.

Each test compound was administered orally as solutions in distilled water (ibuprofen, 1) or polypropylene glycol (3) to three adult male Sprague-Dawley weighing 250-300 g, which were housed singly following jugular vein cannulation prior to administration of compound. Animals were given free access to food and water throughout the study and maintained under a 12-hour light/dark cycle with
temperature and humidity controlled according to Home Office regulations. All compounds were well-tolerated and no-adverse events were reported.

Blood samples (230 µL) were taken from the carotid artery at the following time points and placed in heparinized tubes: predose, 0.25, 0.5, 1, 2, 4, 8 and 24 hours post dose. After the final time point the animals were sacrificed by an overdose of anaesthetic.

Blood samples were centrifuged to obtain the plasma, which was transferred to a separate labelled container. Aliquots from the individual time points for the three animals were analyzed singly. 80 µL of plasma was diluted with 20 µL of 1:1 ACN:water, then 800 µL chilled ACN was added, samples briefly vortex mixed and the centrifuged at 13000 rpm for 5 minutes at 4 °C. 500 µL of the resultant supernatant was further diluted with 500 µL water.

20 µL sample was analyzed by LC-MS/MS using a C18 5 µm Gemini UHPLC column running a gradient of 90% 0.04% acetic acid in water to 90% ACN over 3 minutes at a flow rate of 0.5 mL/min. MS data was acquired under multiple reaction monitoring conditions using a turbo spray ion source. The concentration in the plasma was determined by comparison to standard curves of the administered compound prepared in blank plasma matrices and treated in an identical manner to the samples.

**Synthetic Chemistry**

Anhydrous solvents and reagents were obtained as follows: DMF was dried three times over molecular sieves (3 Å). THF was dried by distillation from sodium benzophenone ketyl, DCM and toluene by distillation from calcium hydride. All reactions were conducted at room temperature in dry glassware under a nitrogen atmosphere, unless otherwise stated. All chemicals were used directly from suppliers’ (Sigma-Aldrich) vessel without further purification, unless otherwise stated. Protected amino acids and HBTU were supplied by Novabiochem. 

^1H NMR spectra were recorded at 300, 400 or 500 MHz and 

^13C NMR spectra at 75, 100 or 125 MHz on a Bruker AC300, AC400, Avance II or Varian Unity INOVA 300 spectrometers. Chemical shifts are denoted in ppm (δ) relative to the internal solvent standard. The splitting patterns for NMR spectra are designated as follows: s (singlet), br (broad), d (doublet), t (triplet), p (pentet), m (multiplet), or combinations thereof. Coupling constants (J) are designated in Hz and reported to 1 decimal place. Assignments were made with the aid of DEPT135, COSY and HMQC experiments. ES-MS (and HRMS) spectra were recorded on a Micromass LCT orthogonal acceleration time-of-flight mass spectrometer (positive ion mode) with flow injection via a Waters 2790 separation module autosampler. IR spectra were obtained using a Nicolet-Nexus 670/680 FT-IR or ATI Mattson Genesis Series FT-IR spectrometer and are quoted in cm⁻¹. Optical rotations were measured at 589 nm in a 1 dm cell using an Optical Activity AA1000 polarimeter and are quoted in 10² deg cm² g⁻¹. Melting point determinations were made using a Stuart Scientific SMP1 apparatus and are uncorrected. Analytical TLC was performed on Merck silica gel 60 F254 aluminium backed plates. The plates were visualised under UV fluorescence (254 nm) or developed using ninhydrin (0.5% w/v butanol), 2-bromocresol or acidified potassium permanganate solution with charring as necessary. Rf values are reported to the nearest 0.01. Mixed solvent system compositions are quoted as volumetric ratios. Column chromatography employed BDH silica gel 50-70 µm. Reverse phase analytical HPLC was performed on a Grace ODS (4.6 x 150 mm) column using a Gilson 306 pump with a flow rate of 1 mL min⁻¹. Detection was at 254 nm by a Gilson 115 UV detector. Reverse phase semi-preparative HPLC was performed on identical apparatus using a Varian ODS 10u (21.2 x 250 mm) column and a 15 mL min⁻¹ flow rate. DataApex Clarity™ software was used for integration and analysis. Retention times (in minutes) are quoted to one decimal place for the analytical system and are followed in parenthesis by the solvent conditions (v/v) used. Silica analytical and semi-preparative HPLC was performed using identical apparatus, software and respective flow rates with Silica PhenoSphere™ (4.6 x 250 mm) and Rainin Dynamax™ 60A (21.4 x 250 mm) columns respectively. Retention times (in minutes) are quoted to
one decimal place for the analytical system and are followed in parenthesis by the solvent conditions (v/v) used.

(S)-3-[2-[[tert-Butoxycarbonylamino-methyl]-cyclohexyl]-acetoxy]-2-[[S]-2-tert-butoxycarbonylamino-thiopropionylamino]-propionic acid tert-butyl ester (14)

Gabapentin (85 mg; 0.5 mmol) and di-tert-butyl dicarbonate (130 mg; 0.6 mmol) were suspended in 1 mL DMF. TEA (0.07 mL; 0.5 mmol) was added and the suspension stirred for four days at room temperature. A solution was formed after the first three hours of stirring. The DMF was removed in vacuo. HBTU (245 mg; 0.6 mmol) and DIPEA (0.11 mL; 0.6 mmol) were added to the residue, which was then redissolved in 1 mL DMF. The resultant solution was stirred at room temperature for 30 minutes.

12 (132 mg; 0.4 mmol) in 1 mL DMF was then added and the solution stirred for three days at room temperature. The DMF was removed and the residue purified by flash column chromatography (4:1 hexane:EtOAc → 1:1 hexane:EtOAc), followed by semi-preparative HPLC (2:1 hexane:EtOAc) to give title compound as a colourless oil (52 mg; 22%). HPLC Rf: 4.2 (2:1 hexane:EtOAc). [α]D19 (CHCl3; c = 3.68): −20.20. υmax (thin film): 3344, 2979, 2931, 2864, 1694, 1514, 1455, 1393, 1367, 1250, 1163, 1102, 1049, 994, 913, 847, 733. δH (500 MHz, CDCl3): 1.51-1.26 (40H, m), 2.31-2.22 (2H, m), 3.07 (1H, dd, J = 14.0 & 6.5), 3.17 (1H, dd, J = 14.0 & 6.5), 4.62-4.40 (3H, m), 4.91 (1H, s), 5.25 (1H, s), 5.50 (1H, s), 8.99 (1H, s). δC (125 MHz, CDCl3): 21.2, 21.8*, 25.7, 27.7, 27.8*, 28.2*, 28.2, 28.3*, 28.3, 33.6, 33.8, 37.4, 39.8, 46.9, 56.1, 57.4, 62.7, 79.2, 79.7, 83.1, 154.9, 156.3, 156.9*, 166.9, 171.6, 206.3, 208.8*. HRMS: Calculated C23H27N2O3S: 624.3289, found: 624.3280. * minor rotamer

(S)-3-[2-[[1-Aminomethyl-cyclohexyl]-acetoxy]-2-[[S]-2-amino-thiopropionylamino]-propionic acid (2)

Deprotection of 44 mg of 14 as for compound 1. Yield of 2 as di-TFA salt = 40 mg; 97%. HPLC Rf: 4.6 (10%, Chromolith³). δH (500 MHz, CD2OD): 1.36-1.45 (3H, m), 1.47-1.59 (10H, m), 2.57 (2H, q, J = 15.5), 3.06 (2H, s), 4.35 (1H, q, J = 6.5), 4.53 (1H, dd, J = 11.5 & 5.5), 4.62 (1H, dd, J = 11.5 & 3.5), 5.30 (0.1H, t, J = 5.0)*, 5.47 (0.9H, t, J = 5.0). δC (100 MHz, CD2OD): 19.5*, 19.7, 20.9*, 21.1, 22.7*, 25.4*, 25.6, 25.7*, 32.9*, 33.0, 33.1*, 35.2, 36.5, 47.2, 53.8, 54.5, 63.1*, 63.3, 171.6, 201.5. MS: C15H27O4S m/z (ES⁺) 361.4 [M+H⁺]+NH3. * minor rotamer

(S)-2-[[S]-2-tert-Butoxycarbonylamino-thiopropionylamino]-succinic acid 1-tert-butyl ester 4-(2,6-diisopropyl-phenyl) ester (15)

13 (120 mg; 0.3 mmol) and DCC (83 mg; 0.4 mmol) were stirred in 1 mL DMF for one hour at 0 °C. A precipitate was formed during this time. DMAP (7 mg; 0.1 mmol) and 2,6-diisopropophenol (0.07 mL; 0.4 mmol) were added. The suspension turned yellow immediately, was warmed to room temperature and stirred for 24 hours. The mixture was filtered and the residue washed with DCM. The solvent was removed in vacuo and the residue purified by flash column chromatography (DCM → 9:1 DCM:Et2O) to title compound as a yellow oil (66 mg; 38%). Rf (95:5 DCM:Et2O): 0.58. [α]D21 (CHCl3; c = 3.00): +60.20. υmax (thin film): 3323, 2980, 2934, 1728, 1514, 1396, 1370, 1335, 1248, 1158, 1053, 912, 846, 734. δH (500 MHz, CDCl3): 1.18 (12H, d, J = 5.5), 1.50-1.41 (21H, m), 2.95-2.70 (2H, m), 3.44-3.35 (1H, m), 3.66-3.55 (1H, m), 4.49 (1H, t, J = 6.5), 5.13 (1H, s), 5.28 (1H, s), 7.15 (2H, d, J = 7.5), 7.22 (1H, t, J = 7.5), 8.56 (0.2 H, br s)*, 8.69 (0.8H, d, J = 7.0). δC (100 MHz, CDCl3): 22.1, 22.8, 24.4, 27.8, 28.2, 34.2, 53.7, 53.9, 83.4, 124.0, 126.8, 140.0, 145.2, 155.1, 168.1, 170.3, 204.9.

(S)-2-[[S]-2-Amino-thiopropionylamino]-succinic acid 4-(2,6-diisopropyl-phenyl) ester (3)

Deprotection of 66 mg of 15 as for compound 1. Yield of 3 as TFA salt = 23 mg; 39%. HPLC Rf: 4.8 (50%, Chromolith³). δH (500 MHz, CD3OD): 1.17 (6H, s), 1.18 (6H, s), 1.49 (0.8H, dd, J = 7.5 & 6.5)*, 1.49 (2.2H, d, J = 6.5), 2.85-3.05 (2H, m), 3.22-3.55 (2H, m), 4.27 (0.8H, q, J = 7.0), 4.33 (0.2, q, J = 7.0)*, 5.53 (1H, br s), 7.15-7.23 (3H, m). δC (100 MHz, CD3OD): 20.9, 23.5, 24.2, 28.3, 28.7, 35.7, 35.2, 55.7, 125.1, 127.9,
4.7), 4.43 (2H, t, J = 4.7), 4.43 (2H, t, J = 4.7), 7.09 (1H, d, J = 8.2), 7.30 (1H, t, J = 7.6), 7.55 (1H, t, J = 8.2), 8.05 (1H, d, J = 7.9). δC (150 MHz, CDCl₃): 20.9, 61.6, 64.0, 69.0, 70.2, 70.6, 72.4, 123.0, 123.7, 125.9, 131.8, 133.9, 150.6, 164.4, 169.7. MS: C₁₅H₂₀O₇ m/z (ES⁺) 335.1 [M+Na⁺]. HRMS: Calculated C₁₅H₂₀O₇Na: 335.1101, found: 335.1092.

(S)-2-{(S)-2-tert-Butyloxycarbonylamino-thiopropionylamino}-succinic acid 4-(2-{2-[2-(2-acetoxy-benzoyloxy)-ethoxy]-ethyl} ester (4)

A suspension of aspirin (345 mg; 1.9 mmol), triphenylphosphine (545 mg; 2.1 mmol) and triethylene glycol (0.27 mL; 2.0 mmol) in 0.7 mL THF was sonicated to give a viscous solution. DIAD (0.40 mL; 2.0 mmol) was then added over 5 minutes to give a yellow solution. This was sonicated for 15 minutes at room temperature. The solution was purified by flash column chromatography (4:1 hexane:EtOAc → EtOAc) to give crude product. This was further purified by flash column chromatography (9:1 DCM:Et₂O → EtOAc) to give the title compound as a colourless oil (294 mg; 49%). Rf (EtOAc): 0.29. υmax (thin film): 3489, 2875, 1768, 1723, 1608, 1486, 1453, 1369, 1295, 1258, 1197, 1123, 1082, 916, 754. δ (CDCl₃): 164.4, 169.7. MS: C₃₃₅.1092.
A mixture of 13 (124 mg; 0.3 mmol) and DCC (68 mg; 0.3 mmol) were dissolved in 0.3 mL DMF and cooled to 0 °C. The reaction was stirred at this temperature for one hour during which time a white precipitate was formed. A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was stirred at 45 °C for one hour. The solution was diluted with 2 mL EtOAc and washed three times with 3 mL water. The EtOAc was removed in vacuo and the residue sequentially taken up in DMF and water, both of which were removed under vacuum. The resultant residue, which was free from phenol (28 mg; 44%).

A mixture of 3 mL water. The EtOAc was removed in vacuo and the residue sequentially taken up in DMF and water, both of which were removed under vacuum. The resultant residue, which was free from phenol contamination, was redissolved in 5 mL water and lyophilised to give di-TFA salt of water, both of which were removed under vacuum. The resultant residue, which was free from phenol (28 mg; 44%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).

A solution of acyclovir (50 mg; 0.2 mmol) and DMAP (10 mg; 0.1 mmol) in 1.5 mL DMF was then added. The suspension was warmed to room temperature and stirred for 24 hours during which time the suspension changed from orange-brown to a brown-purple colour. The suspension was filtered and the filtrate washed with the minimum of DCM. The liquor was concentrated and the residue purified by flash column chromatography (95:5 CHCl₃:MeOH → 9:1 CHCl₃:MeOH) to give the title compound as a brown solid (123 mg; 96%).
5.22 (1H, m), 5.22-5.30 (1H, m), 5.30-5.42 (1H, m), 5.70-5.80 (1H, m), 6.82 (2H, d, J = 8.7), 7.15 (2H, d, J = 8.5). \( \delta_c \) (75 MHz, CDCl3): 17.5, 21.0, 22.1, 22.6, 23.8, 27.8, 28.2, 28.4, 29.3, 29.6, 31.9, 34.9, 42.3, 43.2, 53.9, 56.0, 57.0, 67.4, 80.1, 83.1, 115.1, 127.5, 130.6, 154.8, 157.6, 168.2, 174.1, 205.4. HRMS: Calculated C_{38}H_{40}N_{10}O_{10}S 725.3790, found 725.3795. MS C_{38}H_{39}N_{10}O_{10}S m/z (ES^+) 725.37 [M+H^+].

\((S)-4\)-\((S)-3\)-\((4\)-\(2\)-aminooxoethyl)phenoxy\)-1-(isopropylamino)propan-2-yloxy\)-2-\((S)-2\)-aminopropanethioamido\)-4-oxobutanoic acid (8)

17 (100 mg, 0.138 mmol) was dissolved in 3 mL 97% formic acid. The solution was refluxed at 100 °C for three hours, followed by room temperature for overnight. The excess formic acid was then removed under high vacuum and the residue taken up in 2 mL distilled water. The fine suspension was filtered through a pipette plugged with glass wool and lyophilised to give the formate salt of 8 as a brown solid (53 mg; 76%). M.p. 282-284 °C. [\( \alpha \)]D^{29} (MeOH; c = 0.028): -35.71. \( \nu_{\text{max}} \) (neat)/cm\(^{-1}\) 3648, 3195, 2972, 2116, 1869, 1830, 1738, 1667, 1583, 1510, 1456, 1380, 1346, 1298, 1240, 1176, 1082, 1066, 1046, 920, 879, 798, 763, 668, 567, 518. \( \delta_c \) (300 MHz, CD2OD): 1.1 (6H, d, J = 6.4), 1.42 (3H, d, J = 6.8), 2.73-2.92 (2H, m), 3.22-3.41 (5H, m), 4.01-4.25 (3H, m). HRMS: Calculated C_{22}H_{23}N_{10}O_{10}S m/z (ES^+) 681.27 [M+H^+].

A mixture of 13 (100 mg, 0.26 mmol), HATU (117 mg, 0.30 mmol) and DIPEA (0.15 ml, 0.8 mmol) in anhydrous DMF (10 ml) was stirred under nitrogen at 0 °C for 30 min. then a solution of Relenza (97 mg, 0.29 mmol) in the mixture of dry DMF:DMSO (10 ml, 8:2) was added and stirring was continued for another 3 days at room temperature. The reaction mixture were filtered off and the filtrate, plus a DMF as formate salt = 54 mg; 70%. M.p. 210-212 °C. [\( \alpha \)]D^{29} (H_{2}O; c = 0.057): 17.54. \( \nu_{\text{max}} \) (neat)/cm\(^{-1}\) 3325, 3178, 2924, 2111, 1717, 1680, 1589, 1374, 1324, 1282, 1146, 1041, 945, 768, 665. \( \delta_c \) (300 MHz, CD2OD): 1.42 (3H, s), 1.92 (3H, s), 2.73-2.90 (3H, m), 3.45-3.55 (2H, m), 3.73-3.82 (2H, m), 4.02-4.11 (1H, m), 4.15-4.30 (3H, m), 4.82-4.91 (1H, m), 5.53 (1H, s), 8.22 (1H, s). \( \delta_c \) (75 MHz, CD2OD): 19.2, 21.8, 37.0, 47.5, 50.9, 53.0, 57.2, 62.0, 67.9, 69.6, 75.2, 104.1, 136.0, 150.0, 156.8, 174.2, 180.0, 199.6. MS: C_{19}H_{30}N_{5}O_{10}S m/z (ES^+) 534.2 [M^+]. HRMS: Calculated for C_{19}H_{30}N_{5}O_{10}S 534.1744, found 534.1698.

\((R)-3\)-tert-butoxy-2-\((S)-2\)-\((tert-butoxycarbonyl)propanethioamido\)-3-oxopropyl \(4\)-(\(tert-butoxycarbonyl\))-3-\((4\)-chlorophenyl\)butanoate (19)
A mixture of Boc-baclofen (210 mg, 0.66 mmol), DCC (140.5 mg, 0.68 mmol) and DMAP (5 mg, 0.05 mmol) in anhydrous DCM (20 ml) was stirred at room temperature for 15 min. under the inert atmosphere. Then, the solution of 12 (163 mg, 0.468 mmol) in 10 ml of DCM was slowly added at the same temperature. The mixture was stirred for 5 h at room temperature under the inert atmosphere. The precipitated solid was filtered off and the filtrate was evaporated in vacuo to get viscous liquid, which can further diluted with 30 ml of EtOAc, and washed with 2M HCl (2 x 10 ml), saturated aqueous NaHCO₃ (3 x 10 ml), water (2 x 10 ml) and finally with the brine (20 ml), dried (MgSO₄) and evaporated in vacuo to afford white crude solid. The residue was purified by flash column chromatography (9:1 petrol:EtOAc → 7:3 petrol:EtOAc) to give desired product as a white solid (280 mg; 65%).

Deprotection of 100 mg of 19 as for compound 8. Yield of 10 as formate salt = 54 mg; 80%. M. p. 112-115 °C. [α]D²⁸ (H₂O; c = 0.057): -17.54. u max (neat)/cm⁻¹: 3195, 2979, 2112, 1869, 1730, 1704, 1688, 1582, 1511, 1490, 1456, 1379, 1241, 1164, 1110, 1089, 1050, 1013, 897, 823, 764, 668, 527; δ H (300 MHz, D₂O): 1.42 (3H, s), 2.45-2.62 (1H, m), 2.62-2.75 (1H, m), 3.05-3.15 (1H, m), 3.15-3.35 (2H, m), 4.25-4.35 (1H, dd, J = 11.5 & 2.4), 5.15-5.25 (1H, br m), 5.62 (1H, br s), 7.15 (2H, d, J = 8.5), 7.25 (2H, d, J = 8.3), 8.83 (1H, br s). δ C (75 MHz, CDCl₃): 21.7, 27.8, 28.3, 28.38, 33.8, 37.6, 41.3, 57.1, 57.3, 62.9, 79.4, 82.7, 83.4, 128.9, 132.9, 139.7, 155.9, 167.2, 170.9, 206.2. MS: C₃₀H₄₆Cl₂N₃O₆S m/z (ES⁺) 644.3 [M+H⁺]. HRMS: Calculated for C₃₀H₄₆Cl₂N₃O₆S 644.2195, found 644.2759.

**{(S)-3-((R)-4-amino-3-(4-chlorophenyl)butanoyloxy)-2-((S)-2-aminopropanethioamido)propanoic acid (10)**

**{(E)}-**(S)-3-tert-butoxy-2-**{(S)-2-((tert-butoxycarbonyl)propanethioamido)-3-oxopropyl} 6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoate (20)

A mixture of 12 (94 mg, 0.27 mmol), mycophenolic acid (100 mg, 0.31 mmol) and N-methylmorpholine (137 mg, 1.24 mmol) in anhydrous MeCN (10 ml) was stirred with 3 Å molecular sieves under nitrogen at 20 °C for 2 h, then a solution of HATU (130 mg, 0.34 mmol) in MeCN (5 ml) was added and stirring was continued for another 2 days. The reaction was monitored by TLC (7:3 Petrol : EtOAc). The reaction mixture were filtered off and the filtrate, plus an MeCN washing, was evaporated in vacuo to get crude solid, which can further diluted with 30 ml of EtOAc, and washed with 2M HCl (2 x 10 ml), saturated aqueous NaHCO₃ (3 x 10 ml), water (2 x 10 ml) and finally with brine (20 ml), dried (MgSO₄) and evaporated in vacuo to afford white crude solid, which was further purified by chromatography, eluting with 9:1 (Petrol : EtOAC) to 7.5:1 (Petrol : EtOAC) to give desired product as a white solid (40 mg, 20%). M. p. 69-72 °C. Rf 0.50 [Petrol-EtOAc 1:1]. [α]D²⁰ (CHCl₃; c = 0.028): 35.71. u max (neat)/cm⁻¹: 3326, 2976, 2930, 2115, 1991, 1868, 1738, 1732, 1716, 1699, 1622, 1564, 1506, 1454, 1411, 1393, 1367, 1329, 1245, 1179, 1130, 1049, 1038, 994, 969, 844, 792, 545. δ H (300 MHz, CDCl₃): 1.42 (21H, s), 1.73 (3H, s), 2.14 (3H, s), 2.22 (2H, t, J = 6.8), 2.25-2.35 (2H, m), 3.32 (2H, d, J = 6.8), 3.74 (3H, s), 4.35-4.53 (3H, m), 5.12-5.23 (4H, m), 7.63 (1H, s), 8.53 (1H, m). δ C (75 MHz, CDCl₃): 11.6, 16.1, 21.7, 22.5, 27.8, 28.2, 32.6, 34.3, 57.3, 61.0, 62.8, 70.1, 80.3, 83.5, 106.4, 116.7, 122.02, 122.8, 133.8, 144.1, 153.5, 163.6, 167.3, 172.8, 172.9, 205.6. MS: C₃₂H₄₆N₂O₁₀S m/z (ES⁺) 651.3 [M+H⁺]. HRMS: Calculated for C₃₂H₄₆N₂O₁₀S 651.2951, found 651.2928.
Deprotection of 100 mg of 20 as for compound 8. Yield of 11 as formate salt = 50 mg; 65%. M.p. 140-143 °C. [α]D29 (H2O; c = 0.057): -35.09. υmax (neat)/cm⁻¹: 3746, 3219, 2974, 2934, 2247, 2119, 1830, 1737, 1731, 1688, 1668, 161, 1606, 1558, 1539, 1532, 1516, 1452, 1409, 1380, 1325, 1303, 1251, 1187, 1135, 1107, 1075, 1033, 966, 938, 788, 642, 589, 542. δH (300 MHz, D2O): 1.22 (3H, d, J = 3.8), 1.43 (3H, s), 1.62-1.75 (3H, m), 1.91 (3H, s), 2.31-2.42 (2H, m), 2.52-2.63 (2H, m), 2.73-2.81 (1H, m), 3.02-3.11 (1H, m), 3.72 (3H, s), 4.21-4.43 (4H, m), 5.02-5.11 (3H, m), 8.01 (1H, s). δC (75 MHz, CD3OD): 11.4, 16.5, 20.5, 31.8, 33.2, 55.0, 62.9, 64.0, 70.8, 83.1, 120.0, 123.7, 124.2, 135.0, 162.5, 166.1, 170.8, 173.0, 201.2. MS: C23H30N2O8S m/z (ES⁺) 494.2 [M⁺]. HRMS: Calculated for C23H30N2O8S 494.1723, found 494.1676.

References

[1] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Advanced Drug Delivery Reviews, 46 (2001) 3-26.
[2] T. Tashima, Intriguing possibilities and beneficial aspects of transporter-conscious drug design, Bioorg Med Chem, 23 (2015) 4119-4131.
[3] B.J. Aungst, Absorption Enhancers: Applications and Advances, The AAPS journal, 14 (2012) 10-18.
[4] I.T. Consortium, Membrane transporters in drug development., Nature reviews. Drug discovery, 9 (2010) 215.
[5] C.P. Landowski, B.S. Vig, X. Song, G.L. Amidon, Targeted delivery to PEPT1-overexpressing cells: Acidic, basic, and secondary floxuridine amino acid ester prodrugs, Molecular Cancer Therapeutics, 4 (2005) 659-667.
[6] H. Daniel, G. Kottra, The proton oligopeptide cotransporter family SLC15 in physiology and pharmacology, Pflügers Arch. - Eur. J. Physiol., 447 (2004) 610-618.
[7] I. Rubio-Aliaga, H. Daniel, Peptide transporters and their roles in physiological processes and drug disposition, Xenobiotica, 38 (2008) 1022-1042.
[8] L. Fang, M. Wang, S. Gow, X. Liu, H. Zhang, F. Cao, Combination of amino acid/dipeptide with nitric oxide donating oleandomic acid derivatives as PepT1 targeting antitumor prodrugs, Journal of Medicinal Chemistry, 57 (2014) 1116-1120.
[9] D. Gupta, S. Varughese Gupta, A. Dahan, Y. Tsume, J. Hilfinger, K.-d. Lee, G.L. Amidon, Increasing oral absorption of polar neuraminidase inhibitors: a prodrug transporter approach applied to oseltamivir analogues., Molecular Pharmaceutics, 10 (2013) 512-522.
[10] F. Li, L. Hong, C.-I. Mau, R. Chan, T. Hendricks, C. Dvorak, C. Yee, J. Harris, T. Alfredson, Transport of levovirin prodrugs in the human intestinal Caco-2 cell line, Journal of Pharmaceutical Sciences, 95 (2006) 1318-1325.
[11] T. Nakanishi, I. Tamai, A. Takaki, A. Tсуji, Cancer cell-targeted drug delivery utilizing oligopeptide transport activity, Int. J. Cancer, 88 (2000) 274-280.
[12] Y. Tsume, J.M. Hilfinger, G.L. Amidon, Enhanced cancer cell growth inhibition by dipeptide prodrugs of floxuridine: Increased transporter affinity and metabolic stability, Molecular Pharmaceutics, In Press, Accepted Manuscript (2008).
[13] M.V.S. Varma, A.H. Eriksson, G. Sawada, Y.A. Pak, E.J. Perkins, C.L. Zimmerman, Transepithelial Transport of the Group II Metabotropic Glutamate 2/3 Receptor Agonist (1S,2S,5R,6S)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylate (LY354740) and its Prodrug (1S,2S,5R,6S)-2[(2'S)-(2'-Amino)propionyl]aminobicyclo[3.1.0]hexane-2,6-dicarboxylate (LY544344), Drug Metabolism and Disposition, 37 (2009) 211-220.
[14] P.D. Bailey, Drug delivery system comprising a thiopeptide, in, (The University of Manchester, UK). European Patent Office, 2005.
[15] D. Foley, M. Pieri, R. Pettecrew, R. Price, S. Miles, H.K. Lam, P. Bailey, D. Meredith, The in vitro transport of model thiodipeptide prodrugs designed to target the intestinal oligopeptide transporter, PepT1, Organic & Biomolecular Chemistry, 7 (2009) 3652-3656.
[16] D. Foley, P. Bailey, M. Pieri, D. Meredith, Targeting ketone drugs towards transport by the intestinal peptide transporter, PepT1, Org. Biomol. Chem., 7 (2009) 1064-1067.
[17] The Merck Index - An Encyclopedia of Chemicals, Drugs and Biologicals, 14th ed., Merck & Co. Inc., New Jersey, 2006.
[18] A.P. Bento, A. Gaulton, A. Hersey, L.J. Bellis, J. Chambers, M. Davies, F.A. Krüger, Y. Light, L. Mak, S. McGlinchey, M. Nowotka, G. Papadatos, R. Santos, J.P. Overington, The ChEMBL bioactivity database: an update, Nucleic Acids Res., 42 (2014) D1083-D1090.
[19] S.D. Lepore, Y. He, Use of Sonication for the Coupling of Sterically Hindered Substrates in the Phenolic Mitsunobu Reaction, Journal of Organic Reaction, 68 (2003) 8261-8263.
[20] S. Torii, H. Tanaka, M. Taniguchi, Y. Kameyama, M. Sasaoka, T. Shiroi, R. Kikuchi, I. Kawahara, A. Shimabayashi, S. Nagao, Deprotection of carboxylic esters of b-lactam homologs. Cleavage of p-methoxybenzyl, diphenylmethyl, and tert-butyl esters effected by a phenolic matrix, Journal of Organic Chemistry, 56 (1991) 3633-3637.
[21] D. Meredith, C.A.R. Boyd, J.R. Bronk, P.D. Bailey, K.M. Morgan, I.D. Collier, C.S. Temple, 4-Aminomethylbenzoic acid is a non-translocated competitive inhibitor of the epithelial peptide transporter PepT1, J. Physiol. (London), 512 (1998) 629-634.
[22] C.S. Temple, A.K. Stewart, D. Meredith, N.A. Lister, K.M. Morgan, I.D. Collier, R.D. Vaughan-Jones, C.A.R. Boyd, P.D. Bailey, J.R. Bronk, Peptide Mimics as Substrates for the Intestinal Peptide Transporter, Journal of Biological Chemistry, 273 (1998) 20-22.
[23] I.J. Hidalgo, T.J. Raub, R.T. Borchardt, Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability, Gastroenterology, 96 (1989) 736-749.
[24] P.V. Balimane, S. Chong, K. Patel, Y. Quan, J. Timoszyk, Y.-H. Han, B. Wang, B. Vig, T.N. Faria, Peptide transporter substrate identification during permeability screening in drug discovery: comparison of transfected MDCK-hPepT1 cells to Caco-2 cells, Arch. Pharm. Res., 30 (2007) 507-518.
[25] W. Martin, G. Koselowske, H. Töberich, T. Kerkmann, B. Mangold, J. Augustin, Pharmacokinetics and absolute bioavailability of ibuprofen after oral administration of ibuprofen lysine in man, Biopharmaceutics & Drug Disposition, 11 (1990) 265-278.
[26] K.M. Wozniak, J.J. Vornov, B.M. Mistry, Y. Wu, R. Rais, B.S. Slusher, Gastrointestinal delivery of propofol from fospropropofol: its bioavailability and activity in rodents and human volunteers, Journal of Translational Medicine, 13 (2015) 170.
[27] D.W. Foley, I. Bermudez, P.D. Bailey, D. Meredith, A cyclosporine derivative is a substrate of the oligopeptide transporter PepT1, MedChemComm, 7 (2016) 999-1002.
[28] B.S. Vig, P.J. Lorenzi, S. Mittal, C.P. Landowski, H.-C. Shin, H.I. Mosberg, J.M. Hilfinger, G.L. Amidon, Amino Acid Ester Prodrugs of Floxuridine: Synthesis and Effects of Structure, Stereochemistry, and Site of Esterification on the Rate of Hydrolysis, Pharmaceutical Research, 20 (2003) 1381-1388.