



Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/146983/>

Version: Published Version

Article:

Matos, A.M., Man, T., Idrissi, I. et al. (2019) Discovery of N-methylpiperazinyl flavones as a novel class of compounds with therapeutic potential against Alzheimer's disease : synthesis, binding affinity towards amyloid β oligomers (A β o) and ability to disrupt A β o-PrPC interactions. *Pure and Applied Chemistry*, 91 (7). pp. 1107-1136. ISSN: 0033-4545

<https://doi.org/10.1515/pac-2019-0114>

Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: <https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

Conference paper

Ana M. Matos, Teresa Man, Imane Idrissi, Cleide C. Souza, Emma Mead, Charlotte Dunbar, Joanna Wolak, Maria C. Oliveira, David Evans, James Grayson, Benjamin Partridge, Claire Garwood, Ke Ning, Gary Sharman, Beining Chen and Amélia P. Rauter*

Discovery of *N*-methylpiperazinyl flavones as a novel class of compounds with therapeutic potential against Alzheimer's disease: synthesis, binding affinity towards amyloid β oligomers (A β o) and ability to disrupt A β o-PrP C interactions

<https://doi.org/10.1515/pac-2019-0114>

Abstract: With no currently available disease-modifying drugs, Alzheimer's disease is the most common type of dementia affecting over 47 million people worldwide. In light of the most recent discoveries placing the cellular prion protein (PrP C) as a key player in amyloid β oligomer (A β o)-induced neurodegeneration, we investigated whether the neuroprotective potential of nature-inspired flavonoids against A β -promoted toxicity would translate into the ability to disrupt PrP C -A β o interactions. Hence, we synthesized a small library of flavones and studied their binding affinity towards A β o by STD-NMR. C-glucosyl flavones exhibited improved binding affinity with morpholine, thiomorpholine or *N*-methylpiperazine rings attached to the flavone skeleton in ring B *para* position. Moreover, a *N*-methylpiperazinyl flavone displayed suitable physico-chemical properties and optimal water solubility even without the sugar moiety, and a high interaction with A β o involving the whole flavone core. Its C-glucosyl derivative, was, however, the best compound to inhibit PrP C -A β o interactions in a dose-dependent manner, with 41% of inhibition capacity at 10 μ M. The potential of C-glucosyl flavones and their aglycones as protein-protein interaction inhibitors able to tackle PrP C -A β o interactions is here presented for the first time, and supports this class of compounds as new prototypes for further development in the treatment of Alzheimer's disease.

Keywords: A β o-PrP C interaction disruptors; Alzheimer's disease; C-glucosylation; flavones; ICS-29.

Article note: A collection of invited papers based on presentations at the 29th International Carbohydrate Symposium (ICS-29), held in the University of Lisbon, Portugal, 14-19 July 2018.

***Corresponding author:** Amélia P. Rauter, Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Ed. C8, Campo Grande, 1749-016 Lisboa, Portugal; and Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Ed. C8, Campo Grande, 1749-016 Lisboa, Portugal, e-mail: aprauter@fc.ul.pt

Ana M. Matos: Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Ed. C8, Campo Grande, 1749-016 Lisboa, Portugal; and Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Ed. C8, Campo Grande, 1749-016 Lisboa, Portugal

Teresa Man, David Evans and Gary Sharman: Department of Chemistry, Erl Wood Manor, Eli Lilly, Windlesham, UK

Imane Idrissi: Biofordrug, Via Edoardo Orabona, 4, 70125 – Bari BA, Italy

Cleide C. Souza, James Grayson, Benjamin Partridge, Claire Garwood, Ke Ning and Beining Chen: Department of Chemistry, The University of Sheffield, Dainton Building, Brook Hill, S3 7HF Sheffield, UK

Emma Mead and Charlotte Dunbar: Department of Biology, Erl Wood Manor, Eli Lilly, Windlesham, UK

Joanna Wolak: Department of Chemistry, Erl Wood Manor, Eli Lilly, Windlesham, UK; and Department of Chemistry, The University of Sheffield, Dainton Building, Brook Hill, S3 7HF Sheffield, UK

Maria C. Oliveira: Mass Spectrometry Facility, Centro de Química Estrutural, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

Introduction

Alzheimer's disease (AD) is the most common form of dementia, presently affecting more than 47 million people globally [1]. Symptoms include memory and judgement impairment, disorientation, confusion, mood swings, among others, and their management is associated with a heavy emotional and financial burden to patients, families and national health care systems [2]. With the increase in life expectancy and higher incidence of type 2 diabetes (T2D), which is nowadays generally accepted as a major risk factor for dementia, the number of AD patients is estimated to rise to more than 131 million by 2050 [1, 3]. In spite of these projections, cholinesterase inhibitors including the highly prescribed donepezil, rivastigmine and galantamine, are some of the very few drugs currently available for treating AD. What's more, in addition to a poor risk-benefit relationship [4], these drugs are not supported by robust evidence indicating disease-modifying effects, thus placing the pursuit for new molecular entities able to interfere with the progression of AD as one of the highest priorities of health care providers worldwide.

Since 1992, the amyloid cascade hypothesis has been at the heart of the vast majority of publications concerning the pathophysiology and treatment of AD. Originally postulated by Hardy and Higgins, this hypothesis posits that the accumulation of amyloid β (A β) in the brain is responsible for the formation of neurofibrillary tangles, as well as the increase in intraneuronal calcium (Ca $^{2+}$) concentrations that ultimately result in neuronal death [4, 5]. Even though the initial focus of most AD scientists was on the dense A β fibril networks that constitute amyloid plaques, the past decade has led the scientific community to agree that much smaller A β oligomers (A β o) are actually the most neurotoxic amyloid species of A β . With a spherical shape ranging from 3 nm up to 10 nm in size [6], soluble A β o appear to affect dielectric properties of cell membrane by bilayer insertion and domain formation, and to disrupt neuronal membrane trafficking [7, 8]. More recently, however, new evidence on the role of the cellular prion protein (PrP C) in the molecular mechanisms underpinning the detrimental effects of A β o in AD started to emerge. In 2009, Laurén and co-workers described PrP C as a high affinity cell-surface receptor for soluble A β o, being able to mediate synaptic impairment caused by A β o [9]. Accordingly, *Pmp*^(-/-) mice (genetically engineered prion protein-deficient mice) are resistant to the neurotoxic effects of A β o *in vivo* and *in vitro* [10]. With the subsequent discovery of Fyn kinase as a key mediator in A β o-PrP C -induced neuronal damage [11], it is currently believed that the A β o-PrP C interaction triggers metabotropic glutamate receptor-5 (mGluR5)-dependent signalling events leading to the activation of Fyn kinase, with ensuing NMDA-mediated intracellular Ca $^{2+}$ influx, and activation of kinases such as glycogen synthase kinase 3 β (GSK-3 β) and protein tyrosine kinase 2 (Pyk2), which are ultimately involved in tau hyperphosphorylation [12, 13]. These data strongly suggest that tackling A β o-PrP C interactions with effective protein-protein interaction inhibitors (PPIIs) might lead to new therapeutic approaches against AD progression.

Chicago sky blue, a dye used in fluorescence and immunofluorescence histochemistry, is so far the only small molecule shown to disrupt the binding between A β and PrP C [14]. Given the potential of A β o-PrP C as a therapeutic target in AD, we believe that it is imperative to find new lead scaffolds with the ability to tackle the interaction between both partners, and to investigate whether molecules known to have binding affinity towards A β and/or exert neuroprotective effects could, in fact, act by disrupting A β o-PrP C interactions. In this perspective, we followed a line of investigation that started with the recognition of chrysin (Fig. 1a) as a suitable prototype structure for the interaction with A β and inhibition of small A β o formation [15]. We were furthermore interested in finding out if the insertion of a C-glucosyl moiety could somehow optimize the activity of chrysin derivatives, since vitexin (Fig. 1a) and other glycosyl flavones had been described to exert neuroprotective effects against A β -induced toxicity *in vitro* [16, 17]. Hence, we directed our research efforts to the design and synthesis of a primary series of flavones and corresponding C-glucosyl derivatives, from which our preliminary screening tests selected both morpholinyl derivatives **1** and **2** (Fig. 1b) as the most promising neuroprotective agents against A β ₁₋₄₂-induced toxicity in SHSY-5Y human neuroblastoma cells, with the ability to restore cell viability at 50 μ M (unpublished data).

The present work focuses on the chemical modifications introduced in the lead compounds **1** and **2** that resulted in the discovery of new molecules able to disrupt A β o-PrP C interactions. Figure 2 summarizes

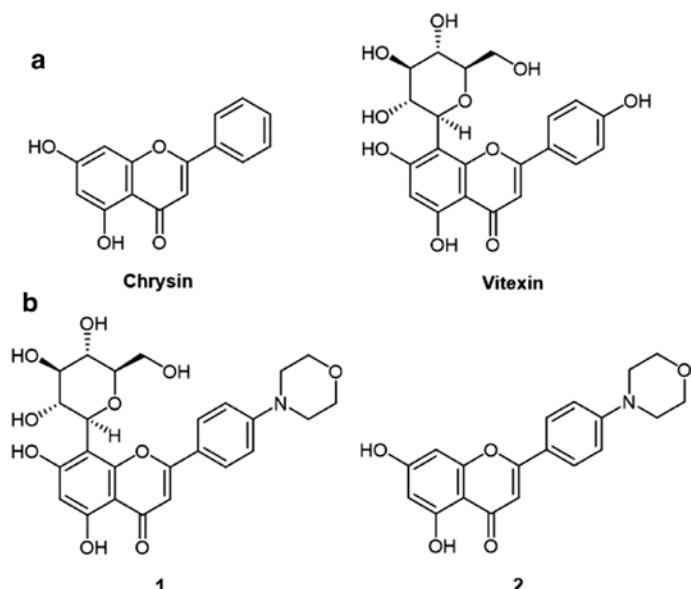


Fig. 1: (a) Chemical structure of chrysin and vitexin, two natural products with the ability to interact with $\text{A}\beta$; (b) lead scaffolds for the present study, compounds 1 and 2.

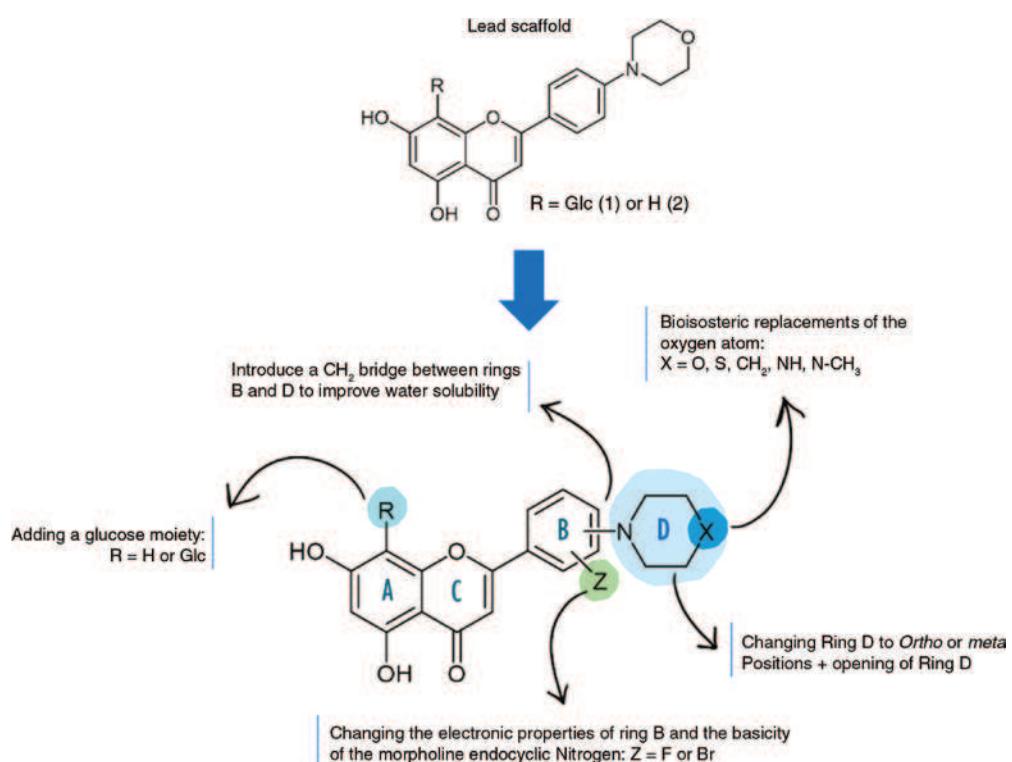


Fig. 2: Strategy for chemical modifications towards the optimization of the lead scaffold.

our strategy for the design and synthesis of a small library of *C*-glucosyl flavone derivatives and their corresponding aglycones, which included a series of bioisosteric replacements of ring D, as well as the insertion of halogen atoms in ring B, among other alterations. Our main goal was to explore the potential of these compounds by STD NMR in order to evaluate their binding affinity towards $\text{A}\beta$, while developing a brief

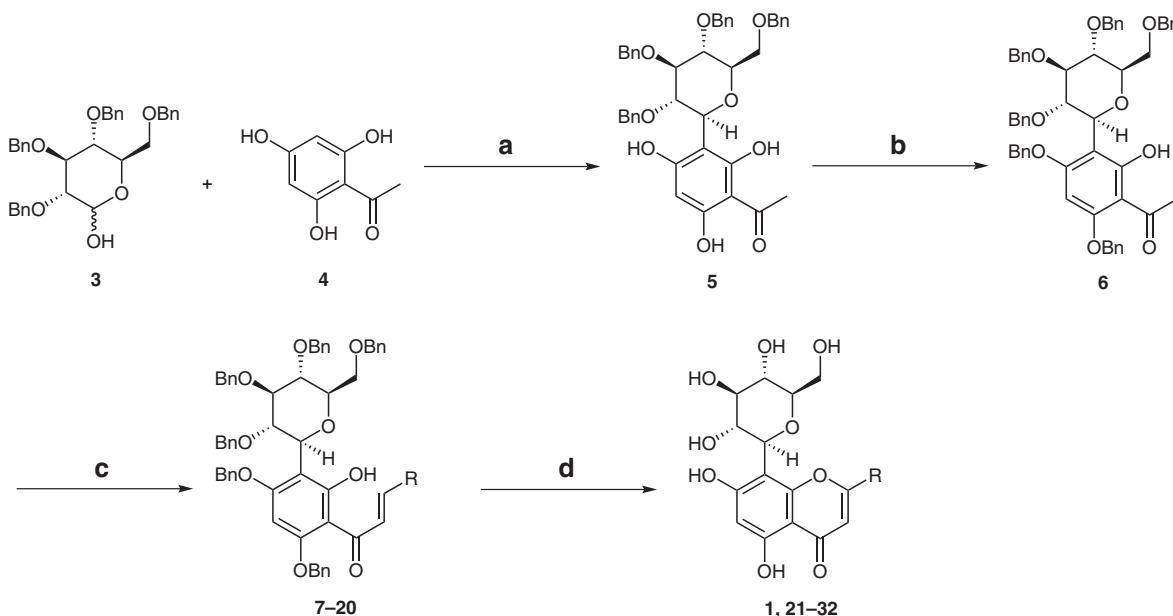
structure-activity relationship study. We subsequently present a functional assay aimed at exploring if some of the most relevant interactions with A β could translate into the highest potential to disrupt A β -PrP^C interactions.

Chemistry

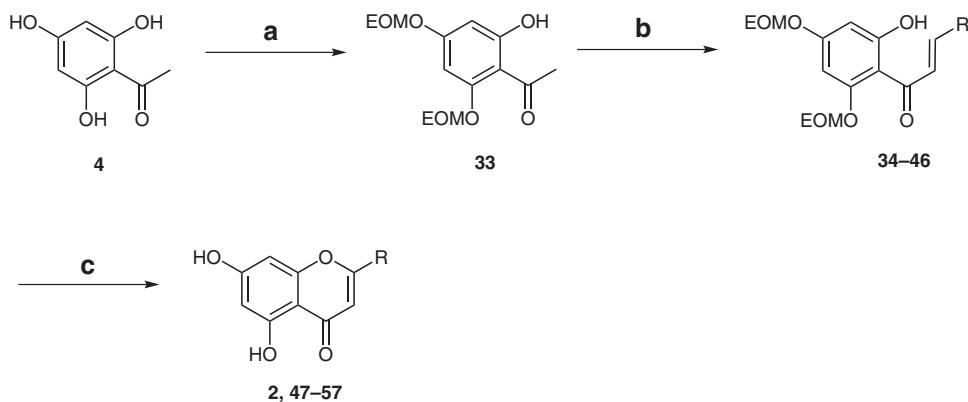
The synthetic route for *C*-glucosyl flavone derivatives started with a TMSOTf-promoted reaction between glycosyl donor **3** and 2,4,6-trihydroxyacetophenone **4**, to give intermediate **5** (Scheme 1). Then, after selective benzylation of two hydroxy groups, chalcone intermediates **7–20** were generated in base-catalysed Claisen-Schmidt aldol condensation reactions under high temperature of compound **6** with different aldehydes. In order to engage in an efficient and highly systematized synthetic process, only commercially available aldehydes or their *N*-Boc protected derivatives were chosen to be used in this step. Lastly, the chalcones were cyclized in the presence of iodine and pyridine, after which all protecting groups were removed using BBr₃ at –78 °C to give the final compounds **1** and **21–32**.

As for the non-glycosylated flavones, 2,4,6-trihydroxyacetophenone (**4**) was selectively protected with ethoxymethyl ether groups (EOM) in a K₂CO₃-promoted reaction to afford compound **33** (Scheme 2). Then, in the presence of each aldehyde, chalcone intermediates **34–46** were generated following the same procedure described for the synthesis of *C*-glucosyl chalcones; however due to the use of smaller protecting groups and absence of the sugar moiety, these reactions proceeded efficiently at room temperature. Finally, each chalcone went through the oxidative cyclization reaction in the presence of iodine and pyridine, followed by deprotection using catalytic amounts of *p*-toluenesulfonic acid (*p*-TSOH) and acetic acid (AcOH) in ethanol under reflux, to give the final compounds **2** and **47–57**.

Notably, compounds **14** and **46** bearing the morpholine group in *ortho* position of ring B, did not afford the corresponding flavones due to chemical instability of the protected intermediates and/or the final compound. Reaction yields of all compounds are provided in Table 1. *C*-Glucosyl chrysins **21** was also synthesized for comparison purposes.



Scheme 1: Synthesis of *C*-glycosyl flavones. Reagents and conditions: (a) ACN/DCM, drierite, TMSOTf, –40 °C → r.t., 18 h, 57%; (b) DMF, K₂CO₃, BnBr, 0 °C → r.t., 4 h, 64%; (c) aldehyde, aq. NaOH 50% (w/v), 1,4-dioxane, 92 °C, 18 h–24 h, 38%–76%; (d) (1) I₂, pyridine, reflux, 48 h–72 h; then, (2) BBr₃, DCM, –78 °C, 2 h–4 h, 24%–95% over two steps.



Scheme 2: Synthesis of non-glycosylated flavones. Reagents and conditions: (a) K_2CO_3 , acetone, EOMCl, reflux, 4 h, 91%*; (b) aldehyde, aq. $NaOH$ 50% (w/v), 1,4-dioxane, r.t., 3 h–24 h, 62%–99%*; (c) (1) I_2 , pyridine, reflux, 24 h–72 h; then, (2) p -TsOH in $AcOH$, $EtOH$, reflux, 2 h–24 h, 63%–99%* over two steps. *Reaction yields determined by LCMS.

Molecular interaction studies and structure-activity relationships

All synthesized compounds were tested for their affinity towards $A\beta_0$ in a screening STD NMR, an assay previously adopted for the study of other polyphenol interactions with $A\beta_0$ [18]. Oligomers were freshly prepared and their size confirmed by dynamic light scattering (DLS), with the vast majority of the amyloid aggregates exhibiting radii between 1 nm and 10 nm (see Supporting Information, Fig. S1). Bexarotene, a high-affinity $A\beta_{1-42}$ ligand with proven ability to block primary and secondary nucleation events in the amyloid aggregation pathway [19], was used in this study as positive control. As negative control, we used 2% DMSO (Fig. S2) – the same DMSO percentage used as vehicle in each assay. Figure 3 presents STD NMR results for the *C*-glucosyl flavones that exhibited any detectable interaction with $A\beta_0$.

When looking into the difference spectrum of lead compound **1**, STD signals corresponding to protons H-3 and H-6 of the flavone core can be observed at 6.53 ppm and 6.13 ppm, respectively. When compared to *C*-glucosyl chrysins **21**, which displays signals corresponding to H-3 (6.71 ppm) and aromatic protons H-2' and H-6' (7.56 ppm), but not H-6, the results suggest that the morpholine moiety in compound **1** is able to change the binding mode of these types of compounds towards $A\beta_0$, by inducing the participation of ring A in the interaction between the flavone and the target protein aggregates.

From all the data presented in Fig. 3, the group of spectra referring to compound **22** stands out for the STD NMR signals corresponding not only to all olefinic and aromatic protons in the flavone core, but also to the anomeric proton H-1'', at 4.89 ppm. This indicates that, on the one hand, the bioisosteric replacement of the endocyclic oxygen with a sulfur atom in the morpholine moiety increases the binding affinity between the flavone skeleton and the target protein by promoting an interaction involving all the protons of the flavone skeleton. On the other hand, this replacement additionally leads to the involvement of the sugar moiety in the established contact. Conversely, the bioisosteric replacement of the morpholine with a piperidine group in compound **23** causes a significant loss in binding affinity. In this case, only the STD signal corresponding to the olefinic proton H-3 is unequivocally detected, while the remaining interactions have a very low and irrelevant signal-to-noise ratio. This result points to the presence of a heteroatom in the terminal end of the morpholine group as a definite structural requirement for the induction of optimal binding affinity of the *C*-glucosyl flavone scaffold towards $A\beta_0$. The lack of observable STD interactions detected for both the *N,N*-dimethyl **31** and the *N,N*-biphenyl **32** derivatives further corroborates this hypothesis. Moreover, the same lack of detectable STD signals in the case of the *ortho*-morpholinyl derivative **30** highlights the need for ring D to be in *para* position of ring B in these *C*-glucosyl flavones.

While the *N*-methylpiperazine in compound **25** still retains some level of interaction with the target protein aggregates, the piperazine group in its analogue **24** completely abolished any observable STD signals (data not shown). However, the *N*-methylpiperazinyl *C*-glucosyl flavone **26** containing an additional CH_2 bridge

Table 1: Reaction yields of chalcone intermediates and final flavones.

R	Yield	C-glucosyl flavones		Non-glucosylated flavones	
		Aldol condensation ^a	Cyclization and deprotection ^b	Aldol condensation ^b	Cyclization and deprotection ^b
		(7) 44 %	(21) 88 %	n.a. ^c	n.a. ^c
		(8) 67 %	(1) 74 %	(34) 76 %	(2) 99 %
		(9) 68 %	(22) 24 %	(35) 77 %	(47) 99 %
		(10) 56 %	(23) 92 %	(36) 75 %	(48) 65 %
	(11) 71 % [R=Boc]	(24) 77 % [R=H]	(37) 62 % [R=Boc]	(49) 99 % [R=H]	
	(12) 63 %	(25) 70 %	(38) 83 %	(50) 79 %	
	(13) 58 %	(26) 84 %	(39) 99 %	(51) 90 %	
	(14) 61 %	(27) 90 %	(40) 73 %	(52) 92 %	
	(15) 76 %	(28) 76 %	(41) 76 %	(53) 93 %	

Table 1 (continued)

R	Yield	C-glucosyl flavones		Non-glucosylated flavones	
		Aldol condensation ^a	Cyclization and deprotection ^b	Aldol condensation ^b	Cyclization and deprotection ^b
		(16) 76 %	(29) 95 %	(42) 77 %	(54) 84 %
	(17) 40 %	(30) 55 %	(43) 81 %	(55) 85 %	
	(18) 55 %	n.d. ^c	(44) 84 %	n.d. ^d	
	(19) 55 %	(31) 91 %	(45) 99 %	(56) 96 %	
	(20) 74 %	(32) 85 %	(46) 61 %	(57) 63 %	

^aIsolated yield; ^breaction yields determined by LCMS; ^cnot applicable; ^dnot detected.

between rings B and D succeeded at further recovering interaction points with A β o in both rings A and B, with STD-NMR signals corresponding to protons H-3 and H-6 being unambiguously detected in the difference spectrum, at 6.70 ppm and 6.11 ppm, respectively.

From all the halogen-containing *C*-glucosyl flavone derivatives, compound **29** was the only analogue presenting a satisfactory binding interaction with A β o by STD NMR. STD signals matching protons H-6' and H-3' in ring B, as well as protons H-3 and H-6 in rings C and A, respectively, are clearly visible. We propose that the difference in bulkiness between the halogens in compounds **28** and **29** might be the underpinning reason for this result, as the larger size of the bromine atom might trigger alterations in the conformation and/or spatial orientation of the morpholine moiety, leading to a more suitable overall conformation of the flavone core. Yet, the introduction of the halogen did not match the full effect of the thiomorpholinyl derivative **22**, as the STD NMR signal for the anomeric proton H-1" failed to be observed in its difference spectrum.

Contrarily to the *C*-glucosyl flavones, aglycones generally displayed relevant water solubility issues. For this reason, the solvent pH had to be increased until signal-to-noise ratio was satisfactory in the reference spectra. However, none of the aglycones succeeded in presenting any signs of interaction with A β o by STD NMR, with the exception of compound **51** (Fig. 4).

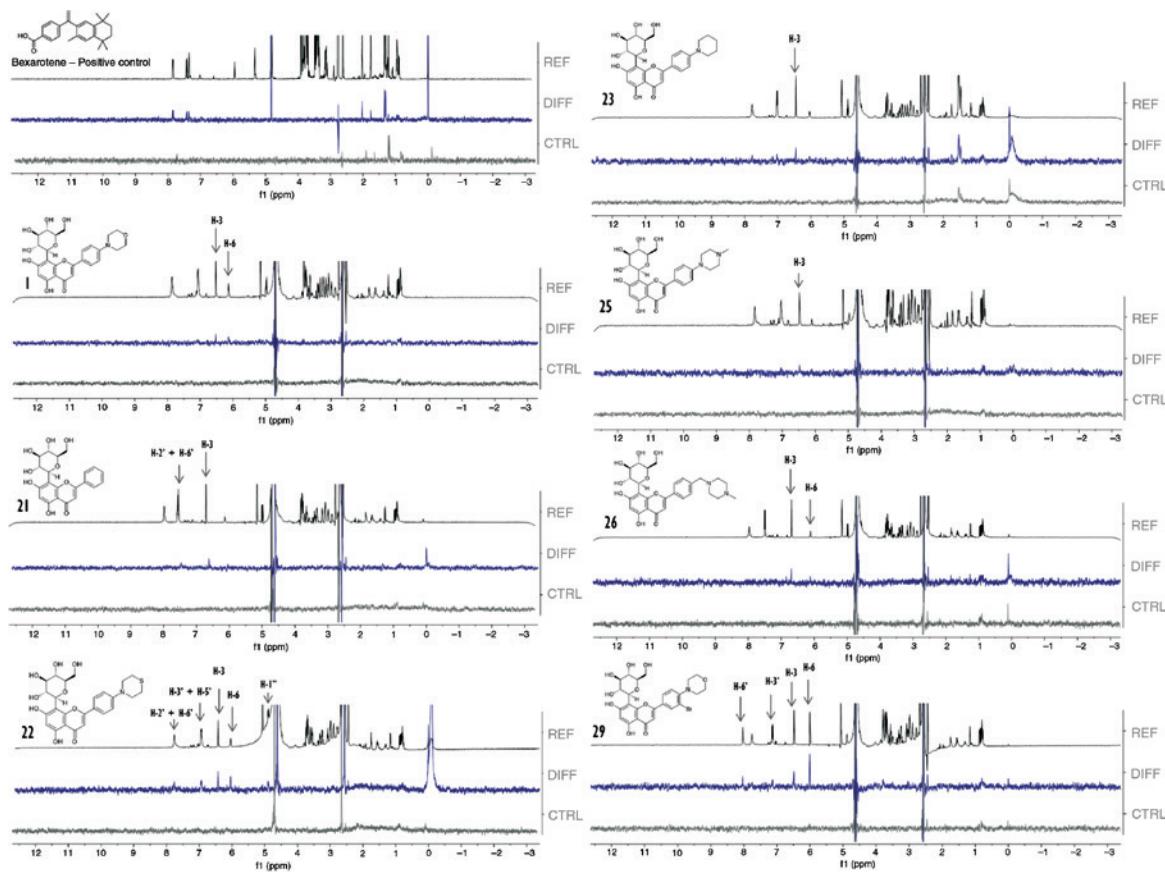


Fig. 3: STD-NMR screening assays to assess the binding affinity of flavones against A β o. For reference (REF) and difference (DIFF) spectra, compounds were added in DMSO to a 2 μ M solution of freshly prepared A β o in a mixture of deuterated phosphate buffer (pH 7.4) and neurobasal medium, to achieve a final compound concentration of 200 μ M (1:100 molar ratio). Control (CTRL) experiments were carried out in the same conditions, but in the absence of A β o.

The design of both compounds **26** and **51** was based on the introduction of a CH₂ bridge between rings B and D to increase molecular flexibility by allowing ring D to rotate around the new C–N bond. Indeed, compound **51** (Fig. 4b) was the only one amongst the synthesized set of aglycones to present good water solubility, despite the highly lipophilic flavone core. It was also the only aglycone presenting any STD interactions with A β o and, overall, one of the most promising molecules of this study. Figure 4a shows that the binding mode of compound **51** towards A β o unequivocally involves both aromatic protons of ring A (H-6 and H-8, at 6.10 ppm and 6.37 ppm), all aromatic protons of ring B (H-2', H-3', H-5' and H-6', at 7.45 ppm and 7.91 ppm), as well as the olefinic proton in ring C, H-3 (at 6.63 ppm). With a much lower signal-to-noise ratio, small peaks that could correspond to the protons of the piperazine moiety appear at 2.16 ppm and 1.85 ppm; however, due to the background spectrum (see Supporting Information, Fig. S2), it is not possible to correctly assign the exact origin of these signals. All in all, the comparison between the signal-to-noise (SNR) relative intensity of the binding epitope points towards H-3 as the most relevant point of interaction between compound **51** and the target amyloid aggregates, followed by both protons in ring A, H-6 and H-8 (Fig. 4c). Although significant, the interaction between ring B and A β o does not appear to contribute as much to the binding epitope of this molecule.

The *Central Nervous System (CNS)-MultiParameter Optimization (MPO)* algorithm is a published mathematical tool that enables the alignment of six key drug-like attributes (partition coefficient, distribution coefficient, pKa, molecular weight, topological polar surface area and the number of hydrogen bond donors), by providing an estimation of a given small molecule to enter the central nervous system, while displaying

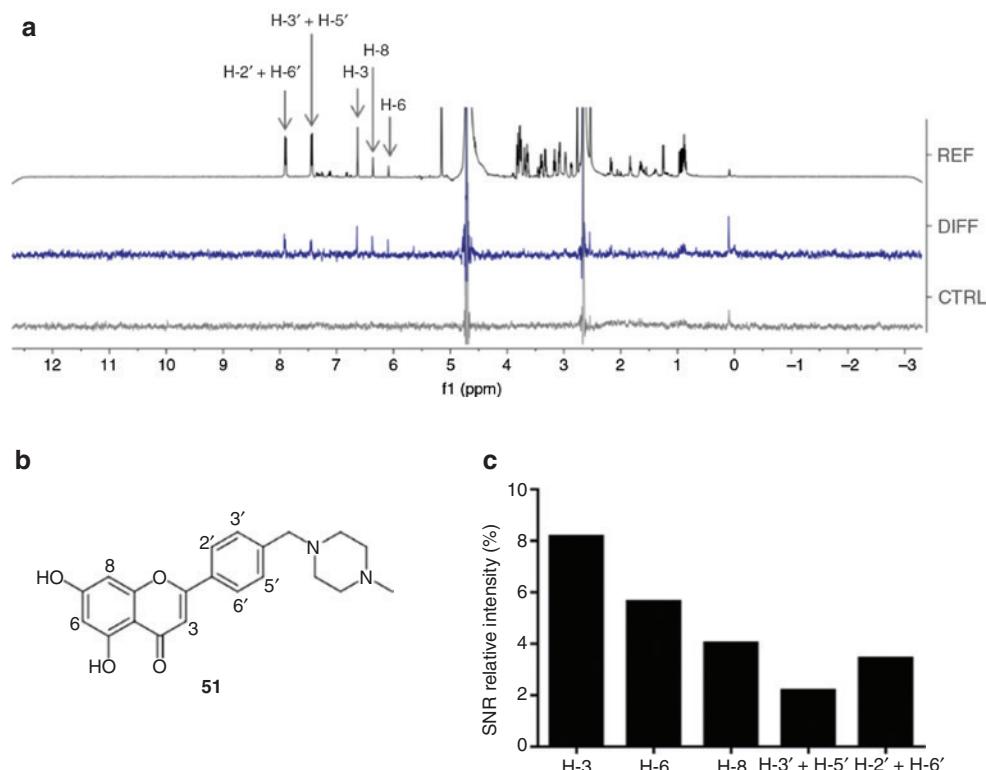


Fig. 4: Binding affinity of flavone **51** against A β , assessed by STD-NMR. (a) STD-NMR results: for the reference (REF) and difference (DIFF) spectra, **51** was added in DMSO to a 2 μ M solution of freshly prepared A β in a mixture of deuterated phosphate buffer (pH 7.4) and neurobasal medium, to achieve a final compound concentration of 200 μ M (1:100 molar ratio); the control (CTRL) experiment was carried out in the same conditions, but in the absence of A β ; (b) chemical structure of flavone **51**; (c) SNR relative intensity for STD-NMR signals of protons H-3, H-6, H-8 and aromatic protons of flavone **51**; results are presented in percentages, calculated using MestReNova SNR script tool; for each STD signal, relative intensities were calculated by dividing the corrected SNR on the difference spectrum by the corresponding SNR on the reference spectrum.

favourable permeability, P-gp efflux, metabolic stability and safety [20]. From a scale of 0–6, compound **51** exhibited a CNS-MPO desirability score of 4.9, which is highly preferable to the 2.5–3.6 scores exhibited by C-glucosyl flavones, at least in view of mere passive diffusion processes. Based on these data, and given that compound **51** was the only water soluble aglycone in this study with proven ability to bind to the target amyloid aggregates, we decided to further explore the potential of our *N*-methylpiperazinyl flavones in this study, particularly focusing on compound **51**.

We thus conducted an STD NMR competition experiment between analogue **51** and the high-affinity A β ligand bexarotene, in order to assess if any of these two compounds could act as an enhancer or inhibitor of each other's binding affinity towards A β . Moreover, based on some level of structure similarity between them, we were also interested in investigating whether they would bind to the same or different binding sites in the target protein. Figure 5 shows that bexarotene displaced compound **51** from binding to A β , with only a very small STD signal corresponding to H-6 of **51** appearing in the difference spectrum. While these data indicate that bexarotene ought to have a higher binding affinity towards the target, they also show that compound **51** possibly interacts with A β at the same binding site as bexarotene. This compound binds to a linear motif at the C-terminal of A β ₁₋₄₂ peptide, in a region encompassing residues 22–35, being therefore able not only to inhibit cholesterol-induced oligomerization of A β ₁₋₄₂ into calcium-permeable ion channels formed by A β ₁₋₄₂ in the neuronal lipid membrane, but also to counteract the disruption of actin cytoskeleton induced by A β [21, 22]. If **51** and bexarotene are both able to bind to the same binding site(s) in A β , it is plausible that **51** and structurally-related compounds might also have an impact in these mechanisms that contribute to the neurodegenerative process in AD.

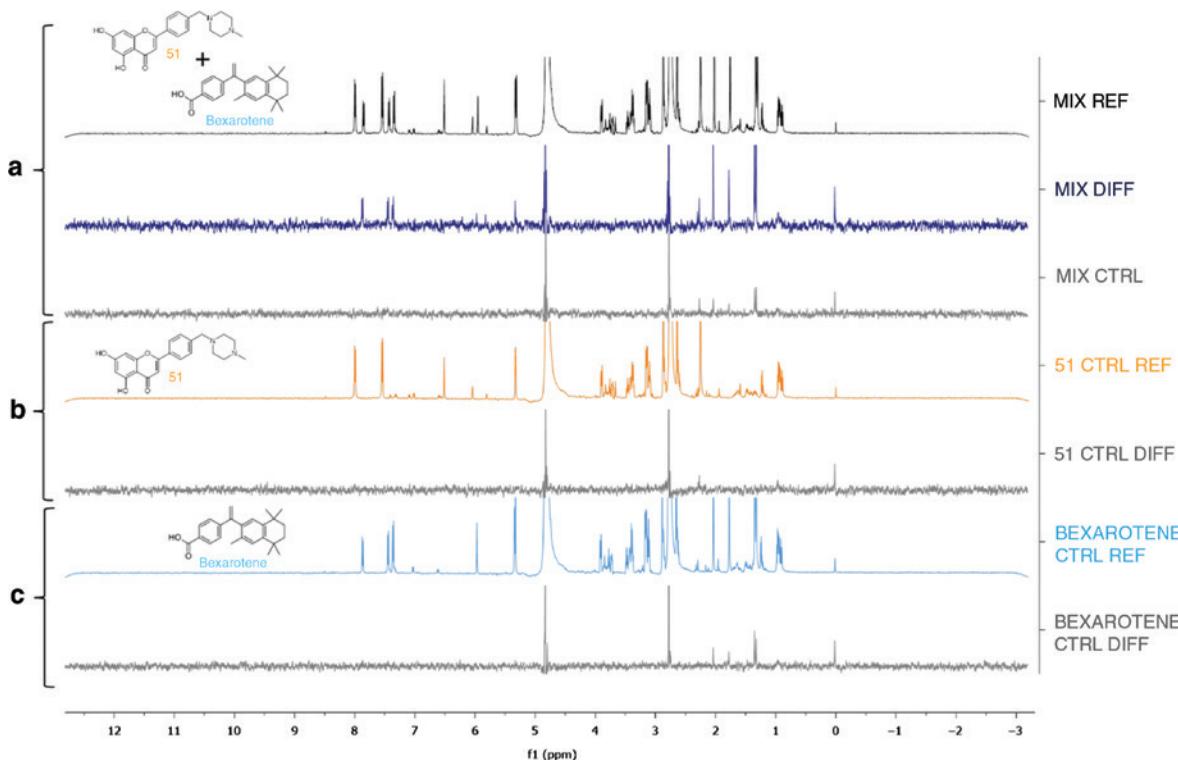


Fig. 5: STD-NMR competition assay between compound 51 and bexarotene towards A β o. (a) Mixture between compound 51 and bexarotene, both at 200 μ M; (b) compound 51 at 200 μ M; (c) bexarotene at 200 μ M. For reference (REF) and difference (DIFF) spectra, compounds were added in DMSO to a 2 μ M solution of freshly prepared A β o in a mixture of deuterated phosphate buffer (pH 7.4) and neurobasal medium. Control (CTRL, CTRL REF or CTRL DIFF) experiments were carried out in the same conditions, with 4 % DMSO, but in the absence of A β o.

Assessment of A β o-PrP C interaction disruption by flavones

The next step in this investigation was to assess if the STD NMR results obtained for compound 51 and related *N*-methylpiperazinyl flavones could translate into a positive outcome in a functional assay. Hence, we tested this small set of molecules on their ability to disrupt A β o-PrP C interactions in human embryonic kidney (HEK) cells, in which PrP C is endogenously expressed.

From all tested compounds, *C*-glucosyl flavone analogue 26 exhibited the highest potential to disrupt A β o-PrP C interactions (Fig. 6a), presenting an inhibition percentage of 41% at 10 μ M, with high statistical significance when compared to A β o-PrP C control (P -value < 0.001). As displayed in Fig. 6a, this compound shows a significantly better prognosis for further development when compared to its aglycone, compound 51: in fact, contrarily to our initial expectation, the higher number of interaction points with A β o in the STD NMR screening experiment did not necessarily translate into the highest interaction-disrupting potential (a similar result was obtained for the bromine-containing *C*-glucosyl derivative 29, with just 26 % of A β o-PrP C binding inhibition percentage; see Supporting Information, Fig. S3). These results seem to indicate: (1) that compound 51 has greater binding affinity for A β o in a region that is not involved in A β o-PrP C interaction; and (2) that compounds 26 and 50, which displayed only moderate or low affinity towards A β o by STD NMR, are nonetheless able to bind to the A β o-PrP C interaction surface, thus competing with A β o for binding towards PrP C . Moreover, in the case of the pair of compounds 26/51, the sugar moiety was clearly able to tune the activity of the aglycone towards a more specific interaction between the compound and the complex A β o-PrP C . What's more, Fig. 6b shows that the effect of 26 is dose-dependent, with statistical significance achieved from 10 μ M to 20 μ M of compound when compared to the A β o-PrP C control.

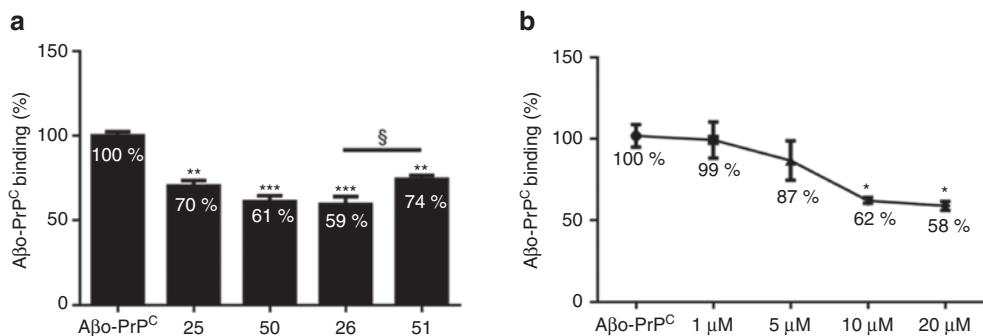


Fig. 6: Ability of *N*-methylpiperazinyl flavones to disrupt binding interactions between A β o-PrP c in HEK cells. (a) screening assay with C-glycosides and their aglycones at 10 μ M; (b) dose-response effects of compound 26. Results are presented as means \pm standard deviation of two experiments preformed in triplicates. Statistical differences between groups were assessed by one-way ANOVA followed by a Tukey's post-test. * P < 0.05, ** P < 0.01 and *** P < 0.001 vs. A β o-PrP c control; \ddagger P < 0.05 vs. another compound.

In a MTT assay performed in the same cell line, none of the four *N*-methylpiperazinyl flavones exhibited relevant signs of cytotoxicity at 20 μ M, with average cell survival rates above 77 % in all cases. However, cells treated with 50 μ M of aglycones **50** and **51** only displayed 56 % \pm 3 % and 36 % \pm 6 % of cell viability, respectively, whereas their C-glycosides displayed 91 % \pm 4 % and 79 % \pm 1 %, in turn. These results indicate that C-glycosylation could be viewed as a valuable tool for reducing the cytotoxicity of flavones as well. Anyhow, all four *N*-methylpiperazinyl flavone derivatives **25–26** and **50–51** presented statistically significant differences in the number of A β o-PrP c units when compared to the non-treated control (Fig. 6a) and, thus, the use of these types of compounds as blockers of A β o-PrP c interactions should ultimately be regarded as a novel strategy against neuronal damage in Alzheimer's disease, with potential for further development and optimization.

Conclusions

In this work, we presented the synthesis of 24 novel C-glucosyl flavones and aglycones with therapeutic potential against AD. Based on the existing robust evidence pointing towards A β o-PrP c complexes as valuable therapeutic targets for the development of PPIs with neuroprotective activity, our goal was to study the synthesized compounds as A β o-binders, and to assess whether the best molecules would also behave as promising A β o-PrP c interaction blockers.

C-glucosyl derivatives displayed optimal water solubility and were generally able to interact with A β o by STD NMR. On the other hand, compound **51** stood out from the set of non-glycosylated derivatives for being the only water-soluble aglycone, and the only one to exhibit the ability to interact with the target amyloid aggregates. Indeed, the presence of the (4-methylpiperazin-1-yl)methyl moiety in this flavone derivative results in higher molecular flexibility when compared to its planar analogues, and allows free rotation of the *N*-methylpiperazinyl group around ring B without compromising brain penetrance estimations by passive diffusion. Ultimately, this feature successfully promoted the establishment of interactions between compound **51** and A β o mediated by the whole flavone core. Our results additionally indicate that compound **51** competes with the bexarotene for the same binding site(s) in A β o. To this point, mostly due to the low stability and high size variability of A β o, it is not clear exactly where bexarotene binds in these small amyloid aggregates; nevertheless, our data suggest that compound **51** and related analogues might have the potential to yield similar effects by interfering with damaging events directly induced by A β o, such as the disruption of the actin cytoskeleton in neurons [21].

A cell-based functional assay in HEK cells revealed that all four *N*-methylpiperazinyl flavone derivatives were able to significantly disrupt the binding between A β o and PrP c at 10 μ M. Compound **26**, the C-glucosyl

derivative of **51**, presented the highest ability to block A β o-PrP^c interactions in a dose-dependent manner, with 41% of inhibition capacity at 10 μ M. Interestingly, significant differences were observed when comparing its activity to that of its aglycone, indicating that, in this particular case, the sugar moiety was able to optimize the affinity of aglycone **51** towards the A β o-PrP^c interaction region, possibly being itself part of the binding epitope. Even though sugars are not the best candidate scaffolds for crossing the blood-brain barrier by passive diffusion due to their physicochemical properties, previous evidence has recognized sugar conjugates as molecules with adequate brain-penetrating capacity (in some cases improved when compared to their aglycones) [23, 24]. In fact, it is thought that the sugar moiety might behave as a shuttle and induce the passage of the compound into the CNS due to its high affinity towards glucose transporter 1 (GLUT-1) located in the blood-brain barrier (BBB), even though the transport can occur in both directions (in and out of the brain). Examples include the C-glucosyl flavone spinosin, and anthocyanin glycosides, among others [25–27]. Ultimately, we show, for the first time in this work, the potential of non-toxic *N*-methylpiperazinyl flavones and their sugar conjugates as A β o-PrP^c interaction disruptors, thus opening a new line of investigation towards the discovery of new drug candidates with neuroprotective potential against AD progression.

Experimental section

Chemistry

HPLC grade solvents and reagents were obtained from commercial suppliers and were used without further purification. Chrysin was purchased from Sigma Aldrich. LCMS experiments were performed in a column XBridge C18 3.5u 2.1 \times 50 mm at 1.2 mL/min and 50 °C; 10 mM ammonium bicarbonate pH 9/ACN, gradient 10 > 95 % ACN in 1.5 min + 0.5 min hold. Flash column chromatography was performed using CombiFlash® Rf200 (Teledyne Isco). Preparative HPLC was performed in a Gilson apparatus using either Phenomenex gemini NX, C18, 5 μ m 30 \times 100 mm or Phenomenex gemini NX, C18, 10 μ m 50 \times 150 mm columns. NMR spectra for compound characterization were recorded on a Bruker AV III HD Nanobay spectrometer running at 400.13 MHz equipped with a room temperature 5 mm BBO Smartprobe with Z-gradients capable of ¹⁹F observation. Chemical shifts are expressed in δ (ppm) and the proton coupling constants J in hertz (Hz). NMR data were assigned using appropriate COSY, DEPT, HMQC, and HMBC spectra. For the characterization of chalcones, protons and carbons in ring A (aromatic ring attached to the carbonyl group) are assigned as H', C'; in ring B (aromatic ring attached to the propenone double bond) as H'', C''; and those belonging to the glucosyl moiety as H''', C''', while propanone atoms are labeled from 1 to 3, to facilitate the description of compound chemical shifts. Melting points were measured with a SMP3 melting point apparatus, Stuart Scientific, Bibby Melting points were obtained with a SMP3 Melting Point Apparatus, Stuart Scientific, Bibby (r.t. < m.p. < 360 °C). Optical rotations were measured with a Perkin–Elmer 343. High resolution mass spectra of new compounds were acquired on a Bruker Daltonics HR QqTOF Impact II mass spectrometer (Billerica, MA, USA). The nebulizer gas (N₂) pressure was set to 1.4 bar, and the drying gas (N₂) flow rate was set to 4.0 L/min at a temperature of 200 °C. The capillary voltage was set to 4500 V and the charging voltage was set to 2000 V. Tested compounds have \geq 95 % purity as determined by LCMS.

Synthesis and characterization of compounds **5** and **6** were carried out according to the previously reported procedures [28].

General procedure for the synthesis of benzyl-protected C-glucosyl chalcones

Compound **6** was dissolved in 1,4-dioxane (0.667 mmol in 8 mL) and the appropriate benzaldehyde (0.734 mmol, 1.1 eq.) was added. The mixture was stirred until fully homogenized. Then, an aqueous solution of NaOH 50 % (w/v, 8 mL) was slowly added and the mixture was stirred under reflux for 18 h–24 h. All reactions were followed by LCMS. Once the starting material was fully consumed, the mixture was allowed to reach room temperature. The reaction was quenched using HCl 2 M, washed with brine and extracted with

EtOAc (3×15 mL). The organic layers were combined, dried over MgSO_4 , filtered and concentrated under vacuum. The residue was purified using the most adequate purification method(s) to afford compounds **7–20**.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl- β -D-glucopyranosyl)]phenyl-3-phenyl-prop-2-en-1-one (7): Purified by column chromatography (cyclohexane/THF 1:0 → 17:3). Isolated yield: 72%; LCMS: r.t. = 1.95 min (high pH method); physical appearance: yellow oil; $^1\text{H NMR}$ (CDCl_3) δ (ppm) 14.86, 14.62 (s, 1H, OH-2')*, 7.86–7.75, 7.73–7.59 (olefinic AB system, 2H, $J_{\text{trans}} = 15.4$ Hz, H-2 and H-3)*, 7.49–7.92 (m, 35H, benzyl aromatics, H-2'', H-3'', H-4'', H-5'', H-6''), 6.07, 6.02 (s, 1H, H-5')*, 5.14–4.83 (m, 8H, Ph- CH_2 , H-1'''), 4.72–4.48 (m, 5H, Ph- CH_2 ; part A of AB system, H-4''), 4.36–4.24 (m, 2H, Ph- CH_2 ; part B of AB system, H-2'''), 3.84–3.60 (m, 4H, H-3''', H-5''', H-6''a and H-6''b). $^{13}\text{C NMR}$ (CDCl_3) δ (ppm) 193.1, 193.0 (C-1)*, 166.9, 166.1 (C-2')*, 164.4, 163.6 (C-4')*, 162.2, 162.0 (C-6')*, 142.9, 142.6 (C-3)*, 139.1, 139.0, 138.6, 138.5, 138.4, 136.4, 136.2, 135.4, 135.3 (benzyl C_q -aromatics)*, 129.9–127.0 (benzyl CH-aromatics, C-1'', C-2'', C-3'', C-4'', C-5'', C-6'' and C-2), 107.5, 107.1 (C-3')*, 106.7, 106.1 (C-1')*, 89.3, 89.1 (C-5')*, 87.9 (C-5'''), 79.8, 79.4 (C-2'')*, 79.3, 79.1 (C-4'')*, 78.6, 78.4 (C-3'')*, 75.7, 75.5, 75.2, 75.0, 74.4, 74.3, 73.5, 73.4 ($\text{CH}_2\text{-Ph}$)*, 72.9, 72.6 (C-1'')*, 71.4, 71.3, 70.8, 70.6 ($\text{CH}_2\text{-Ph}$)*, 69.6, 69.5 (C-6'')*. *Two peaks were observed due to the presence of rotamers.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl- β -D-glucopyranosyl)]phenyl-3-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (8): Purified by column chromatography (cyclohexane/THF 1:0 → 3:1). Isolated yield: 67%; LCMS: r.t. = 1.86 min (high pH method); physical appearance: orange oil; $^1\text{H NMR}$ (CDCl_3) δ (ppm) 14.68 (s, 1H, OH-2'), 7.68–7.58 (s, 2H, H-2 and H-3)*, 7.43–6.85 (m, 32H, benzyl aromatics, H-2'' and H-6''), 6.63–6.58 (m, 2H, H-3'' and H-5'')*, 5.98, 5.93 (s, 1H, H-5')*, 5.06–4.74 (m, 8H, Ph- CH_2 , H-1'''), 4.64–4.40 (m, 5H, Ph- CH_2 ; part A of AB system, H-4''), 4.28–4.17 (m, 2H, Ph- CH_2 ; part B of AB system, H-2'''), 3.74–3.53 (m, 8H, H-3''', H-5''', H-6''a, H-6''b, $\text{NCH}_2\text{CH}_2\text{O}$), 3.26–3.13 (m, 4H, $\text{NCH}_2\text{CH}_2\text{O}$)*. $^{13}\text{C NMR}$ (CDCl_3) δ (ppm) 191.9, 191.7 (C-1)*, 165.8, 165.0 (C-2')*, 163.0, 162.2 (C-4')*, 160.9, 160.7 (C-6')*, 151.3, 151.2 (C-4'')*, 142.3, 142.0 (C-3)*, 138.0, 137.6, 137.5, 137.4, 135.4, 135.3, 134.6, 134.5 (benzyl C_q -aromatics)*, 129.1–125.5 (benzyl CH-aromatics, C-1'', C-2'' and C-6''), 123.4, 123.2 (C-2)*, 119.3 (benzyl CH-aromatics), 113.5 (C-3'' and C-5''), 106.4, 106.1 (C-3')*, 105.5, 105.1 (C-1')*, 88.2, 88.1 (C-5')*, 86.8 (C-5'''), 78.8, 78.3 (C-2'')*, 78.2, 78.1 (C-4'')*, 77.6, 77.3 (C-3'''), 74.5, 74.1, 73.9, 73.3, 73.2, 72.4, 72.3 ($\text{CH}_2\text{-Ph}$)*, 71.9, 71.5 (C-1'')*, 70.3, 70.1, 69.9, 69.1, 68.4 ($\text{CH}_2\text{-Ph}$)*, 65.2 ($\text{NCH}_2\text{CH}_2\text{O}$), 65.6, 65.5 (C-6'')*, 47.1, 46.3 ($\text{NCH}_2\text{CH}_2\text{O}$)*. *Two peaks were observed due to the presence of rotamers.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl- β -D-glucopyranosyl)]phenyl-3-[4-(1,4-thiamorpholin-4-yl)phenyl]prop-2-en-1-one (9): Purified by column chromatography (cyclohexane/THF 1:0 → 1:1). Isolated yield: 68%; LCMS: r.t. = 1.86 min (lipophilic high pH method); physical appearance: orange oil; $^1\text{H NMR}$ (CDCl_3) δ (ppm) 14.62, 14.33 (s, 1H, OH-2')*, 7.68, 7.60 (s, 2H, H-2 and H-3)*, 7.43–6.88 (m, 32H, benzyl aromatics, H-2'' and H-6''), 6.60–6.56 (m, 2H, H-3'' and H-5'')*, 5.98, 5.93 (s, 1H, H-5')*, 5.04–4.74 (m, 8H, Ph- CH_2 , H-1'''), 4.65–4.16 (m, 7H, Ph- CH_2 , H-2'' and H-4''), 3.83–3.49 (m, 8H, H-3''', H-5''', H-6''a, H-6''b, $\text{NCH}_2\text{CH}_2\text{S}$), 3.66–3.63 (m, 4H, $\text{NCH}_2\text{CH}_2\text{S}$). $^{13}\text{C NMR}$ (CDCl_3) δ (ppm) 191.9, 191.7 (C-1)*, 165.7, 165.0 (C-2')*, 162.9, 162.0 (C-4'')*, 160.9, 160.7 (C-6')*, 151.4, 151.3 (C-4'')*, 142.3, 142.1 (C-3)*, 138.0, 137.8, 137.7, 137.5, 137.4, 136.9, 135.4, 135.3, 134.6, 134.5 (benzyl C_q -aromatics)*, 129.3–124.7 (benzyl CH-aromatics, C-1'', C-2'' and C-6''), 123.2, 122.9 (C-2)*, 119.3 (benzyl CH-aromatics), 114.2 (C-3'' and C-5''), 106.5, 106.2 (C-3')*, 105.9, 105.3 (C-1')*, 88.3, 88.2 (C-5')*, 86.8 (C-5'''), 78.8, 78.4 (C-2'')*, 78.3, 78.1 (C-4'')*, 77.5, 77.3 (C-3'')*, 75.2, 75.0, 74.6, 74.4, 74.1, 73.9, 73.4, 72.4, 72.3 ($\text{CH}_2\text{-Ph}$)*, 71.9, 71.5 (C-1'')*, 71.0, 70.8, 70.2 ($\text{CH}_2\text{-Ph}$)*, 69.7, 69.2 (C-6'')*, 49.8, 49.2 ($\text{NCH}_2\text{CH}_2\text{S}$)*, 25.9 ($\text{NCH}_2\text{CH}_2\text{S}$). *Two peaks were observed due to the presence of rotamers.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl- β -D-glucopyranosyl)]phenyl-3-[4-(piperidin-1-yl)phenyl]prop-2-en-1-one (10): Purified by column chromatography (iso-hexane/THF 1:0 → 1:1). Isolated yield: 56%; LCMS: r.t. = 1.89 min (lipophilic high pH method); physical appearance: orange oil; $[\alpha]_D^{20} = -11^\circ$ (c 0.1 CHCl_3); $^1\text{H NMR}$ (CDCl_3) δ (ppm) 14.68 (s, 1H, OH-2'), 7.73–7.57 (olefinic AB system, 2H, $J_{\text{trans}} = 15.4$ Hz, H-2 and H-3)*, 7.44–6.81 (m, 32H, benzyl aromatics, H-2'' and H-6''), 6.64–6.58 (m, 2H, H-3'' and H-5'')*, 5.98, 5.93 (s, 1H, H-5')*, 5.05–4.16 (m, 15H, Ph- CH_2 , H-1''', H-2'' and H-4''), 3.76–3.45 (m, 4H, H-3''', H-5''', H-6''a and H-6''b), 3.23–3.15 (m, 4H, NCH_2), 1.62–1.54 (m, 6H, $\text{NCH}_2\text{CH}_2\text{CH}_2$). $^{13}\text{C NMR}$ (CDCl_3) δ (ppm)

191.9, 191.7 (C-1)*, 165.7, 165.0 (C-2')*, 162.8, 162.0 (C-4')*, 160.9, 160.6 (C-6')*, 152.2, 151.8 (C-4'')*, 142.9, 142.7 (C-3)*, 138.1, 138.0, 137.9, 137.7, 137.4, 137.3, 136.0, 135.6, 134.7, 134.6 (benzyl C_q-aromatics)*, 129.3–124.1 (benzyl CH-aromatics, C-1", C-2" and C-6"), 123.2, 122.9 (C-2)*, 119.3 (benzyl CH-aromatics), 113.7 (C-3" and C-5"), 106.5, 106.2 (C-3')*, 105.9, 105.4 (C-1')*, 88.3, 88.2 (C-5')*, 86.8 (C-5''), 78.8, 78.4 (C-2'')*, 78.3, 78.2 (C-4'')*, 77.5, 77.3 (C-3'')*, 74.5, 74.3, 74.1, 73.9, 73.5, 73.1, 72.5, 72.3 (CH₂-Ph)*, 71.9, 71.5 (C-1'')*, 70.5, 70.2, 69.5, 69.4 (CH₂-Ph)*, 69.3, 69.2 (C-6'')*, 48.3 (NCH₂)*, 24.4 (NCH₂CH₂CH₂), 23.4 (NCH₂CH₂CH₂). *Two peaks were observed due to the presence of rotamers.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)]phenyl-3-[4-(4-tert-butyloxycarbonylpiperazin-1-yl)phenyl]prop-2-en-1-one (11): Purified by column chromatography (cyclohexane/THF 1:0 → 3:1). Isolated yield: 71%; LCMS: r.t. = 1.91 min (lipophilic high pH method); physical appearance: orange oil. ¹H NMR (CDCl₃) δ (ppm) 14.64 (s, 1H, OH-2'), 7.68–7.60 (s, 2H, H-2 and H-3)*, 7.42–6.82 (m, 32H, benzyl aromatics, H-2" and H-6"), 6.64–6.60 (m, 2H, H-3" and H-5")*, 5.99, 5.94 (s, 1H, H-5')*, 5.07–4.16 (m, 15H, Ph-CH₂, H-1'", H-2'" and H-4''), 3.89–3.46 (m, 8H, H-3'', H-5'', H-6'"a, H-6'"b and NCH₂CH₂N-Boc), 3.32–3.30, 3.18–3.15 (m, 4H, NCH₂CH₂N-Boc)*, 1.43 [s, 9H, C(CH₃)₃]. ¹³C NMR (CDCl₃) δ (ppm) 192.0, 191.7 (C-1)*, 165.8, 165.0 (C-2')*, 163.0, 162.2 (C-4')*, 160.9, 160.7 (C-6')*, 153.7 (C=O Boc), 151.2, 151.1 (C-4'')*, 143.3, 142.9 (C-3)*, 138.0, 137.6, 137.4, 135.4, 135.3, 136.6, 134.5, 130.8 (benzyl C_q-aromatics)*, 129.1–125.5 (benzyl CH-aromatics, C-1", C-2" and C-6"), 123.5, 123.2 (C-2)*, 114.2, 112.8 (C-3" and C-5''), 106.9, 106.5 (C-3')*, 105.8, 105.2 (C-1''), 88.3, 88.2 (C-5')*, 86.8 (C-5''), 79.1 [C(CH₃)₃], 78.8, 78.3 (C-2'')*, 78.3, 78.1 (C-4'')*, 77.5, 77.3 (C-3'')*, 74.6, 74.4, 74.1, 73.3, 73.2, 72.4, 72.3 (CH₂-Ph)*, 71.9, 71.5 (C-1'')*, 70.2 (CH₂-Ph)*, 69.7, 69.2 (C-6''), 47.0, 46.0 (NCH₂CH₂N-Boc)*, 27.4 [C(CH₃)₃]. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for C₇₅H₇₅NO₁₁ 1143.5365, found 1143.5368; [M + Na]⁺ calcd for C₇₅H₇₄NNaO₁₁ 1165.6184, found 1165.6178.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)]phenyl-3-[4-(4-methylpiperazin-1-yl)phenyl]prop-2-en-1-one (12): Purified by precipitation in cold methanol, followed by filtration under reduced pressure. Isolated yield: 63%; LCMS: r.t. = 1.87 min (lipophilic high pH method); physical appearance: orange solid; m.p. = 52.7–55.2°C; [α]_D²⁰ = -14° (c 0.1 CHCl₃); ¹H NMR (CDCl₃) δ (ppm) 14.33, 14.14 (s, 1H, OH-2'), 7.69–7.61 (s, 2H, H-2 and H-3)*, 7.43–6.83 (m, 32H, benzyl aromatics, H-2" and H-6"), 6.65–6.62 (m, 2H, H-3" and H-5")*, 5.99, 5.94 (s, 1H, H-5')*, 5.05–4.13 (m, 15H, Ph-CH₂, H-1'", H-2'" and H-4''), 3.76–3.50 (m, 4H, H-3'', H-5'', H-6'"a and H-6'"b) 3.35–3.33, 3.24–3.21 (m, 4H, NCH₂CH₂NCH₃)*, 2.51–2.46 (m, 4H, NCH₂CH₂NCH₃)*, 2.28 (s, 3H, NCH₃). ¹³C NMR (CDCl₃) δ (ppm) 192.0, 191.7 (C-1)*, 165.6, 165.1 (C-2')*, 162.9, 162.2 (C-4')*, 161.9, 160.9 (C-6')*, 151.3, 151.2 (C-4'')*, 142.5, 142.3 (C-3)*, 138.1, 137.9, 137.5, 137.4, 136.7, 135.4, 135.2, 134.6, 134.5, 130.8 (benzyl C_q-aromatics and C-1")*, 129.1–124.9 (benzyl CH-aromatics, C-2" and C-6"), 123.1, 122.8 (C-2)*, 113.7, 112.5 (C-3" and C-5''), 109.9, 107.8 (C-3')*, 106.5, 105.9 (C-1''), 88.3, 88.2 (C-5')*, 86.8 (C-5''), 78.8, 78.4 (C-2'')*, 78.3, 78.1 (C-4'')*, 77.5, 77.3 (C-3'')*, 74.6, 74.4, 74.1, 73.4, 73.3, 73.2, 72.4, 72.3 (CH₂-Ph)*, 71.9, 71.5 (C-1'')*, 70.2 (CH₂-Ph), 69.6, 69.2 (C-6''), 53.8, 53.7 (NCH₂CH₂NCH₃)*, 46.8, 46.1 (NCH₂CH₂NCH₃)*, 45.2, 45.1 (NCH₃)*. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for C₆₈H₆₉N₂O₉ 1057.4998, found 1057.5002; [M + Na]⁺ calcd for C₆₈H₆₈N₂NaO₉ 1079.4817, found 1079.4825.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)]phenyl-3-[4-(4-methylpiperazin-1-yl)methylphenyl]prop-2-en-1-one (13): Purified by filtration through an Isolute SCX-2 column (Biotage). Isolated yield: 58%; LCMS: r.t. = 1.89 min (lipophilic high pH method); physical appearance: orange oil. ¹H NMR (CDCl₃) δ (ppm) 14.85, 14.25 (s, 1H, OH-2')*, 7.86–7.72 (olefinic AB system, 2H, J_{trans} = 15.6 Hz, H-2 and H-3)*, 7.50–6.95 (m, 34H, benzyl aromatics, H-2", H-3", H-5" and H-6")*, 6.07, 6.02 (s, 1H, H-5')*, 5.15–4.83 (m, 8H, Ph-CH₂, H-1''), 4.70–4.45 (m, 5H, Ph-CH₂; part A of AB system, H-4''), 4.36–4.24 (m, 2H, Ph-CH₂; part B of AB system, H-2''), 3.84–3.59 (m, 4H, H-3'', H-5'', H-6'"a and H-6'"b), 3.48 (s, 2H, PhCH₂N), 2.46 (br s, 8H, NCH₂CH₂NCH₃), 2.27 (s, 3H, NCH₃). ¹³C NMR (CDCl₃) δ (ppm) 192.9, 192.0 (C-1)*, 166.8, 166.1 (C-2')*, 162.9, 162.2 (C-4')*, 162.1, 161.9 (C-6')*, 143.3, 142.7 (C-3)*, 141.3, 140.3, 139.1, 139.0, 138.5, 138.4, 137.3, 136.9, 136.3, 136.2, 135.5, 135.3, (benzyl C_q-aromatics and C-4")*, 134.1, 133.3 (C-2)*, 129.8–126.6 (benzyl CH-aromatics, C-1", C-2", C-3", C-5" and C-6"), 107.5, 107.2 (C-3')*, 106.8, 106.1 (C-1''), 89.3, 89.2 (C-5')*, 87.9

(C-5''), 79.8, 79.3 (C-2'')*, 79.3, 79.1 (C-4'')*, 78.6, 78.3 (C-3'')*, 75.6, 75.5, 75.1, 75.0, 74.4, 74.3, 73.5, 73.3 (CH₂-Ph)*, 72.8, 72.5 (C-1'')*, 71.4, 71.3, 70.7, 70.2 (CH₂-Ph)*, 69.3 (C-6''), 62.7, 62.6 (PhCH₂N)*, 55.1 (NCH₂CH₂N)*, 46.0 (NCH₃)*. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₆₉H₇₁N₂O₉ 1071.5154, found 1071.5152; [M + Na]⁺ calcd for C₆₉H₇₀N₂NaO₉ 1093.4974, found 1093.4965.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)]phenyl-3-[2-fluoro-4-(morpholin-4-yl)phenyl]prop-2-en-1-one (14): Purified by column chromatography (cyclohexane/THF 1:0 → 3:2). Isolated yield: 61%; LCMS: r.t. = 1.85 min (lipophilic high pH method); physical appearance: orange oil; $[\alpha]_D^{20} = -10^\circ$ (c 0.1 CHCl₃); ¹H NMR (CDCl₃) δ (ppm) 14.87, 14.55 (s, 1H, OH-2'')*, 7.93–7.68 (olefinic AB system, 2H, $J_{\text{trans}} = 15.9$ Hz, H-2 and H-3''), 7.48–6.83 (m, 31H, benzyl aromatics and H-6''), 6.49–6.39 (m, 2H, H-3'' and H-5''), 6.04, 5.99 (s, 1H, H-3''), 5.11–5.83 (m, 8H, Ph-CH₂, H-1''), 4.70–4.48 (m, 5H, Ph-CH₂; part A of AB system, H-4''), 4.35–4.24 (m, 2H, Ph-CH₂; part B of AB system, H-2''), 3.86–3.57 (m, 8H, H-3'', H-5'', H-6''a, H-6''b, NCH₂CH₂O), 3.23–3.20, 3.15–3.13 (m, 4H, NCH₂CH₂O)*. ¹³C NMR (CDCl₃) δ (ppm) 193.0, 192.8 (C-1)*, 166.6, 165.9 (C-2''), 164.1, 163.3 (C-4''), 163.0, 162.7 (d, $J_{\text{C-F}} = 252.7$ Hz, C-2''), 161.9, 161.7 (C-6''), 153.7, 153.6 (d, $J_{\text{C-F}} = 11.1$ Hz, C-4''), 139.1, 139.0, 138.6, 138.5, 138.4, 136.4, 136.3, 135.8, 135.7 (benzyl C_q-aromatics)*, 135.5, 135.2 (C-3''), 129.6–127.0 (benzyl CH-aromatics, C-6''), 126.0, 125.9 (d, $J_{\text{C-F}} = 4.3$ Hz C-2''), 114.0 (d, $J_{\text{C-F}} = 12.4$ Hz, C-1''), 110.2 (C-5''), 107.5, 107.1 (C-3''), 107.0, 106.4 (C-1''), 101.3, 101.2 (d, $J_{\text{C-F}} = 26.3$ Hz, C-3''), 89.5, 89.4 (C-5''), 87.8 (C-5''), 79.8, 79.4 (C-2''), 79.3, 79.1 (C-4''), 78.6, 78.3 (C-3''), 75.6, 75.5, 75.2, 75.0, 74.3, 73.5, 73.3 (CH₂-Ph)*, 72.9, 72.5 (C-1''), 71.2, 71.1, 70.7, 70.1 (CH₂-Ph)*, 69.4 (C-6''), 66.7 (NCH₂CH₂O), 48.8, 47.8 (NCH₂CH₂O). ¹⁹F NMR (CDCl₃) δ (ppm) -112.57, -112.77 (dd, $J_{\text{F-H-5''}} = 13.7$ Hz, $J_{\text{F-H-2''}} = 8.2$ Hz). **Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₆₇H₆₅FNO₁₀ 1062.4584, found 1062.4594; [M + Na]⁺ calcd for C₆₇H₆₄FNNaO₁₀ 1084.4406, found 1084.4406.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)]phenyl-3-[3-fluoro-4-(morpholin-4-yl)phenyl]prop-2-en-1-one (15): Purified by column chromatography (cyclohexane/THF 1:0 → 3:2). Isolated yield: 76%; LCMS: r.t. = 1.89 min (lipophilic high pH method); physical appearance: yellow solid; m.p. = 68.6–70.4 °C; $[\alpha]_D^{20} = -9^\circ$ (c 0.1 CHCl₃); ¹H NMR (CDCl₃) δ (ppm) 14.85, 14.52 (s, 1H, OH-2''), 7.74–7.53 (olefinic AB system, 2H, $J_{\text{trans}} = 15.6$ Hz, H-2 and H-3''), 7.49–6.90 (m, 31H, benzyl aromatics and H-5''), 6.78–6.72 (m, 2H, H-2'' and H-6''), 6.07, 6.01 (s, 1H, H-5''), 5.14–5.82 (m, 8H, Ph-CH₂, H-1''), 4.73–4.45 (m, 5H, Ph-CH₂; part A of AB system, H-4''), 4.36–4.24 (m, 2H, Ph-CH₂; part B of AB system, H-2''), 3.89–3.57 (m, 8H, H-3'', H-5'', H-6''a, H-6''b, NCH₂CH₂O), 3.27–3.24, 3.15–3.13 (m, 4H, NCH₂CH₂O)*. ¹³C NMR (CDCl₃) δ (ppm) 193.2, 192.6 (C-1)*, 166.5, 166.9 (C-2''), 164.2, 164.1 (C-4''), 162.8, 162.7 (d, $J_{\text{C-F}} = 253.3$ Hz, C-3''), 162.1, 161.7 (C-6''), 151.1, 150.9 (d, $J_{\text{C-F}} = 11.2$ Hz, C-4''), 141.6, 141.4 (C-3''), 139.1, 139.0, 138.7, 138.6, 138.5, 138.4, 136.4, 136.2, 135.8, 135.6 (benzyl C_q-aromatics)*, 129.7–126.9 (benzyl CH-aromatics and, C-1'' and C-6''), 126.4, 126.2 (d, $J_{\text{C-F}} = 4.5$ Hz C-2''), 118.1 (C-5''), 115.0, 114.9 (d, $J_{\text{C-F}} = 22.3$ Hz, C-2''), 107.4, 107.0 (C-3''), 106.8, 106.5 (C-1''), 89.6, 89.4 (C-5''), 87.8 (C-5''), 79.9, 79.3 (C-2''), 79.3, 79.1 (C-4''), 78.5, 78.3 (C-3''), 75.4, 75.4, 75.1, 74.9, 74.3, 73.5, 73.3 (CH₂-Ph)*, 72.8, 72.5 (C-1''), 71.4, 71.1, 70.2, 69.4 (CH₂-Ph)*, 69.4 (C-6''), 66.9 (NCH₂CH₂O), 50.7, 50.5 (NCH₂CH₂O)*. ¹⁹F NMR (CDCl₃) δ (ppm) -123.20 (dd, $J_{\text{F-H-6''}} = 13.8$ Hz, $J_{\text{F-H-3''}} = 8.1$ Hz). **Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₆₇H₆₅FNO₁₀ 1062.4584, found 1062.4593; [M + Na]⁺ calcd for C₆₇H₆₄FNNaO₁₀ 1084.4406, found 1084.4414.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)]phenyl-3-[3-bromo-4-(morpholin-4-yl)phenyl]prop-2-en-1-one (16): Purified by column chromatography (cyclohexane/THF 1:0 → 13:7). Isolated yield: 76%; LCMS: r.t. = 2.01 min (lipophilic high pH method); physical appearance: red solid; m.p. = 65.8–68.5 °C; $[\alpha]_D^{20} = -6^\circ$ (c 0.1 CHCl₃); ¹H NMR (CDCl₃) δ (ppm) 14.67, 14.34 (s, 1H, OH-2''), 7.75–7.56 (olefinic AB system, 2H, $J_{\text{trans}} = 15.6$ Hz, H-2 and H-3''), 7.53–6.93 (m, 31H, benzyl aromatics and H-5''), 6.85–6.81 (m, 2H, H-2'' and H-6''), 6.06, 6.01 (s, 1H, H-5''), 5.15–5.83 (m, 8H, Ph-CH₂, H-1''), 4.71–4.48 (m, 5H, Ph-CH₂; part A of AB system, H-4''), 4.35–4.23 (m, 2H, Ph-CH₂; part B of AB system, H-2''), 3.91–3.57 (m, 8H, H-3'', H-5'', H-6''a, H-6''b, NCH₂CH₂O), 3.11–3.08 (m, 4H, NCH₂CH₂O)*. ¹³C NMR (CDCl₃) δ (ppm) 192.8, 192.6 (C-1)*, 166.5, 166.8 (C-2''), 164.5, 164.4 (C-4''), 162.0, 161.8 (C-6''), 151.6 (C-4''), 140.8, 140.5 (C-3''), 139.1, 139.0, 138.5,

138.4, 136.3, 136.2, 135.4, 135.3, 134.0, 131.7 (benzyl C_q-aromatics)*, 129.0–127.0 (benzyl CH-aromatics and, C-1'', C-2'', C-6'' and C-2), 120.5 (C-5'')*, 119.3 (C-3'')*, 107.5, 107.2 (C-3'')*, 106.3, 106.1 (C-1'')*, 89.5, 89.3 (C-5'')*, 87.9, 87.8 (C-5''')*, 79.8, 79.3 (C-2'')*, 79.3, 79.1 (C-4'')*, 78.5, 78.3 (C-3'')*, 75.6, 75.5, 75.1, 75.0, 74.3, 73.5, 73.3, (CH₂-Ph)*, 72.8, 72.5 (C-1'')*, 71.4, 71.2, 70.7, 70.3 (CH₂-Ph)*, 69.4 (C-6'''), 67.0 (NCH₂CH₂O), 51.8 (NCH₂CH₂O)*. *Two peaks were observed due to the presence of rotamers.

(2E)-1-[4,6-dibenzoyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)]phenyl-3-[3-(morpholin-4-yl)phenyl]prop-2-en-1-one (17): Purified by column chromatography (cyclohexane/THF 1:0 → 3:2). Isolated yield: 40 %; LCMS: r.t. = 1.89 min (lipophilic high pH method); physical appearance: orange oil; $[\alpha]_D^{20} = -8^\circ$ (c 0.1 CHCl₃); ¹H NMR (CDCl₃) δ (ppm) 14.70, 14.36 (s, 1H, OH-2''), 7.86–7.72 (olefinic AB system, 2H, $J_{trans} = 16.5$ Hz, H-2 and H-3''), 7.49–6.94 (m, 32H, benzyl aromatics, H-5''), 6.89–6.85 (m, 2H, H-2'', H-4''), 6.62–6.59 (m, 1H, H-6''), 6.06, 6.01 (s, 1H, H-5'')*, 5.12–4.86 (m, 8H, Ph-CH₂, H-1''), 4.69–4.48 (m, 5H, Ph-CH₂; part A of AB system, H-4''), 4.35–4.23 (m, 2H, Ph-CH₂; part B of AB system, H-2''), 3.83–3.57 (m, 8H, H-3'', H-5'', H-6''a, H-6''b, NCH₂CH₂O), 3.13–3.10, 3.06–3.03 (m, 4H, NCH₂CH₂O)*. ¹³C NMR (CDCl₃) δ (ppm) 193.2, 193.0 (C-1''), 166.5, 165.8 (C-2''), 164.3, 163.5 (C-4''), 162.1, 161.9 (C-6''), 151.5, 151.4 (C-3''), 143.3 (C-3), 143.1 (C-1''), 139.1, 139.0, 138.6, 138.5, 138.4, 138.3, 136.4, 136.2, 135.5, 135.4 (benzyl C_q-aromatics)*, 127.4–127.0 (benzyl CH-aromatics, C-2 and C-5''), 119.4, 119.3 (C-6''), 117.4, 117.3 (C-4''), 116.4, 116.3 (C-2''), 107.5, 107.1 (C-3''), 107.0, 106.4 (C-1''), 89.5, 89.3 (C-5''), 87.8 (C-5''), 79.8, 79.3 (C-2''), 79.3, 79.1 (C-4''), 78.6, 78.3 (C-3''), 75.6, 75.5, 75.2, 75.0, 74.4, 74.3 (CH₂-Ph)*, 72.9, 72.5 (C-1''), 71.2 (CH₂-Ph)*, 70.8, 70.2 (C-6''), 69.4 (CH₂-Ph), 66.9 (NCH₂CH₂O), 49.1 (NCH₂CH₂O). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₆₇H₆₆NO₁₀ 1044.4681, found 1044.4681; [M + Na]⁺ calcd for C₆₇H₆₅NNaO₁₁ 1066.4501, found 1066.4500.

(2E)-1-[4,6-dibenzoyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)]phenyl-3-[2-(morpholin-4-yl)phenyl]prop-2-en-1-one (18): Purified by column chromatography (cyclohexane/THF 1:0 → 4:1). Isolated yield: 55 %; LCMS: r.t. = 1.89 min (lipophilic high pH method); physical appearance: orange solid; m.p. = 70.1–71.1 °C; $[\alpha]_D^{20} = -7^\circ$ (c 0.1 CHCl₃); ¹H NMR (CDCl₃) δ (ppm) 14.66, 14.34 (s, 1H, OH-2''), 8.10–7.97 (part A of olefinic AB system, 1H, $J_{trans} = 15.4$ Hz, H-3''), 7.72–7.59 (part B of olefinic AB system, 1H, $J_{trans} = 15.8$ Hz, H-2''), 7.42–6.89 (m, 33H, benzyl aromatics, H-3'', H-4'' and H-5''), 6.78–6.72 (m, 1H, H-6''), 6.00, 5.94 (s, 1H, H-5''), 5.06–4.73 (m, 8H, Ph-CH₂, H-1''), 4.64–4.40 (m, 5H, Ph-CH₂; part A of AB system, H-4''), 4.30–4.18 (m, 2H, Ph-CH₂; part B of AB system, H-2''), 3.84–3.50 (m, 8H, H-3'', H-5'', H-6''a, H-6''b, NCH₂CH₂O), 2.93–2.90, 2.85–2.79 (m, 4H, NCH₂CH₂O)*. ¹³C NMR (CDCl₃) δ (ppm) 192.4, 192.1 (C-1''), 165.4, 164.8 (C-2''), 163.2, 162.3 (C-4''), 160.9, 160.7 (C-6''), 151.7, 151.6 (C-2''), 139.4, 138.8 (C-3''), 138.6, 138.0, 137.9, 137.6, 137.5, 137.4, 135.3, 135.2, 134.3, 134.2, 129.5, 128.5 (benzyl C_q-aromatics)*, 127.9–124.1 (benzyl CH-aromatics, C-2, C-1'' and C-4''), 122.3, 122.0 (C-6''), 119.6, 119.4 (C-5''), 117.4 (C-3''), 106.5, 106.2 (C-3''), 106.0, 105.3 (C-1''), 88.2, 88.1 (C-5''), 86.8 (C-5''), 78.8, 78.3 (C-2''), 78.3, 78.1 (C-4''), 77.5, 77.1 (C-3''), 74.6, 74.5, 74.1, 73.9, 73.3, 72.4, 72.3 (CH₂-Ph)*, 71.9, 71.5 (C-1''), 70.3, 70.2 (CH₂-Ph)*, 69.7, 69.2 (C-6''), 68.4 (CH₂-Ph), 66.2, 66.1 (NCH₂CH₂O)*, 52.2 (NCH₂CH₂O). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₆₇H₆₆NO₁₀ 1044.4681, found 1044.4677; [M + Na]⁺ calcd for C₆₇H₆₅NNaO₁₁ 1066.4501, found 1066.4497.

(2E)-1-[4,6-dibenzoyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)]phenyl-3-[(4-dimethylamino)phenyl]prop-2-en-1-one (19): Purified by column chromatography (cyclohexane/THF 1:0 → 3:2). Isolated yield: 55 %; LCMS: r.t. = 1.91 min (lipophilic high pH method); physical appearance: orange solid; m.p. = 168.8–169.4 °C; $[\alpha]_D^{20} = -15^\circ$ (c 0.1 CHCl₃); ¹H NMR (CDCl₃) δ (ppm) 14.83 (s, 1H, OH-2''), 7.83–7.64 (olefinic AB system, 2H, $J_{trans} = 15.5$ Hz, H-2 and H-3''), 7.52–6.95 (m, 32H, benzyl aromatics, H-2'' and H-6''), 6.52–6.49 (m, 2H, H-3'' and H-5''), 6.05, 6.01 (s, 1H, H-5''), 5.15–4.83 (m, 8H, Ph-CH₂, H-1''), 4.74–4.46 (m, 5H, Ph-CH₂; part A of AB system, H-4''), 4.36–4.25 (m, 2H, Ph-CH₂; part B of AB system, H-2''), 3.84–3.58 (m, 4H, H-3'', H-5'', H-6''a, H-6''b), 3.01, 3.00 [N(CH₃)₂]⁺. ¹³C NMR (CDCl₃) δ (ppm) 192.9, 192.7 (C-1''), 166.8, 166.1 (C-2''), 163.7, 163.0 (C-4''), 161.9, 161.7 (C-6''), 151.7, 151.6 (C-4''), 144.4, 144.2 (C-3''), 139.1, 139.0, 138.8, 138.6, 138.5, 138.3, 136.5, 136.4, 135.7, 130.5, 130.4 (benzyl C_q-aromatics)*, 129.0–126.9 (benzyl CH-aromatics),

C-2" and C-6"), 123.3 (C-1"), 122.5, 122.3 (C-2)*, 111.7, 111.0 (C-3" and C-5")*, 107.5, 107.2 (C-3')*, 107.0, 106.4 (C-1')*, 89.4, 89.2 (C-5')*, 87.8 (C-5"), 79.9, 79.4 (C-2")*, 79.3, 79.1 (C-4")*, 78.6, 78.4 (C-3")*, 75.6, 75.5, 75.1, 75.0, 74.4, 74.3, 73.5, 73.3 (CH₂-Ph)*, 73.0, 72.6 (C-1")*, 73.1, 71.2, 70.7, 70.2 (CH₂-Ph)*, 69.4 (C-6"), 40.2, 40.1 [N(CH₂)₃]. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₆₅H₆₄NO₉, 1002.4576, found 1002.4579; [M + Na]⁺ calcd for C₆₅H₆₃NNaO₉, 1024.4395, found 1024.4399.

(2E)-1-[4,6-dibenzylxyloxy-2-hydroxy-3-(2,3,4,6-tetra-O-benzyl- β -D-glucopyranosyl)]phenyl-3-[(4-diphenylamino)phenyl]prop-2-en-1-one (20): Purified by column chromatography (cyclohexane/THF 1:0 → 7:3). Isolated yield: 74 %; LCMS: r.t. = 1.92 min (lipophilic high pH method); physical appearance: orange oil; $[\alpha]_D^{20} = -5^\circ$ (c 0.1 CHCl₃); ¹H NMR (CDCl₃) δ (ppm) 14.68, 14.40 (s, 1H, OH-2")*, 7.78–7.71, 7.69–7.63 (olefinic AB system, 2H, J_{trans} = 15.6 Hz, H-2 and H-3)*, 7.49–6.95 (m, 42H, benzyl aromatics, NPh₂, H-2" and H-6"), 6.84–6.80 (m, 2H, H-3" and H-5")*, 6.05, 6.00 (s, 1H, H-5")*, 5.13–4.81 (m, 8H, Ph-CH₂, H-1"), 4.73–4.45 (m, 5H, Ph-CH₂; part A of AB system, H-4"), 4.36–4.24 (m, 2H, Ph-CH₂; part B of AB system, H-2"), 3.83–3.56 (m, 4H, H-3", H-5", H-6" a, H-6" b). ¹³C NMR (CDCl₃) δ (ppm) 192.9, 192.7 (C-1)*, 166.8, 166.1 (C-2)*, 164.1, 164.0 (C-4)*, 163.3, 162.0 (C-6)*, 149.7, 149.6 (C-4")*, 146.9 (C_q-Ph), 142.8, 142.6 (C-3)*, 139.0, 138.7, 138.5, 136.5, 136.3, 135.4, 135.3 (benzyl C_q-aromatics)*, 129.6–126.9 (benzyl CH-aromatics, C-1", C-2" and C-6"), 125.3 (C-2)*, 124.0, 123.9 (benzyl CH-aromatics)*, 121.7, 121.3 (C-3" and C-5")*, 107.9, 107.5 (C-3")*, 106.2, 106.0 (C-1")*, 89.3 (C-5")*, 87.8 (C-5"), 79.9, 79.4 (C-2")*, 79.3, 79.1 (C-4")*, 78.5, 78.4 (C-3")*, 75.6, 75.5, 75.1, 75.0, 74.4, 74.3, 73.5, 73.3 (CH₂-Ph)*, 73.1, 72.9 (C-1")*, 71.3, 71.2, 70.7, 70.2 (CH₂-Ph)*, 69.4 (C-6"). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₇₅H₆₈NO₉, 1126.4884, found 1126.4890.

General procedure for the synthesis of C-glucosyl flavones

Each *C*-glucosyl chalcone **7–20** was dissolved in dry pyridine (0.172 mmol in 5.11 mL). Then, catalytic amounts of I₂ (0.060 mmol, 0.35 eq.) were added and the mixture was stirred under reflux for 48 h–72 h. All reactions were followed by LCMS. Once the starting material was fully consumed, the mixture was allowed to reach room temperature and the pyridine was co-evaporated with toluene under reduced pressure. The residue was resuspended in dichloromethane, washed first with a saturated solution of sodium thiosulfate, and then with brine. The flavone was extracted with dichloromethane (3 × 30 mL), dried over MgSO₄, and the solution filtered and concentrated under vacuum. The residue was then resuspended in extra dry dichloromethane (7.10 mL) and stirred at –78 °C under N₂ saturated atmosphere. A 1 M solution of BBr₃ in dichloromethane (1.72 mmol, 10 eq.) was added in a dropwise manner over 5 min, and the reaction stirred for 2 h–4 h. After having reached completion by LCMS, the reaction was quenched with a 1:1 mixture of dichloromethane/methanol (ca. 15 mL) and the reaction was stirred for approximately 20 min at room temperature. The solvent was evaporated under vacuum and the residue purified using the most adequate purification method(s) to afford compounds **1** and **21–32**.

5,7-Dihydroxy-8-(β -D-glucopyranosyl)-4'-(morpholin-4-yl)flavone (1): Purified by preparative HPLC. Reaction yield over two steps: 74 %; LCMS: r.t. = 0.57 min, *m/z* = 500.0 [M–H][–] (high pH method); physical appearance: orange solid; m.p. = 210.5–211.4 °C; $[\alpha]_D^{20} = +10^\circ$ (c 0.5 MeOH); ¹H NMR (MeOD) δ (ppm) 7.94, 7.84 (d, 2H, J_{ortho} = 8.3 Hz, H-2" and H-6")*, 7.01 (d, 2H, J_{ortho} = 8.6 Hz, H-3" and H-5"), 6.52 (s, 1H, H-3), 6.26 (s, 1H, H-6), 5.05, 4.99 (d, 1H, $J_{1",2"} = 9.9$ Hz, H-1")*, 4.14 (t, 1H, $J_{2",1"-2",3"} = 9.5$ Hz, H-2"), 3.98–3.69 (m, 6H, H-6" a, H-6" b and NCH₂CH₂O), 3.70 (t, 1H, $J_{4",3"-4",5"} = 9.6$ Hz, H-4"), 3.56–3.54 (m, 1H, H-3"), 3.50–3.45 (m, 1H, H-5"), 3.32–3.20 (NCH₂CH₂O, superimposed with the MeOD peak). ¹³C NMR (MeOD) δ (ppm) 184.8 (C-4), 166.7 (C-2), 164.7 (C-7), 162.8 (C-5), 152.3 (C-8a), 155.3 (C-4'), 129.7 (C-2" and C-6"), 122.3 (C-1'), 115.5 (C-3" and C-5"), 106.0 (C-4a), 105.4 (C-8), 103.2 (C-3), 99.6 (C-6), 83.1 (C-5"), 80.5 (C-3"), 75.5 (C-1"), 73.1 (C-2"), 72.3 (C-4"), 67.9 (NCH₂CH₂O), 63.0 (C-6"), 47.1 (NCH₂CH₂O). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₂₅H₂₈NO₁₀, 502.1708, found 502.1695.

5,7-Dihydroxy-8-(β -D-glucopyranosyl)flavone (21): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 88%; LCMS: r.t. = 0.49 min, m/z = 414.80 [$M - H$]⁺ (high pH method); physical appearance: yellow solid; m.p. = 188.1–189.2 °C. **¹H NMR** (MeOD) δ (ppm) 8.12, 8.02 (d, 2H, $J_{\text{ortho}} = 7.1$ Hz, H-2' and H-6')*, 7.58–7.54 (m, 3H, H-3', H-4' and H-5'), 6.75 (s, 1H, H-3), 6.30 (s, 1H, H-6), 5.09, 5.02 (d, 1H, $J_{1''-2''} = 9.9$ Hz, H-1')*, 4.13 (t, 1H, $J_{2''-1''-2''-3''} = 9.3$ Hz, H-2''), 3.99, 3.93 (br d, 1H, $J_{6''-\text{a}-6''-\text{b}} = 12.1$ Hz, H-6''a)*, 3.83 (dd, 1H, $J_{6''-\text{b}-6''-\text{a}} = 12.1$ Hz, $J_{6''-\text{b}-5''} = 5.3$ Hz, H-6''a), 3.69 (t, 1H, $J_{4''-3''-4''-5''} = 9.2$ Hz, H-4''), 3.57–3.48 (m, 2H, H-3'' and H-5''). **¹³C NMR** (MeOD) δ (ppm) 182.8 (C-4), 164.6 (C-2), 163.4 (C-7), 161.3 (C-5), 156.8 (C-8a), 131.6 (C-3' and C-5'), 131.3 (C-1'), 128.8 (C-4'), 126.6, 126.3 (C-2' and C-6')*, 104.6 (C-4a), 104.3 (C-3), 103.9 (C-8), 98.2 (C-6), 81.5 (C-5''), 78.8 (C-3''), 73.9 (C-1''), 71.4 (C-2''), 70.9, 70.1 (C-4'')*, 61.7, 61.2 (C-6'')*. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for $C_{21}H_{21}O_9$ 417.1180, found 417.1174.

5,7-Dihydroxy-8-(β -D-glucopyranosyl)-4'-(1,4-thiazinane-4-yl)flavone (22): Purified by preparative HPLC followed by SFCMS (naphthyl column). Reaction yield over two steps: 24%; LCMS: r.t. = 0.70 min, m/z = 518.2 [$M + H$]⁺ (high pH method); physical appearance: yellow solid; **¹H NMR** (MeOD) δ (ppm) 7.84, 7.74 (d, 2H, $J_{\text{ortho}} = 8.5$ Hz, H-2' and H-6')*, 6.99, 6.88 (d, 2H, $J_{\text{ortho}} = 8.5$ Hz, H-3' and H-5')*, 6.42, 6.41 (s, 1H, H-3)*, 6.15 (s, 1H, H-6), 4.96, 4.89 (d, 1H, $J_{1''-2''} = 9.9$ Hz, H-1'')*, 4.04 (t, 1H, $J_{2''-1''-2''-3''} = 9.6$ Hz, H-2''), 3.84 (t, 1H, $J_{6''-\text{a}-6''-\text{b}} = 11.8$ Hz, H-6''a), 3.74–3.68 (m, 5H, H-6''b and $\text{NCH}_2\text{CH}_2\text{S}$), 3.61 (t, 1H, $J_{4''-3''-4''-5''} = 9.4$ Hz, H-4''), 3.44 (t, 1H, $J_{3''-2''-3''-4''} = 8.9$ Hz, H-3''), 3.40–3.35 (m, 1H, H-5''), 2.59–2.57 (m, 4H, $\text{NCH}_2\text{CH}_2\text{S}$). **¹³C NMR** (MeOD) δ (ppm) 182.6 (C-4), 165.2 (C-2), 163.3 (C-7), 161.2 (C-5), 156.8 (C-8a), 152.5 (C-4'), 128.4 (C-2' and C-6'), 119.5 (C-1'), 114.2 (C-3' and C-5'), 103.4, 103.3 (C-8, C-4a), 101.2 (C-3), 98.0 (C-6), 81.4 (C-5''), 78.9 (C-3''), 73.9 (C-1''), 71.5 (C-2''), 70.9 (C-4''), 61.6 (C-6''), 50.1 ($\text{NCH}_2\text{CH}_2\text{S}$), 25.3 ($\text{NCH}_2\text{CH}_2\text{S}$). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for $C_{25}H_{28}\text{NO}_9\text{S}$ 518.1479, found 518.1478; [M + Na]⁺ calcd for $C_{25}H_{27}\text{NNaO}_9\text{S}$ 540.1299, found 540.1298.

5,7-Dihydroxy-8-(β -D-glucopyranosyl)-4'-(piperidin-1-yl)flavone (23): Purified by preparative HPLC followed by SFCMS (naphthyl column). Reaction yield over two steps: 92%; LCMS: r.t. = 0.76 min, m/z = 500.2 [$M + H$]⁺ (high pH method); physical appearance: yellow solid; m.p. = 203.0–204.5 °C; $[\alpha]_D^{20} = +14^\circ$ (c 0.2 MeOH); **¹H NMR** (MeOD) δ (ppm) 7.93, 7.85 (d, 2H, $J_{\text{ortho}} = 8.0$ Hz, H-2' and H-6')*, 7.01 (d, 2H, $J_{\text{ortho}} = 8.8$ Hz, H-3' and H-5'), 6.52 (s, 1H, H-3), 6.26 (s, 1H, H-6), 5.02 (d, 1H, $J_{1''-2''} = 9.8$ Hz, H-1'')*, 4.16 (t, 1H, $J_{2''-1''-2''-3''} = 9.2$ Hz, H-2''), 3.97 (br d, 1H, $J_{6''-\text{a}-6''-\text{b}} = 12.1$ Hz, H-6''a), 3.82 (dd, 1H, $J_{6''-\text{b}-6''-\text{a}} = 12.1$ Hz, $J_{6''-\text{b}-5''} = 5.7$ Hz, H-6''a), 3.72 (t, 1H, $J_{4''-3''-4''-5''} = 9.2$ Hz, H-4''), 3.56 (t, 1H, $J_{3''-2''-3''-4''} = 8.8$ Hz, H-3''), 3.51–3.47 (m, 1H, H-5''), 3.41–3.38 (m, 4H, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 1.70 (s, 6H, $\text{NCH}_2\text{CH}_2\text{CH}_2$). **¹³C NMR** (MeOD) δ (ppm) 182.5 (C-4), 165.3 (C-2), 163.7 (C-7), 161.2 (C-5), 156.7 (C-8a), 153.9 (C-4'), 128.1 (C-2' and C-6'), 119.1 (C-1'), 114.0 (C-3' and C-5'), 104.1, 103.8 (C-8 and C-4a), 101.0 (C-3), 98.2 (C-6), 81.5 (C-5''), 78.9 (C-3''), 73.9 (C-1''), 71.5 (C-2''), 70.9 (C-4''), 61.7 (C-6''), 48.3 ($\text{NCH}_2\text{CH}_2\text{CH}_2$), 25.2 ($\text{NCH}_2\text{CH}_2\text{CH}_2$), 24.1 ($\text{NCH}_2\text{CH}_2\text{CH}_2$). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for $C_{26}H_{30}\text{NO}_9$ 500.1915, found 500.1914; [M + Na]⁺ calcd for $C_{26}H_{29}\text{NNaO}_9$ 522.1753, found 522.1731.

5,7-Dihydroxy-8-(β -D-glucopyranosyl)-4'-(piperazin-1-yl)flavone (24): Purified by filtration through an Isolute SCX-2 column (Biotage), followed by preparative HPLC. Reaction yield over two steps: 77%; LCMS: r.t. = 0.47 min, m/z = 501.2 [$M + H$]⁺ (high pH method); physical appearance: orange solid; m.p. > 360 °C; $[\alpha]_D^{20} = +16^\circ$ (c 0.3 MeOH); **¹H NMR** [(CD₃)₂SO] δ (ppm) 8.01, 7.92 (d, 2H, $J_{\text{ortho}} = 8.5$ Hz, H-2' and H-6')*, 7.07, 7.01 (d, 2H, $J_{\text{ortho}} = 8.9$ Hz, H-3' and H-5')*, 6.79, 6.76 (s, 1H, H-3)*, 6.26 (s, 1H, H-6), 4.69, 4.61 (d, 1H, $J_{1''-2''} = 9.9$ Hz, H-1'')*, 3.85 (t, 1H, $J_{2''-1''-2''-3''} = 9.2$ Hz, H-2''), 3.77 (br d, 1H, $J_{6''-\text{a}-6''-\text{b}} = 11.7$ Hz, H-6''a), 3.56–3.22 (m, 8H, H-3'', H-4'', H-5'', H-6''b and $\text{NCH}_2\text{CH}_2\text{N}$), 2.91–2.89 (m, 4H, $\text{NCH}_2\text{CH}_2\text{N}$). **¹³C NMR** [(CD₃)₂SO] δ (ppm) 182.4 (C-4), 164.8 (C-2), 163.6 (C-7), 161.5 (C-5), 157.4 (C-8a), 153.5 (C-4'), 128.8 (C-2' and C-6'), 121.3 (C-1'), 114.2 (C-3' and C-5')*, 105.8 (C-8), 104.5 (C-4a), 102.2 (C-3), 98.6 (C-6), 82.1 (C-5''), 79.2 (C-3''), 74.2 (C-1''), 71.6 (C-2''), 71.4 (C-4''), 62.0 (C-6''), 47.8 ($\text{NCH}_2\text{CH}_2\text{NH}$), 45.5 ($\text{NCH}_2\text{CH}_2\text{NH}$). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for $C_{25}H_{29}\text{N}_2\text{O}_9$ 501.1868, found 501.1873; [M + Na]⁺ calcd for $C_{25}H_{28}\text{N}_2\text{NaO}_9$ 523.1687, found 523.1687.

5,7-Dihydroxy-8-(β -D-glucopyranosyl)-4'-(4-methylpiperazin-1-yl)flavone (25): Purified by preparative HPLC. Reaction yield over two steps: 70 %; LCMS: r.t. = 0.71 min, m/z = 515.0 [M + H]⁺ (high pH method); physical appearance: yellow solid; m.p. = 213.3–214.7 °C; $[\alpha]_D^{20} = +13^\circ$ (c 0.4 MeOH); ¹H NMR [(CD₃)₂SO] δ (ppm) 13.25 (s, 1H, OH-5), 8.09 (d, 2H, $J_{\text{ortho}} = 8.4$ Hz, H-2' and H-6'), 7.02 (d, 2H, $J_{\text{ortho}} = 8.6$ Hz, H-3' and H-5'), 6.75 (s, 1H, H-3), 6.24 (s, 1H, H-6), 4.98–4.93 (m, 2H, OH), 4.70 (d, 1H, $J_{1''-2''} = 9.8$ Hz, H-1''), 4.62–4.58 (m, 1H, OH), 3.85 (t, 1H, $J_{2''-3''-2''-3''} = 9.4$ Hz, H-2''), 3.79–3.79 (m, 1H, H-6'a), 3.55–3.50 (m, 1H, H-6'b), 3.41–3.22 (m, 7H, H-3'', H-4'', H-5'' and NCH₂CH₂NCH₃), 2.46–2.43 (NCH₂CH₂NCH₃), 2.23 (s, 3H, NCH₃). ¹³C NMR [(CD₃)₂SO] δ (ppm) 181.3 (C-4), 163.4 (C-2), 162.5 (C-7), 159.8 (C-5), 155.3 (C-8a), 152.6 (C-4'), 127.7 (C-2' and C-6'), 118.9 (C-1'), 113.2 (C-3' and C-5'), 103.9 (C-8), 103.3 (C-4a), 101.1 (C-3), 99.8 (C-6), 81.3 (C-5''), 78.1 (C-3''), 72.8 (C-1''), 70.3 (C-2''), 70.0 (C-4''), 60.8 (C-6''), 53.7 (NCH₂CH₂NCH₃), 46.0 (NCH₂CH₂NCH₃), 45.1 (NCH₃). HRMS-ESI (m/z): [M + H]⁺ calcd for C₂₅H₂₈NO₁₀ 502.1708, found 502.1700; [M + Na]⁺ calcd for C₂₅H₂₇NNaO₁₀ 524.1527, found 524.1518.

5,7-Dihydroxy-8-(β -D-glucopyranosyl)-4'-(4-methylpiperazin-1-yl)methyl]flavone (26): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 84 %; LCMS: r.t. = 0.53 min, m/z = 529.2 [M + H]⁺ (high pH method); physical appearance: orange solid; m.p. = 191.7–192.8 °C; $[\alpha]_D^{20} = +18^\circ$ (c 0.1 MeOH); ¹H NMR [MeOD] δ (ppm) 8.11, 8.01 (d, 2H, $J_{\text{ortho}} = 8.1$ Hz, H-2' and H-6')*, 7.56 (d, 2H, $J_{\text{ortho}} = 8.5$ Hz, H-3' and H-5'), 6.75 (s, 1H, H-3), 6.30 (s, 1H, H-6), 5.02 (d, 1H, $J_{1''-2''} = 9.9$ Hz, H-1''), 4.12 (t, 1H, $J_{2''-3''-2''-3''} = 9.6$ Hz, H-2''), 4.00–3.94 (m, 1H, H-6'a), 3.85–3.79 (m, 1H, H-6'b), 3.72–3.48 (m, 5H, H-3'', H-4'', H-5'' and PhCH₂N), 2.66–2.51 (NCH₂CH₂NCH₃), 2.35 (s, 3H, NCH₃). ¹³C NMR [MeOD] δ (ppm) 182.7 (C-4), 164.3 (C-2), 164.0 (C-7), 161.3 (C-5), 157.1 (C-8a), 142.0 (C-4'), 130.4 (C-1'), 129.7 (C-3' and C-5'), 126.7 (C-2' and C-6'), 104.2, 104.2 (C-8, C-4a and C-3), 98.8 (C-6), 81.5 (C-5''), 78.8 (C-3''), 73.9 (C-1''), 71.5 (C-2''), 70.9 (C-4''), 61.8 (PhCH₂N), 61.6 (C-6''), 54.3 (NCH₂CH₂NCH₃), 52.0 (NCH₂CH₂NCH₃), 44.4 (NCH₃). HRMS-ESI (m/z): [M + H]⁺ calcd for C₂₇H₃₃N₂O₉ 529.2181, found 529.2184; [M + Na]⁺ calcd for C₂₇H₃₂N₂NaO₉ 551.2000, found 551.1996.

2'-Fluoro-5,7-dihydroxy-8-(β -D-glucopyranosyl)-4'-(morpholin-4-yl)flavone (27): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 90 %; LCMS: r.t. = 0.65 min, m/z = 520.2 [M + H]⁺ (low pH method); physical appearance: orange solid; m.p. 225.1–227.2 °C; $[\alpha]_D^{20} = +8^\circ$ (c 0.1 MeOH); ¹H NMR (MeOD) δ (ppm) 8.23, 7.91 (t, 1H, $J_{\text{ortho-H-F}} = 8.8$ Hz, H-6')*, 6.93 (d, 1H, $J_{\text{ortho}} = 8.7$ Hz, H-5'), 6.80 (d, 1H, $J_{\text{H-F}} = 16.1$ Hz, H-3'), 6.65 (s, 1H, H-3), 6.29 (s, 1H, H-6), 4.99 (d, 1H, $J_{1''-2''} = 9.7$ Hz, H-1''), 4.09 (t, 1H, $J_{2''-3''-2''-3''} = 9.3$ Hz, H-2''), 3.98–3.79 (m, 6H, H-6'a, H-6'b and NCH₂CH₂O), 3.60 (t, 1H, $J_{4''-3''-4''-5''} = 9.6$ Hz, H-4''), 3.53 (t, 1H, $J_{3''-2''-3''-4''} = 8.5$ Hz, H-3''), 3.49–3.44 (m, 1H, H-5''), 3.39–3.29 (NCH₂CH₂O, superimposed with the MeOD peak). ¹³C NMR (MeOD) δ (ppm) 182.6 (C-4), 163.2 (C-7), 162.5 (d, $J_{\text{C-F}} = 250.0$ Hz, C-2'), 161.4 (C-5), 160.3 (C-2), 156.6 (C-8a), 155.1 (d, $J_{\text{C-F}} = 11.1$ Hz, C-4'), 129.9 (C-6'), 109.8 (C-5'), 108.2 (d, $J_{\text{C-F}} = 10.3$ Hz, C-1'), 106.4 (C-3), 104.5 (C-4a), 103.7 (C-8), 100.8 (d, $J_{\text{C-F}} = 28.5$ Hz, C-3'), 98.0 (C-6), 81.5 (C-5''), 78.8 (C-3''), 73.9 (C-1''), 71.4 (C-2''), 70.6, 70.2 (C-4'')*, 66.1 (NCH₂CH₂O), 61.4 (C-6''), 47.0 (NCH₂CH₂O). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for C₂₅H₂₇FNO₁₀ 520.1614, found 520.1613; [M + Na]⁺ calcd for C₂₅H₂₆FNNaO₁₀ 542.1433, found 542.1428.

3'-Fluoro-5,7-dihydroxy-8-(β -D-glucopyranosyl)-4'-(morpholin-4-yl)flavone (28): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 76 %; LCMS: r.t. = 0.59 min, m/z = 518.0 [M - H]⁻ (high pH method); physical appearance: orange solid; m.p. = 206.0–207.0 °C; $[\alpha]_D^{20} = +15^\circ$ (c 0.5 MeOH); ¹H NMR (MeOD) δ (ppm) 7.88–7.69 (m, 2H, H-2' and H-6')*, 7.12 (t, 1H, $J_{\text{ortho-H-F}} = 8.7$ Hz, 1H, H-5'), 6.65 (s, 1H, H-3), 6.28 (s, 1H, H-6), 5.06, 5.01 (d, 1H, $J_{1''-2''} = 9.9$ Hz, H-1''), 4.09 (t, 1H, $J_{2''-1''-2''-3''} = 9.6$ Hz, H-2''), 4.01 (br d, 1H, $J_{6''\text{a}-6''\text{b}} = 12.4$ Hz, H-6'a), 3.89–3.82 (m, 5H, H-6'b and NCH₂CH₂O), 3.71 (t, 1H, $J_{4''-3''-4''-5''} = 9.2$ Hz, H-4''), 3.56 (t, 1H, $J_{3''-2''-3''-4''} = 8.6$ Hz, H-3''), 3.53–3.49 (m, 1H, H-5''), 3.28–3.19 (m, 4H, NCH₂CH₂O). ¹³C NMR (MeOD) δ (ppm) 182.6 (C-4), 163.3 (C-7 and C-2), 161.3 (C-5), 156.6 (C-8a), 154.0 (d, $J_{\text{C-F}} = 245.7$ Hz, C-3'), 143.0 (d, $J_{\text{C-F}} = 8.2$ Hz, C-4'), 124.5 (d, $J_{\text{C-F}} = 7.8$ Hz, C-1'), 123.6 (C-6'), 118.4 (C-5'), 114.2 (d, $J_{\text{C-F}} = 22.1$ Hz, C-2'), 104.5 (C-4a), 103.8 (C-8), 103.2 (C-3), 98.1 (C-6), 81.6 (C-5''), 78.8 (C-3''), 73.9 (C-1''), 71.5 (C-2''), 71.1 (C-4''), 66.4 (NCH₂CH₂O), 61.8 (C-6''), 50.0 (NCH₂CH₂O). ¹⁹F NMR (MeOD) δ (ppm) -122.45, -122.62 (dd, $J_{\text{F-H-5''}} = 14.2$ Hz,

$J_{\text{F}-\text{H},2''} = 8.9$ Hz). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for $\text{C}_{25}\text{H}_{27}\text{FNO}_{10}$ 520.1614, found 520.1615; [M + Na]⁺ calcd for $\text{C}_{25}\text{H}_{26}\text{FNNaO}_{10}$ 542.1433, found 542.1431.

3'-Bromo-5,7-dihydroxy-8-(β -D-glucopyranosyl)-4'-(morpholin-4-yl)flavone (29): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 95%; LCMS: r.t. = 0.69 min, m/z = 581.0, 582.0 [M + H]⁺ (Br isotopes, high pH method); physical appearance: yellow solid; m.p. = 181.1–182.2 °C; $[\alpha]_D^{20} = +11^\circ$ (c 0.2 MeOH); ¹H NMR (MeOD) δ (ppm) 8.24, 8.17 (s, 1H, H-2')*, 7.97, 7.92 (d, 1H, $J_{\text{ortho}} = 8.6$ Hz, H-6')*, 7.20 (d, 1H, $J_{\text{ortho}} = 8.5$ Hz, H-5'), 6.64 (s, 1H, H-3), 6.26 (s, 1H, H-6), 5.04, 5.01 (d, 1H, $J_{1''-2''} = 9.9$ Hz, H-1')*, 4.13–4.03 (m, 2H, H-2' and H-6'a), 3.96–3.81 (m, 6H, H-4'', H-6''b and $\text{NCH}_2\text{CH}_2\text{O}$), 3.60–3.53 (m, 2H, H-3'' and H-5''), 3.17–3.10 (m, 4H, $\text{NCH}_2\text{CH}_2\text{O}$). ¹³C NMR (MeOD) δ (ppm) 182.4 (C-4), 163.4 (C-7), 162.7 (C-2), 161.2 (C-5), 156.5 (C-8a), 153.4 (C-4'), 131.4 (C-2'), 127.0 (C-1'), 126.7 (C-6'), 120.8 (C-5'), 118.9 (C-3'), 104.5 (C-4a), 103.8 (C-8), 103.7 (C-3), 98.3 (C-6), 82.0 (C-5''), 78.9 (C-3''), 73.9 (C-1''), 71.5 (C-2''), 71.4 (C-4''), 66.6 ($\text{NCH}_2\text{CH}_2\text{O}$), 62.3 (C-6''), 51.5 ($\text{NCH}_2\text{CH}_2\text{O}$). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for $\text{C}_{25}\text{H}_{27}\text{BrNO}_{10}$ 580.0813, found 580.0811; [M + Na]⁺ calcd for $\text{C}_{25}\text{H}_{26}\text{BrNNaO}_{10}$ 602.0632, found 602.0624.

5,7-Dihydroxy-8-(β -D-glucopyranosyl)-3'-(morpholin-4-yl)flavone (30): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 99%; LCMS: r.t. = 0.56 min, m/z = 502.0 [M + H]⁺ and m/z = 524.0 [M + Na]⁺ (high pH method); physical appearance: orange solid; m.p. = 188.9–190.1 °C; $[\alpha]_D^{20} = +11^\circ$ (c 0.1 MeOH); ¹H NMR (MeOD) δ (ppm) 7.65 (br s, 1H, H-2'), 7.48–7.42 (m, 2H, H-5' and H-4'), 7.19 (d, 1H, $J_{\text{ortho}} = 7.8$ Hz, H-6'), 6.73 (s, 1H, H-3), 6.30 (s, 1H, H-6), 5.10, 5.01 (d, 1H, $J_{1''-2''} = 9.9$ Hz, H-1')*, 4.22–4.17, 4.10–4.04 (m, 1H, H-2'')*, 3.98–3.88 (m, 5H, H-6''a and $\text{NCH}_2\text{CH}_2\text{O}$), 3.81–3.74 (m, 1H, H-6''b), 3.62–3.46 (m, 3H, H-3'', H-4'' and H-5''), 3.29, 3.25 (s, 4H, $\text{NCH}_2\text{CH}_2\text{O}$)*. ¹³C NMR (MeOD) δ (ppm) 182.7 (C-4), 165.6 (C-2), 163.7 (C-7), 161.4 (C-5), 157.8 (C-8a), 152.2 (C-3'), 132.3 (C-1'), 129.5 (C-5'), 118.9 (C-6'), 113.3 (C-4' and C-2'), 104.6 (C-4a and C-3), 99.7 (C-8), 98.2 (C-6), 81.6 (C-5''), 78.9 (C-3''), 73.8 (C-1''), 71.5 (C-2''), 71.2 (C-4''), 66.6 ($\text{NCH}_2\text{CH}_2\text{O}$), 61.9, 61.1 (C-6'')*, 49.2, 48.9 ($\text{NCH}_2\text{CH}_2\text{O}$)*. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for $\text{C}_{25}\text{H}_{28}\text{NO}_{10}$ 502.1708, found 502.1709; [M + Na]⁺ calcd for $\text{C}_{25}\text{H}_{27}\text{NNaO}_{10}$ 524.1527, found 524.1530.

5,7-Dihydroxy-8-(β -D-glucopyranosyl)-4'-dimethylaminoflavone (31): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 91%; LCMS: r.t. = 0.66 min, m/z = 460.2 [M + H]⁺ (high pH method); physical appearance: orange solid; m.p. = 204.8–205.7 °C; $[\alpha]_D^{20} = +16^\circ$ (c 0.5 MeOH); ¹H NMR (MeOD) δ (ppm) 7.94, 7.84 (d, 2H, $J_{\text{ortho}} = 8.4$ Hz, H-2' and H-6')*, 6.81 (d, 2H, $J_{\text{ortho}} = 8.7$ Hz, H-3' and H-5'), 6.50 (s, 1H, H-3), 6.26 (s, 1H, H-6), 5.08, 5.01 (d, 1H, $J_{1''-2''} = 9.6$ Hz, H-1')*, 4.18 (t, 1H, $J_{2''-1''-2''-3''} = 9.6$ Hz, H-2''), 3.99, 3.94 (d, 1H, $J_{6''\text{a}-6''\text{b}} = 12.3$ Hz, H-6''a)*, 3.82 (dd, 1H, $J_{6''\text{b}-6''\text{a}} = 12.2$ Hz, $J_{6''\text{b}-5''} = 6.1$ Hz, H-6''b), 3.73 (t, 1H, $J_{4''-3''-4''-5''} = 9.4$ Hz, H-4''), 3.57 (t, 1H, $J_{3''-2''-3''-4''} = 9.1$ Hz, H-3''), 3.52–3.47 (m, 1H, H-5''), 3.01 [s, 6H, $\text{N}(\text{CH}_3)_2$]. ¹³C NMR (MeOD) δ (ppm) 182.4 (C-4), 165.8 (C-2), 163.1 (C-7), 161.2 (C-5), 156.6 (C-8a), 153.0 (C-4'), 128.1 (C-2' and C-6'), 117.4 (C-1'), 111.3 (C-3' and C-5'), 103.8 (C-4a), 103.7 (C-8), 100.4 (C-3), 97.9 (C-6), 81.5 (C-5''), 73.9 (C-3''), 73.8 (C-1''), 71.5 (C-2''), 71.0 (C-4''), 61.8 (C-6''), 38.7 [$\text{N}(\text{CH}_3)_2$]. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (m/z): [M + H]⁺ calcd for $\text{C}_{23}\text{H}_{26}\text{NO}_9$ 460.1602, found 460.1605; [M + Na]⁺ calcd for $\text{C}_{23}\text{H}_{25}\text{NNaO}_9$ 482.1422, found 482.1422.

5,7-Dihydroxy-8-(β -D-glucopyranosyl)-4'-diphenylamino)flavone (32): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 85%; LCMS: r.t. = 0.99 min, m/z = 584.2 [M + H]⁺ (high pH method); physical appearance: yellow solid; m.p. = 155.5–157.2 °C; $[\alpha]_D^{20} = +14^\circ$ (c 0.8 MeOH); ¹H NMR (MeOD) δ (ppm) 7.95–7.86 (d, 2H, $J_{\text{ortho}} = 8.8$ Hz, H-2' and H-6')*, 7.40–7.35 (m, 4H, N-Ph_2), 7.19–7.15 (m, 6H, N-Ph_2), 7.06 (d, 2H, $J_{\text{ortho}} = 8.8$ Hz, H-3' and H-5'), 6.61 (s, 1H, H-3), 6.30 (s, 1H, H-6), 5.05, 4.98 (d, 1H, $J_{1''-2''} = 9.9$ Hz, H-1')*, 4.13 (t, 1H, $J_{2''-1''-2''-3''} = 9.3$ Hz, H-2''), 3.89, 3.83 (d, 1H, $J_{6''\text{a}-6''\text{b}} = 12.1$ Hz, H-6''a)*, 3.78–3.41 (m, 4H, H-3'', H-4'', H-5'' and H-6''b)*. ¹³C NMR (MeOD) δ (ppm) 182.6 (C-4), 164.7 (C-2), 163.2 (C-7), 161.3 (C-5), 156.7 (C-8a), 151.5 (C-4'), 146.7 ($\text{C}_q\text{-Ph}$), 129.4 (CH-Ph), 127.9 (C-2' and C-6'), 125.8, 125.7

(CH-Ph), 124.5 (C-1'), 120.1 (C-3' and C-5'), 104.5 (C-4a), 103.8 (C-8), 102.3 (C-3), 98.0 (C-6), 81.5, 81.4 (C-5'')*, 78.7 (C-3''), 73.9 (C-1''), 71.4 (C-2''), 70.8, 70.7 (C-4'')*, 61.5, 60.8 (C-6'')*. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₃₃H₃₀NO₉ 584.1915, found 584.1912; [M + Na]⁺ calcd for C₃₃H₃₀NNaO₉ 606.1735, found 606.1730.

Synthesis of 1-[2,4-bis(ethoxymethoxy)-6-hydroxyphenyl]ethan-1-one (33): 2,4,6-trihydroxyacetophenone (**4**, 3 g, 16.12 mmol) was dissolved in dry acetone (60 mL), after which anhydrous potassium carbonate (4.90 g, 35.45 mmol, 2.2 eq.) was added at room temperature. The mixture was stirred at room temperature for 5 min, and then methyl ethoxymethyl chloride (EOMCl, 3.30 mL, 2.2 eq.) was added in a dropwise manner. The mixture was stirred under reflux for the next 4 h and followed by LCMS. After complete disappearance of the starting material (91% reaction yield), the reaction mixture was allowed to cool down to room temperature and the solvent was evaporated. Then, the residue was resuspended in EtOAc, washed with brine and extracted with EtOAc (3 × 50 mL). The organic layers were combined, dried over MgSO₄, filtered and concentrated under vacuum. The residue was purified by column chromatography (0:1 → 1:0 iso-hexane/ethyl acetate), affording compound **33** as a colorless solid; m.p. = 47.2–49.2 °C. LCMS: r.t. = 1.26 min, *m/z* = 285.2 [M + H]⁺, 307.2 [M + Na]⁺, 323.2 [M + K]⁺ (high pH method). ¹H NMR (CDCl₃) δ (ppm) 13.66 (s, 1H, OH-6), 6.19 (d, 1H, *J*_{meta} = 2.4 Hz, H-3), 6.18 (d, 1H, *J*_{meta} = 2.4 Hz, H-5), 5.21 (s, 2H, OCH₂O), 5.13 (s, 2H, OCH₂O), 3.70–3.60 (m, 4H, OCH₂CH₃), 2.55 (s, 3H, CH₃-Ac), 1.21–1.10 (m, 6H, OCH₂CH₃). ¹³C NMR (CDCl₃) δ (ppm) 202.2 (C=O), 165.8 (C-6), 162.6 (C-4), 159.5 (C-2), 105.8 (C-1), 96.0 (C-5), 93.1 (C-3), 92.2, 91.7 (OCH₂O), 64.0, 63.8 (OCH₂CH₃), 32.0 (CH₃-Ac), 14.0 (OCH₂CH₃). HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₁₄H₂₁O₆ 285.2333, found 285.1324; [M + Na]⁺ calcd for C₁₄H₂₀NNaO₆ 307.1152, found 307.1149.

General procedure for the synthesis of non-glycosylated EOM-protected chalcones

Compound **33** was dissolved in 1,4-dioxane (1.055 mmol in 4 mL) and the appropriate benzaldehyde (1.583 mmol, 1.5 eq.) was added. The mixture was stirred until fully homogenized. Then, an aqueous solution of NaOH 50 % (w/v, 4 mL) was slowly added and the mixture was stirred at room temperature for 3 h–24 h. All reactions were followed by LCMS; once the starting material was fully consumed, the reaction was quenched using HCl 2 M, washed with brine and extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over MgSO₄, filtered and concentrated under vacuum. The residue was purified using the most adequate purification method(s) to afford compounds **34–46**.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxyphenyl]-3-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (34): Purified by preparative HPLC. Reaction yield: 76 %; LCMS: r.t. 1.42 min, *m/z* = 458.2 [M + H]⁺ (high pH method); physical appearance: yellow solid; m.p. = 206.2–208.3 °C; ¹H NMR (CDCl₃) δ (ppm) 13.98 (s, 1H, OH-6'), 7.76 (part A of AB system, 1H, *J*_{trans} = 15.5 Hz, H-2), 7.70 (part B of AB system, 1H, *J*_{trans} = 15.5 Hz, H-3), 7.46 (d, 2H, *J*_{ortho} = 8.8 Hz, H-2'' and H-6''), 6.82 (d, 2H, *J*_{ortho} = 8.9 Hz, H-3'' and H-5''), 6.24 (d, 1H, *J*_{meta} = 2.3 Hz, H-5'), 6.18 (d, 1H, *J*_{meta} = 2.3 Hz, H-3'), 5.25 (s, 2H, OCH₂O), 5.16 (s, 2H, OCH₂O), 3.79 (t, *J* = 5.0 Hz, 4H, NCH₂CH₂O), 3.73–3.63 (m, 4H, OCH₂CH₃), 3.19 (t, *J* = 5.0 Hz, 4H, NCH₂CH₂O), 1.19–1.14 (m, 6H, OCH₂CH₃). ¹³C NMR (CDCl₃) δ (ppm) 191.8 (C-1), 166.3 (C-6'), 162.3 (C-4'), 158.9 (C-2'), 150.5 (C-4''), 142.0 (C-3), 129.0 (C-2'', C-6''), 125.1 (C-1''), 122.9 (C-2), 113.7 (C-3'', C-5''), 106.5 (C-1'), 96.4 (C-5'), 93.8 (C-3'), 92.9, 91.8, (OCH₂O), 65.6 (NCH₂CH₂O), 64.1, 63.8 (OCH₂CH₃), 47.0 (NCH₂CH₂O), 14.1 (OCH₂CH₃). HRMS-ESI (*m/z*): [M + H]⁺ calcd for C₂₅H₃₂NO₇ 458.2173, found 458.2177; [M + Na]⁺ calcd for C₂₅H₃₁NNaO₇ 480.1993, found 480.1998.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxyphenyl]-3-[4-(1,4-thiamorpholin-4-yl)phenyl]prop-2-en-1-one (35): Purified by preparative HPLC. Reaction yield: 77 %; LCMS: r.t. = 1.52 min, *m/z* = 474.2 [M + H]⁺ (high pH method); physical appearance: yellow solid; m.p. = 123.3–125.2 °C; ¹H NMR (CDCl₃) δ (ppm) 14.02 (s, 1H, OH-6'), 7.75 (part A of AB system, 1H, *J*_{trans} = 15.5 Hz, H-2), 7.70 (part B of AB system, 1H, *J*_{trans} = 15.5 Hz, H-3), 7.44 (d, 2H, *J*_{ortho} = 8.8 Hz, H-2'' and H-6''), 6.78 (d, 2H, *J*_{ortho} = 8.9 Hz, H-3'' and H-5''), 6.24 (d, 1H, *J*_{meta} = 2.3 Hz, H-5'),

6.18 (d, 1H, $J_{meta} = 2.3$ Hz, H-3'), 5.25 (s, 2H, OCH_2O), 5.16 (s, 2H, OCH_2O), 3.76–3.63 (m, 6H, OCH_2CH_3 , NCH_2CH_2S), 2.66–2.63 (m, 4H, NCH_2CH_2S), 1.20–1.14 (m, 6H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ (ppm) 191.7 (C-1), 166.3 (C-6'), 162.3 (C-4'), 158.9 (C-2'), 150.5 (C-4''), 142.1 (C-3), 129.3 (C-2'', C-6''), 124.7 (C-1''), 122.6 (C-2), 114.3 (C-3'', C-5''), 106.5 (C-1'), 96.4 (C-5'), 93.8 (C-3'), 92.9, 91.8, (OCH_2O), 64.2, 63.8 (OCH_2CH_3), 49.8 (NCH_2CH_2S), 25.0 (NCH_2CH_2S), 14.1 (OCH_2CH_3). HRMS-ESI (m/z): [M + H]⁺ calcd for $C_{25}H_{32}NO_6S$ 474.1945, found 474.1953; [M + Na]⁺ calcd for $C_{25}H_{31}NNaO_6S$ 496.1764, found 496.1770.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-3-[4-(piperidine-1-yl)phenyl]prop-2-en-1-one (36): Purified by preparative HPLC. Reaction yield: 75 %; LCMS: r.t. = 1.65 min, m/z = 456.2 [M + H]⁺ (low pH method); physical appearance: orange solid; m.p. = 110.4–112.3 °C; 1H NMR ($CDCl_3$) δ (ppm) 14.09 (s, 1H, OH-6'), 7.73 (s, 2H, H-2 and H-3), 7.42 (d, 2H, $J_{ortho} = 8.9$ Hz, H-2'' and H-6''), 6.81 (d, 2H, $J_{ortho} = 8.9$ Hz, H-3'' and H-5''), 6.24 (d, 1H, $J_{meta} = 2.3$ Hz, H-5'), 6.18 (d, 1H, $J_{meta} = 2.3$ Hz, H-3'), 5.25 (s, 2H, OCH_2O), 5.16 (s, 2H, OCH_2O), 3.71 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 3.65 (q, 2H, $J = 7.1$ Hz, OCH_2CH_3), 3.25–3.22 (m, 4H, NCH_2), 1.62–1.56 (m, 6H, $NCH_2CH_2CH_2$), 1.20–1.14 (m, 6H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ (ppm) 191.7 (C-1), 166.2 (C-6'), 162.2 (C-4'), 158.9 (C-2'), 152.0 (C-4''), 142.0 (C-3), 129.2 (C-2'', C-6''), 124.0 (C-1''), 121.9 (C-2), 113.8 (C-3'', C-5''), 106.6 (C-1'), 96.4 (C-5'), 93.8 (C-3'), 92.9, 91.8, (OCH_2O), 64.2, 63.7 (OCH_2CH_3), 48.1 (NCH_2), 24.4 (NCH_2CH_2), 23.3 ($NCH_2CH_2CH_2$), 14.1 (OCH_2CH_3). HRMS-ESI (m/z): [M + H]⁺ calcd for $C_{26}H_{34}NO_6$ 456.2381, found 456.2387; [M + Na]⁺ calcd for $C_{26}H_{33}NNaO_6$ 478.2200, found 478.2204.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-3-[4-(4-tert-butoxycarbonylpiperazin-1-yl)phenyl]prop-2-en-1-one (37): Purified by column chromatography (cyclohexane/THF 1:0 → 3:2). Reaction yield: 62 %; LCMS: r.t. = 1.59 min, m/z = 557.2 [M + H]⁺ and m/z = 579.2 [M + Na]⁺ (low pH method); physical appearance: orange oil. 1H NMR ($CDCl_3$) δ (ppm) 14.04 (s, 1H, OH-6'), 7.83 (part A of AB system, 1H, $J_{trans} = 15.5$ Hz, H-2), 7.77 (part B of AB system, 1H, $J_{trans} = 15.5$ Hz, H-3), 7.53 (d, 2H, $J_{ortho} = 8.8$ Hz, H-2'' and H-6''), 6.90 (d, 2H, $J_{ortho} = 8.9$ Hz, H-3'' and H-5''), 6.31 (d, 1H, $J_{meta} = 2.3$ Hz, H-5'), 6.25 (d, 1H, $J_{meta} = 2.3$ Hz, H-3'), 5.33 (s, 2H, OCH_2O), 5.23 (s, 2H, OCH_2O), 3.80–3.70 (m, 4H, OCH_2CH_3), 3.59 (t, $J = 5.0$ Hz, 4H, NCH_2 -2 and NCH_2 -6) 3.28 (t, $J = 5.0$ Hz, 4H, NCH_2 -3 and NCH_2 -5), 1.49 [s, 9H, $C(CH_3)_3$], 1.27–1.22 (m, 6H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ (ppm) 192.8 (C-1), 167.3 (C-6'), 163.2 (C-4'), 159.9 (C-2'), 154.7 (Boc-C=O), 152.3 (C-4''), 143.0 (C-3), 130.1 (C-2'', C-6''), 126.5 (C-1''), 124.0 (C-2), 115.3 (C-3'', C-5''), 107.6 (C-1'), 97.5 (C-5'), 94.8 (C-3'), 93.9, 92.8, (OCH_2O), 80.1 ($C(CH_3)_3$), 65.2, 64.8 (OCH_2CH_3), 48.0, 43.2 (NCH_2), 28.4 [$C(CH_3)_3$], 15.1 (OCH_2CH_3). HRMS-ESI (m/z): [M + H]⁺ calcd for $C_{30}H_{41}N_2O_8$ 557.2857, found 557.2859; [M + Na]⁺ calcd for $C_{30}H_{40}N_2NaO_8$ 579.2677, found 579.2679.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-3-[4-(4-methylpiperazin-1-yl)phenyl]prop-2-en-1-one (38): Purified by precipitation in cold methanol, followed by filtration under reduced pressure. Reaction yield: 83 %; LCMS: r.t. = 1.39 min, m/z = 471.2 [M + H]⁺ (high pH method); physical appearance: orange solid; m.p. = 122.6–123.2 °C; 1H NMR ($CDCl_3$) δ (ppm) 14.07 (s, 1H, OH-6'), 7.84, 7.80(2) (part A of AB system, 1H, $J_{trans} = 15.5$ Hz, H-2), 7.79(6), 7.76 (part B of AB system, 1H, $J_{trans} = 15.5$ Hz, H-3), 7.52 (d, 2H, $J_{ortho} = 8.8$ Hz, H-2'' and H-6''), 6.90 (d, 2H, $J_{ortho} = 8.9$ Hz, H-3'' and H-5''), 6.31 (d, 1H, $J_{meta} = 2.3$ Hz, H-5'), 6.25 (d, 1H, $J_{meta} = 2.3$ Hz, H-3'), 5.32 (s, 2H, OCH_2O), 5.23 (s, 2H, OCH_2O), 3.81–3.70 (m, 4H, OCH_2CH_3), 3.33 (t, $J = 5.0$ Hz, 4H, NCH_2 -2 and NCH_2 -6) 2.57 (t, $J = 5.0$ Hz, 4H, NCH_2 -3 and NCH_2 -5), 2.36 (s, 3H, $N-CH_3$), 1.27–1.21 (m, 6H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ (ppm) 192.8 (C-1), 167.3 (C-6'), 163.3 (C-4'), 159.9 (C-2'), 152.5 (C-4''), 143.2 (C-3), 130.1 (C-2'', C-6''), 125.9 (C-1''), 123.6 (C-2), 114.9 (C-3'', C-5''), 107.6 (C-1'), 97.5 (C-5'), 94.8 (C-3'), 93.9, 92.8, (OCH_2O), 65.2, 64.8 (OCH_2CH_3), 54.8, 47.8 (NCH_2), 46.2 (NCH_3), 15.1 (OCH_2CH_3). HRMS-ESI (m/z): [M + H]⁺ calcd for $C_{26}H_{35}N_2O_6$ 471.2490, found 471.2496; [M + Na]⁺ calcd for $C_{26}H_{34}N_2NaO_6$ 493.2309, found 493.2309.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-3-[4-(4-methylpiperazin-1-yl)methylphenyl]prop-2-en-1-one (39): Purified by preparative HPLC. Reaction yield: 99 %; LCMS: r.t. = 1.35 min, m/z = 485.2 [M + H]⁺ (high pH method); physical appearance: yellow oil; 1H NMR [$(CD_3)_2CO$] δ (ppm) 8.29 (s, 1H, OH-6'), 8.07, 8.03 (part A of AB system, 1H, $J_{trans} = 15.5$ Hz, H-2), 7.80, 7.76 (part B of AB system, 1H, $J_{trans} = 15.5$ Hz, H-3), 7.69 (d, 2H, $J_{ortho} = 8.8$ Hz, H-2'' and H-6''), 7.44 (d, 2H, $J_{ortho} = 8.9$ Hz, H-3'' and H-5''), 6.35 (d, 1H, $J_{meta} = 1.8$ Hz,

H-3'), 6.27 (d, 1H, $J_{meta} = 1.8$ Hz, H-5'), 5.47 (s, 2H, OCH_2O), 5.31 (s, 2H, OCH_2O), 3.82 (q, 4H, $J = 7.1$ Hz, OCH_2CH_3), 3.73 (q, 4H, $J = 7.2$ Hz, OCH_2CH_3), 3.58 (s, 2H, $PhCH_2N$), 2.72 (br s, 4H, NCH_2 -2 and NCH_2 -6), 2.57 (br s, 4H, NCH_2 -3 and NCH_2 -5), 1.22–1.17 (m, 6H, OCH_2CH_3). ^{13}C NMR [(CD₃)₂CO] δ (ppm) 192.8 (C-1), 167.1 (C-6'), 163.9 (C-4'), 160.3 (C-2'), 142.1 (C-3), 141.1 (C-4''), 134.4 (C-1''), 129.5 (C-3'', C-5''), 128.4 (C-2'', C-6''), 127.1 (C-2), 107.1 (C-1'), 96.8 (C-5'), 94.8 (C-3'), 94.1, 92.8, (OCH_2O), 65.0, 64.4 (OCH_2CH_3), 61.7 ($PhCH_2N$), 54.0, 51.6 (NCH_2), 44.0 (NCH_3), 14.6 (OCH_2CH_3). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₂₇H₃₇N₂O₆ 485.2646, found 485.2651; [M+Na]⁺ calcd for C₂₇H₃₆N₂NaO₆ 507.2466, found 507.2469.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxyphenyl-3-[2-fluoro-4-(morpholin-4-yl)phenyl]prop-2-en-1-one (40): Purified by column chromatography (cyclohexane/acetone 1:0 → 4:1) followed by precipitation in cold methanol and filtration under reduced pressure. Reaction yield: 73%; LCMS: r.t. = 1.45 min, *m/z* = 476.2 [M+H]⁺ (high pH method); physical appearance: yellow solid; m.p. = 111.0–111.9 °C; 1H NMR (CDCl₃) δ (ppm) 13.95 (s, 1H, OH-6'), 7.89, 7.85 (part A of AB system, 1H, $J_{trans} = 16.4$ Hz, H-2), 7.77, 7.73 (part B of AB system, 1H, $J_{trans} = 16.2$ Hz, H-3), 7.39 (t, 1H, $J_{ortho-H-F} = 8.8$ Hz, H-6''), 6.59 (dd, 1H, $J_{ortho} = 8.8$ Hz, $J_{meta} = 2.3$ Hz, H-5''), 6.50 (dd, 1H, $J_{H-F} = 14.5$ Hz, $J_{meta} = 2.2$ Hz, H-3''), 6.24 (d, 1H, $J_{meta} = 2.3$ Hz, H-5'), 6.21 (d, 1H, $J_{meta} = 2.3$ Hz, H-3'), 5.25 (s, 2H, OCH_2O), 5.16 (s, 2H, OCH_2O), 3.79 (t, $J = 5.0$ Hz, 4H, NCH_2CH_2O), 3.73–3.63 (m, 4H, OCH_2CH_3), 3.19 (t, $J = 5.0$ Hz, 4H, NCH_2CH_2O), 1.19–1.15 (m, 6H, OCH_2CH_3). ^{13}C NMR (CDCl₃) δ (ppm) 191.9 (C-1), 166.3 (C-6'), 162.4 (C-4'), 152.2 (d, $J_{C-F} = 252.8$ Hz, C-2''), 159.0 (C-2'), 152.6 (d, $J_{C-F} = 11.2$ Hz, C-4''), 135.2 (C-3), 130.3 (d, $J_{C-F} = 5.6$ Hz, C-6''), 125.2 (C-2), 113.1 (d, $J_{C-F} = 12.1$ Hz, C-1''), 109.0 (d, $J_{C-F} = 2.0$ Hz, C-5''), 106.6 (C-1'), 100.6 (d, $J_{C-F} = 26.2$ Hz, C-3''), 96.3 (C-5'), 93.7 (C-3'), 92.6, 91.6 (OCH_2O), 65.5 (NCH_2CH_2O), 64.1, 63.8 (OCH_2CH_3), 46.7 (NCH_2CH_2O), 14.0 (OCH_2CH_3). ^{19}F NMR (CDCl₃) δ (ppm) –111.20 (dd, $J_{F-H-5''} = 14.4$ Hz, $J_{F-H-2''} = 8.7$ Hz). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₂₅H₃₁FNO₇ 476.2079, found 476.2085; [M+Na]⁺ calcd for C₂₅H₃₀FNNaO₇ 498.1899, found 498.1904.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxyphenyl-3-[3-fluoro-4-(morpholin-4-yl)phenyl]prop-2-en-1-one (41): Purified by column chromatography (cyclohexane/tetrahydrofuran 1:0 → 3:2). Reaction yield: 76%; LCMS: r.t. = 1.46 min, *m/z* = 476.2 [M+H]⁺ (high pH method); physical appearance: yellow solid; m.p. = 108.7–110.2 °C; 1H NMR (CDCl₃) δ (ppm) 13.89 (s, 1H, OH-6'), 7.84, 7.80 (part A of AB system, 1H, $J_{trans} = 15.6$ Hz, H-2), 7.72, 7.68 (part B of AB system, 1H, $J_{trans} = 15.6$ Hz, H-3), 7.31–7.26 (m, 2H, H-2'' and H-6''), 6.92 (t, 1H, $J_{ortho-H-F} = 8.3$ Hz, H-5''), 6.32 (d, 1H, $J_{meta} = 2.3$ Hz, H-5'), 6.24 (d, 1H, $J_{meta} = 2.3$ Hz, H-3'), 5.33 (s, 2H, OCH_2O), 5.24 (s, 2H, OCH_2O), 3.88 (t, $J = 5.0$ Hz, 4H, NCH_2CH_2O), 3.81–3.69 (m, 4H, OCH_2CH_3), 3.17 (t, $J = 5.0$ Hz, 4H, NCH_2CH_2O), 1.28–1.20 (m, 6H, OCH_2CH_3). ^{13}C NMR (CDCl₃) δ (ppm) 192.6 (C-1), 167.4 (C-6'), 163.7 (C-4'), 159.9 (C-2'), 155.2 (d, $J_{C-F} = 241.6$ Hz, C-3''), 141.4 (d, $J_{C-F} = 11.2$ Hz, C-4''), 141.3 (C.3), 129.9 (d, $J_{C-F} = 7.7$ Hz, C-1''), 126.2 (C-2), 125.9 (d, $J_{C-F} = 2.7$ Hz, C-6''), 118.3 (d, $J_{C-F} = 3.5$ Hz, C-5''), 115.0 (d, $J_{C-F} = 21.6$ Hz, C-2''), 107.4 (C-1'), 97.4 (C-5'), 94.8 (C-3'), 94.0, 92.8 (OCH_2O), 66.8 (NCH_2CH_2O), 65.4, 64.9 (OCH_2CH_3), 50.5, 50.4 (NCH_2CH_2O), 15.1, 15.0 (OCH_2CH_3). ^{19}F NMR (CDCl₃) δ (ppm) –123.20 (dd, $J_{F-H-6''} = 14.4$ Hz, $J_{F-H-3''} = 8.6$ Hz). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₂₅H₃₁FNO₇ 476.2079, found 476.2081; [M+Na]⁺ calcd for C₂₅H₃₀FNNaO₇ 498.1899, found 498.1897.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxyphenyl-3-[3-bromo-4-(morpholin-4-yl)phenyl]prop-2-en-1-one (42): Purified by preparative HPLC. Reaction yield: 77%; LCMS: r.t. = 1.54 min, *m/z* = 536.0, 537.0, 538.0 [M+H]⁺ (Br isotopes; high pH method); physical appearance: orange solid; m.p. = 112.0–112.8 °C; 1H NMR (CDCl₃) δ (ppm) 13.82 (s, 1H, OH-6'), 7.86–7.83 (m, 2H, H-2, part A of AB system, and H-2''), 7.69, 7.65 (part B of AB system, 1H, $J_{trans} = 15.7$ Hz, H-3), 7.49 (dd, 1H, $J_{ortho} = 8.3$ Hz, $J_{meta} = 2.0$ Hz, H-6''), 7.04 (d, 1H, $J_{ortho} = 8.3$ Hz, H-5''), 6.32 (d, 1H, $J_{meta} = 2.3$ Hz, H-5'), 6.25 (d, 1H, $J_{meta} = 2.4$ Hz, H-3'), 5.34 (s, 2H, OCH_2O), 5.24 (s, 2H, OCH_2O), 3.90 (t, $J = 5.0$ Hz, 4H, NCH_2CH_2O), 3.82–3.70 (m, 4H, OCH_2CH_3), 3.11 (t, $J = 5.0$ Hz, 4H, NCH_2CH_2O), 1.29–1.22 (m, 6H, OCH_2CH_3). ^{13}C NMR (CDCl₃) δ (ppm) 192.5 (C-1), 167.4 (C-6'), 163.8 (C-4'), 160.0 (C-2'), 151.8 (C-4''), 140.5 (C-3), 133.4 (C-2''), 131.9 (C-1''), 128.9 (C-6''), 127.1 (C-2), 120.7 (C-5''), 119.6 (C-3''), 107.4 (C-1'), 97.4 (C-5'), 94.7 (C-3'), 93.9, 92.8 (OCH_2O), 67.0 (NCH_2CH_2O), 65.4, 64.9 (OCH_2CH_3), 51.8 (NCH_2CH_2O), 15.1 (OCH_2CH_3). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₂₅H₃₁BrNO₇ 536.1278, found 536.1282; [M+Na]⁺ calcd for C₂₅H₃₀BrNNaO₇ 558.1098, found 558.1102.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-3-[3-(morpholin-4-yl)phenyl]prop-2-en-1-one (43):

Purified by column chromatography (cyclohexane/tetrahydrofuran 1:0 → 3:2). Reaction yield: 81%; LCMS: r.t. = 1.43 min, m/z = 458.2 [M + H]⁺ (high pH method); physical appearance: orange oil. ¹H NMR (CDCl₃) δ (ppm) 13.84 (s, 1H, OH-6'), 7.93, 7.89 (part A of AB system, 1H, J_{trans} = 15.6 Hz, H-2), 7.76, 7.72 (part B of AB system, 1H, J_{trans} = 15.7 Hz, H-3), 7.31 (t, 1H, J_{ortho} = 7.9 Hz, H-5"), 7.16 (br d, 1H, J_{ortho} = 7.7 Hz, H-6"), 7.10 (br s, 1H, H-2"), 6.95 (dd, 1H, J_{ortho} = 8.1 Hz, J_{meta} = 2.2 Hz, H-4"), 6.32 (d, 1H, J_{meta} = 2.2 Hz, H-5'), 6.26 (d, 1H, J_{meta} = 2.3 Hz, H-3'), 5.32 (s, 2H, OCH₂O), 5.24 (s, 2H, OCH₂O), 3.89–3.87 (m, 4H, NCH₂CH₂O), 3.79–3.70 (m, 4H, OCH₂CH₃), 3.21–3.18 (m, 4H, NCH₂CH₂O), 1.23 (t, 6H, J = 7.1 Hz, OCH₂CH₃). ¹³C NMR (CDCl₃) δ (ppm) 192.9 (C-1), 167.3 (C-6'), 163.7 (C-4'), 160.0 (C-2'), 151.7 (C-3'), 142.9 (C-3), 136.4 (C-1"), 129.7 (C-5"), 127.5 (C-2), 119.5 (C-6"), 117.6 (C-4"), 115.9 (C-2"), 107.5 (C-1'), 97.4 (C-5'), 94.8 (C-3'), 94.0, 92.8, (OCH₂O), 66.9 (NCH₂CH₂O), 65.3, 64.8 (OCH₂CH₃), 49.2 (NCH₂CH₂O), 15.1 (OCH₂CH₃). HRMS-ESI (m/z): [M + H]⁺ calcd for C₂₅H₃₂NO₇ 458.2173, found 458.2176; [M + Na]⁺ calcd for C₂₅H₃₁NNaO₇ 480.1993, found 480.1990.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-3-[2-(morpholin-4-yl)phenyl]prop-2-en-1-one (44):

Purified by preparative HPLC. Reaction yield: 84%; LCMS: r.t. = 1.46 min, m/z = 458.2 [M + H]⁺ (high pH method); physical appearance: yellow solid; m.p. = 75.9–76.3 °C; ¹H NMR (CDCl₃) δ (ppm) 13.89 (s, 1H, OH-6'), 8.14, 8.10 (part A of AB system, 1H, J_{trans} = 15.6 Hz, H-3), 7.82, 7.78 (part B of AB system, 1H, J_{trans} = 15.8 Hz, H-2), 7.55 (dd, 1H, J_{ortho} = 7.6 Hz, J_{meta} = 1.3 Hz, H-6"), 7.30 (td, 1H, J_{ortho} = 8.1 Hz, J_{meta} = 1.5 Hz, H-4"), 7.05–6.99 (m, 2H, H-3" and H-5"), 6.25 (d, 1H, J_{meta} = 2.3 Hz, H-5'), 6.19 (d, 1H, J_{meta} = 2.3 Hz, H-3'), 5.26 (s, 2H, OCH₂O), 5.16 (s, 2H, OCH₂O), 3.86–3.83 (m, 4H, NCH₂CH₂O), 3.72–3.63 (m, 4H, OCH₂CH₃), 2.93–2.91 (m, 4H, NCH₂CH₂O), 1.16 (t, 6H, J = 7.1 Hz, OCH₂CH₃). ¹³C NMR (CDCl₃) δ (ppm) 192.1 (C-1), 166.4 (C-6'), 162.6 (C-4'), 160.0 (C-2'), 151.7 (C-2"), 138.6 (C-3), 129.8 (C-4"), 126.8 (C-6"), 126.3 (C-2), 122.1 (C-5"), 117.8 (C-3"), 106.4 (C-1'), 96.4 (C-5'), 93.7 (C-3'), 92.8, 91.8, (OCH₂O), 66.2 (NCH₂CH₂O), 64.2, 63.8 (OCH₂CH₃), 52.2 (NCH₂CH₂O), 14.1, 14.0 (OCH₂CH₃). HRMS-ESI (m/z): [M + H]⁺ calcd for C₂₅H₃₂NO₇ 458.2173, found 458.2177; [M + Na]⁺ calcd for C₂₅H₃₁NNaO₇ 480.1993, found 480.1998.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-3-(4-dimethylaminophenyl)prop-2-en-1-one (45):

Purified by preparative HPLC. Reaction yield: 99%; LCMS: r.t. = 1.48 min, m/z = 416.2 [M + H]⁺ (high pH method); physical appearance: red solid; m.p. = 100.0–100.5 °C; ¹H NMR (CDCl₃) δ (ppm) 14.19 (s, 1H, OH-6'), 7.85, 7.81 (part A of AB system, 1H, J_{trans} = 15.4 Hz, H-3), 7.80, 7.76 (part B of AB system, 1H, J_{trans} = 15.1 Hz, H-2), 7.51 (d, 2H, J_{ortho} = 8.8 Hz, H-2" and H-6"), 6.82 (d, 2H, J_{ortho} = 8.9 Hz, H-3" and H-5"), 6.31 (d, 1H, J_{meta} = 2.4 Hz, H-5'), 6.26 (d, 1H, J_{meta} = 2.4 Hz, H-3'), 5.33 (s, 2H, OCH₂O), 5.23 (s, 2H, OCH₂O), 3.79 (q, 2H, J = 7.1 Hz, OCH₂CH₃), 3.73 (q, 2H, J = 7.1 Hz, OCH₂CH₃), 3.04 [s, 6H, N(CH₃)₂], 1.27–1.22 (m, 6H, OCH₂CH₃). ¹³C NMR (CDCl₃) δ (ppm) 192.7 (C-1), 167.2 (C-6'), 163.1 (C-4'), 160.0 (C-2'), 151.9 (C-4"), 144.1 (C-3), 130.4 (C-2", C-6"), 123.3 (C-1"), 122.0 (C-2), 111.9 (C-3", C-5"), 107.7 (C-1'), 97.5 (C-5'), 94.8 (C-3'), 93.9, 92.8, (OCH₂O), 65.2, 64.7 (OCH₂CH₃), 40.2 (N(CH₃)₂), 15.1 (OCH₂CH₃). HRMS-ESI (m/z): [M + H]⁺ calcd for C₂₃H₃₀NO₆ 416.2068, found 416.2074; [M + Na]⁺ calcd for C₂₃H₂₉NNaO₆ 438.1887, found 438.1892.

(2E)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-3-[4-(diphenylamino)phenyl]prop-2-en-1-one (46):

Purified by precipitation in cold methanol, followed by column chromatography (cyclohexane/acetone 1:0 → 9:1). Reaction yield: 61%; LCMS: r.t. = 1.75 min, m/z = 540.2 [M + H]⁺ (high pH method); physical appearance: orange oil. ¹H NMR (CDCl₃) δ (ppm) 14.04 (s, 1H, OH-6'), 7.86, 7.82 (part A of AB system, 1H, J_{trans} = 15.7 Hz, H-2), 7.78, 7.74 (part B of AB system, 1H, J_{trans} = 15.4 Hz, H-3), 7.45 (d, 2H, J_{ortho} = 8.6 Hz, H-2" and H-6"), 7.32–7.28 (m, 4H, NPh₂), 7.15–7.08 (m, 6H, NPh₂), 7.02 (d, 2H, J_{ortho} = 8.9 Hz, H-3" and H-5"), 6.31 (d, 1H, J_{meta} = 2.4 Hz, H-3'), 6.26 (d, 1H, J_{meta} = 2.4 Hz, H-5'), 5.31 (s, 2H, OCH₂O), 5.23 (s, 2H, OCH₂O), 3.78–3.69 (m, 4H, OCH₂CH₃), 1.23 (t, 6H, J = 7.1 Hz, OCH₂CH₃). ¹³C NMR (CDCl₃) δ (ppm) 192.7 (C-1), 167.4 (C-6'), 163.4 (C-4'), 160.0 (C-2'), 149.9 (C-4"), 146.9 (C_q-Ph), 142.7 (C-3), 129.6 (C-2", C-6"), 129.5 (CH-Ph), 128.5 (C-1"), 125.5, 124.7 (CH-Ph), 124.1 (C-2), 121.7 (C-3", C-5"), 107.5 (C-1'), 97.5 (C-5'), 94.8 (C-3'), 93.9, 92.8, (OCH₂O), 65.2, 64.8 (OCH₂CH₃), 15.1 (OCH₂CH₃). HRMS-ESI (m/z): [M + H]⁺ calcd for C₃₃H₃₄NO₆ 540.2381, found 540.2387; [M + Na]⁺ calcd for C₃₃H₃₃NNaO₆ 562.2200, found 562.2213.

General procedure for the synthesis of non-glycosylated flavones

Each chalcone **34–46** was dissolved in dry pyridine (0.248 mmol in 7.33 mL). Then, catalytic amounts of I₂ (0.087 mmol, 0.35 eq.) were added and the mixture was stirred under reflux for 24 h–72 h. All reactions were followed by LCMS. Once the starting material was fully consumed, the mixture was allowed to reach room temperature and the pyridine was co-evaporated with toluene under reduced pressure. The residue was resuspended in dichloromethane, washed first with a saturated solution of sodium thiosulfate, and then with brine. The flavone was extracted with dichloromethane (3 × 30 mL), dried over MgSO₄, and the solution filtered and concentrated under vacuum. The residue was then resuspended in ethanol (15 mL) and *p*-TsOH (12% in AcOH, 0.1 mL) was added. The reaction was stirred under reflux for 2 h–24 h. After having reached completion by LCMS, the solvent was evaporated under vacuum and the residue purified using the most adequate purification method(s) to afford compounds **2** and **47–57**.

5,7-Dihydroxy-4'-(morpholin-4-yl)flavone (2): Purified by preparative HPLC. Reaction yield over two steps: 68%; LCMS: r.t.=1.07 min, *m/z*=340.0 [M+H]⁺ (low pH method); physical appearance: orange solid; m.p.=234.0–235.6 °C. ¹H NMR [(CD₃)OD] δ (ppm) 7.88 (d, 2H, *J*_{ortho}=9.0 Hz, H-2' and H-6'), 7.07 (d, 2H, *J*_{ortho}=9.1 Hz, H-3' and H-5'), 6.59 (s, 1H, H-3), 6.46 (d, 1H, *J*_{meta}=2.2 Hz, H-8), 6.22 (d, 1H, *J*_{meta}=2.1 Hz, H-6), 3.86–3.84 (m, 4H, NCH₂CH₂O), 3.35–3.35 (NCH₂CH₂O, overlapped with metanol-*d*₄ peak). ¹³C NMR [(CD₃)OD] δ (ppm) 183.9 (C-4), 166.2 (C-7), 163.5 (C-5), 159.6 (C-8a), 155.5 (C-4'), 129.1 (C-2', C-6'), 122.0 (C-1'), 115.6 (C-3', C-5'), 103.5 (C-4a), 100.3 (C-6), 95.2 (C-8), 67.7 (NCH₂CH₂O), 49.2 (NCH₂CH₂O). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₁₉H₁₈NO₅ 340.1179, found 340.1175.

5,7-Dihydroxy-4'-(1,4-thiamorpholin-4-yl)flavone (47): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 99%; LCMS: r.t.=1.04 min, *m/z*=356.0 [M+H]⁺ (high pH method); physical appearance: yellow solid; m.p.=245.4–247.3 °C; ¹H NMR [(CD₃)₂CO] δ (ppm) 12.98 (s, 1H, OH-5), 7.76 (d, 2H, *J*_{ortho}=8.7 Hz, H-2' and H-6'), 6.92 (d, 2H, *J*_{ortho}=9.2 Hz, H-3' and H-5'), 6.45 (s, 1H, H-3), 6.39 (d, 1H, *J*_{meta}=2.2 Hz, H-8), 6.11 (d, 1H, *J*_{meta}=2.1 Hz, H-6), 3.72–3.69 (m, 4H, NCH₂CH₂O), 2.58–2.56 (m, 4H, NCH₂CH₂O). ¹³C NMR [(CD₃)₂CO] δ (ppm) 182.1 (C-4), 164.3 (C-2), 163.9 (C-7), 162.5 (C-5), 157.9 (C-8a), 152.3 (C-4'), 128.0 (C-2' and C-6'), 119.4 (C-1'), 114.5 (C-3' and C-5'), 104.5 (C-4a), 102.2 (C-3), 98.7 (C-6), 93.8 (C-8), 50.2 (NCH₂CH₂O), 47.4 (NCH₂CH₂O). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₁₉H₁₈NO₄S 356.0951, found 356.0949; [M+Na]⁺ calcd for C₁₉H₁₇NNaO₄S 378.0770, found 378.0767.

5,7-Dihydroxy-4'-(piperidin-1-yl)flavone (48): Purified by preparative HPLC. Reaction yield over two steps: 65%; LCMS: r.t.=1.26 min, *m/z*=338.0 [M+H]⁺ (low pH method); physical appearance: golden solid; m.p.=233.2–234.4 °C; ¹H NMR [CO(CD₃)₂] δ (ppm) 13.14 (s, 1H, OH-5), 7.87 (d, 2H, *J*_{ortho}=8.7 Hz, H-2' and H-6'), 7.04 (d, 2H, *J*_{ortho}=8.8 Hz, H-3' and H-5'), 6.57 (s, 1H, H-3), 6.52 (br s, 1H, H-8), 6.24 (br s, 1H, H-6), 3.40 (br s, 4H, NCH₂), 1.66 (br s, 6H, NCH₂CH₂CH₂). ¹³C NMR [CO(CD₃)₂] δ (ppm) 182.0 (C-4), 164.5 (C-2), 163.8 (C-7), 162.5 (C-5), 157.8 (C-8a), 153.8 (C-4'), 127.7 (C-2' and C-6'), 118.9 (C-1'), 114.1 (C-3' and C-5'), 104.4 (C-4a), 101.9 (C-3), 98.7 (C-6), 93.7 (C-8), 48.2 (NCH₂), 25.2 (NCH₂CH₂CH₂), 24.2 (NCH₂CH₂CH₂). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₂₀H₂₀NO₄ 338.1387, found 338.1385; [M+Na]⁺ calcd for C₂₀H₂₀NNaO₄ 360.1206, found 360.1194.

5,7-Dihydroxy-4'-(piperazin-1-yl)flavone (49): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 99%; LCMS: r.t.=0.65 min, *m/z*=339.0 [M+H]⁺ (low pH method); physical appearance: orange solid; m.p.>360 °C; ¹H NMR [(CD₃)₂SO] δ (ppm) 7.94 (d, 2H, *J*_{ortho}=8.8 Hz, H-2' and H-6'), 7.10 (d, 2H, *J*_{ortho}=8.3 Hz, H-3' and H-5'), 6.81 (s, 1H, H-3), 6.55 (d, 1H, *J*_{meta}=2.0 Hz, H-8), 6.23 (d, 1H, *J*_{meta}=2.0 Hz, H-6), 3.55–3.47 (m, 4H, NCH₂CH₂NH), 3.13–3.07 (m, 4H, NCH₂CH₂NH). ¹³C NMR [(CD₃)₂SO] δ (ppm) 182.1 (C-4), 164.8 (C-2), 164.1 (C-7), 161.8 (C-5), 157.7 (C-8a), 153.1 (C-4'), 128.3 (C-2' and C-6'), 120.9 (C-1'), 114.9 (C-3' and C-5'), 104.1 (C-4a), 102.9 (C-3), 99.4 (C-6), 94.5 (C-8), 45.5 (NCH₂CH₂NH), 45.4 (NCH₂CH₂NH). HRMS-ESI (*m/z*): [M+H]⁺ calcd for C₁₉H₁₉N₂O₄ 339.1339, found 339.1335.

5,7-Dihydroxy-4'-(4-methylpiperazin-1-yl)flavone (50): Purified by preparative HPLC. Reaction yield over two steps: 79 %; LCMS: r.t. = 0.81 min, m/z = 353.0 [M + H]⁺ (low pH method); physical appearance: orange solid; m.p. = 225.8–226.6 °C; ¹H NMR [MeOD/CO(CD₃)₂ 10:1] δ (ppm) 7.77 (d, 2H, $J_{\text{ortho}} = 8.6$ Hz, H-2' and H-6'), 6.96 (d, 2H, $J_{\text{ortho}} = 8.7$ Hz, H-3' and H-5'), 6.47 (s, 1H, H-3), 6.35 (br s, 1H, H-8), 6.09 (br s, 1H, H-6), 3.29–3.27 (m, 4H, NCH₂CH₂NCH₃), 2.44–2.42 (m, 4H, NCH₂CH₂NCH₃), 2.18 (s, 3H, NCH₃). ¹³C NMR [MeOD/CO(CD₃)₂ 10:1] δ (ppm) 182.0 (C-4), 165.2 (C-7), 164.3 (C-2), 162.0 (C-5), 158.0 (C-8a), 153.6 (C-4'), 127.6 (C-2' and C-6'), 120.0 (C-1), 114.3 (C-3' and C-5'), 103.9 (C-4a), 102.1 (C-3), 99.0 (C-6), 94.0 (C-8), 54.5 (NCH₂CH₂NCH₃), 46.9 (NCH₂CH₂NCH₃), 45.2 (NCH₃). HRMS-ESI (m/z): [M + H]⁺ calcd for C₂₀H₂₁N₂O₄ 353.1496, found 353.1495; [M + Na]⁺ calcd for C₂₀H₂₀N₂NaO₄ 375.1315, found 375.1312.

5,7-Dihydroxy-4'-(4-methylpiperazin-1-yl)methyl]flavone (51): Purified by preparative HPLC, followed by Isolute SCX-2 column chromatography (Biotage). Reaction yield over two steps: 90 %; LCMS: r.t. = 0.71 min, m/z = 367.0 [M + H]⁺ (low pH method); physical appearance: brownish oil. ¹H NMR [CO(CD₃)₂] δ (ppm) 7.83 (d, 2H, $J_{\text{ortho}} = 8.1$ Hz, H-2' and H-6'), 7.38 (d, 2H, $J_{\text{ortho}} = 7.9$ Hz, H-3' and H-5'), 6.58 (s, 1H, H-3), 6.41 (br s, 1H, H-8), 6.13 (br s, 1H, H-6), 3.42 (s, 2H, PhCH₂N), 3.39–3.21 (m, 8H, NCH₂CH₂NCH₃), 2.07 (s, 3H, NCH₃). ¹³C NMR [CO(CD₃)₂] δ (ppm) 182.1 (C-4), 165.6 (C-7), 163.6 (C-2), 162.2 (C-5), 158.1 (C-8a), 143.2 (C-4'), 130.0 (C-1'), 129.5 (C-3' and C-5'), 126.2 (C-2' and C-6'), 104.8 (C-3), 104.1 (C-4a), 99.3 (C-6), 94.2 (C-8), 62.0 (PhCH₂N), 54.9 (NCH₂CH₂NCH₃), 52.8 (NCH₂CH₂NCH₃), 45.3 (NCH₃). HRMS-ESI (m/z): [M + H]⁺ calcd for C₂₁H₂₃N₂O₄ 367.1652, found 367.1649.

2'-Fluoro-5,7-dihydroxy-4'-(morpholin-4-yl)flavone (52): Purified by preparative HPLC. Reaction yield over two steps: 92 %; LCMS: r.t. = 0.89 min, m/z = 358.2 [M + H]⁺ (high pH method); physical appearance: orange solid; m.p. = 243.2–244.0 °C; ¹H NMR [(CD₃)₂CO] δ (ppm) 12.98 (s, 1H, OH-5), 7.90 (t, 1H, $J_{\text{ortho-H-F}} = 9.0$ Hz, H-6'), 6.95 (d, 1H, $J_{\text{ortho}} = 8.8$ Hz, H-5'), 6.85 (d, 1H, $J_{\text{H-F}} = 16.0$ Hz, H-3'), 6.57 (s, 1H, H-3), 6.52 (br s, 1H, H-8), 6.27 (br s, 1H, H-6), 3.81–3.79 (m, 4H, NCH₂CH₂O), 3.40–3.37 (m, 4H, NCH₂CH₂O). ¹³C NMR [(CD₃)₂CO] δ (ppm) 181.9 (C-4), 164.0 (C-7), 162.2 (d, $J_{\text{C-F}} = 250.8$ Hz, C-2'), 162.1 (C-5), 159.9 (C-2), 157.9 (C-8a), 155.1 (d, $J_{\text{C-F}} = 11.9$ Hz, C-4'), 129.6 (d, $J_{\text{C-F}} = 3.70$ Hz, C-6'), 110.0 (d, $J_{\text{C-F}} = 1.8$ Hz, C-5'), 108.1 (d, $J_{\text{C-F}} = 10.2$ Hz, C-1'), 107.2 (d, $J_{\text{C-F}} = 13.3$ Hz, C-3), 101.2 (d, $J_{\text{C-F}} = 27.5$ Hz, C-3'), 104.4 (C-4a), 98.7 (C-6), 93.8 (C-8), 66.1 (NCH₂CH₂O), 47.1 (NCH₂CH₂O). ¹⁹F NMR (CDCl₃) δ (ppm) -110.42 (dd, $J_{\text{F-H-5''}} = 15.7$ Hz, $J_{\text{F-H-2''}} = 8.8$ Hz). HRMS-ESI (m/z): [M + H]⁺ calcd for C₁₉H₁₇FNO₅ 358.1085, found 358.1081; [M + Na]⁺ calcd for C₁₉H₁₆FNNaO₅ 380.0905, found 380.0903.

3'-Fluoro-5,7-dihydroxy-4'-(morpholin-4-yl)flavone (53): Purified by preparative HPLC. Reaction yield over two steps: 93 %; LCMS: r.t. = 1.10 min, m/z = 358.0 [M + H]⁺ (low pH method); physical appearance: orange solid; m.p. = 241.6–242.8 °C; ¹H NMR [(CD₃)₂CO] δ (ppm) 7.84–7.77 (m, 2H, H-2' and H-6'), 7.18 (t, 1H, $J_{\text{ortho-H-F}} = 8.7$ Hz, H-5'), 6.72 (s, 1H, H-3), 6.60 (br s, 1H, H-8), 6.27 (br s, 1H, H-6), 3.83–3.81 (m, 4H, NCH₂CH₂O), 3.24–3.22 (m, 4H, NCH₂CH₂O). ¹³C NMR [(CD₃)₂CO] δ (ppm) 182.0 (C-4), 164.1 (C-7), 162.6 (d, $J_{\text{C-F}} = 2.5$ Hz, C-2), 162.2 (C-5), 157.9 (C-8a), 154.8 (d, $J_{\text{C-F}} = 246.5$ Hz, C-3'), 143.0 (d, $J_{\text{C-F}} = 8.0$ Hz, C-4'), 124.4 (d, $J_{\text{C-F}} = 8.2$ Hz, C-1'), 123.3 (d, $J_{\text{C-F}} = 2.9$ Hz, C-6'), 118.7 (d, $J_{\text{C-F}} = 3.7$ Hz, C-5'), 114.1 (d, $J_{\text{C-F}} = 24.1$ Hz, C-2'), 104.5 (C-4a), 104.2 (C-3), 98.8 (C-6), 94.0 (C-8), 66.5 (NCH₂CH₂O), 50.2 (d, $J_{\text{C-F}} = 4.4$ Hz, NCH₂CH₂O). ¹⁹F NMR (CDCl₃) δ (ppm) -122.29 (dd, $J_{\text{F-H-6''}} = 14.2$ Hz, $J_{\text{F-H-3''}} = 8.8$ Hz). HRMS-ESI (m/z): [M + H]⁺ calcd for C₁₉H₁₇FNO₅ 358.1085, found 358.1083; [M + Na]⁺ calcd for C₁₉H₁₆FNNaO₅ 380.0905, found 380.0900.

3'-Bromo-5,7-dihydroxy-4'-(morpholin-4-yl)flavone (54): Purified by preparative HPLC followed by SFC-MS (PPU column). Reaction yield over two steps: 84 %; LCMS: r.t. = 0.96 min, m/z = 417.8, 419.8, 420.8 [M + H]⁺ (Br isotopes; high pH method); physical appearance: brownish oil. ¹H NMR [(CD₃)₂CO] δ (ppm) 12.90 (s, 1H, OH-7), 8.27 (s, 1H, H-2'), 8.05 (d, 1H, $J_{\text{ortho}} = 8.6$ Hz, H-6'), 7.32 (d, 1H, $J_{\text{ortho}} = 8.6$ Hz, H-5'), 6.77 (s, 1H, H-3), 6.60 (br s, 1H, H-8), 6.28 (br s, 1H, H-6), 3.85–3.82 (m, 4H, NCH₂CH₂O), 3.17–3.15 (m, 4H, NCH₂CH₂O). ¹³C NMR [(CD₃)₂CO] δ (ppm) 182.0 (C-4), 164.2 (C-7), 163.3 (C-5), 162.2 (C-2), 157.9 (C-8a), 153.5 (C-4'), 131.7 (C-2'), 127.0 (C-1'), 126.9 (C-6'), 121.1 (C-5'), 118.9 (C-3'), 104.9 (C-4a and C-3), 98.9 (C-6), 94.1 (C-8), 66.5 (NCH₂CH₂O), 51.7 (NCH₂CH₂O). HRMS-ESI (m/z): [M + H]⁺ calcd for C₁₉H₁₇BrNO₅ 418.0285, found 418.0280; [M + Na]⁺ calcd for C₁₉H₁₆BrNNaO₅ 440.0104, found 440.0102.

5,7-Dihydroxy-3'-(morpholin-4-yl)flavone (55): Purified by Isolute SCX-2 column chromatography (Biotope) followed by preparative HPLC. Reaction yield over two steps: 85 %; LCMS: r.t. = 1.06 min, m/z = 340.2 [$M + H$]⁺ and m/z = 362.2 [$M + Na$]⁺ (low pH method); physical appearance: orange solid; m.p. = 135.1–137.6 °C; ¹H NMR [(CD₃)₂CO] δ (ppm) 12.93 (s, 1H, OH-5), 7.60 (s, 1H, H-2'), 7.50–7.42 (m, 2H, H-5' and H-4'), 7.21 (d, 1H, J_{ortho} = 8.1 Hz, H-6'), 6.79 (s, 1H, H-3), 6.57 (br s, 1H, H-8), 6.28 (br s, 1H, H-6), 3.83–3.81 (m, 4H, NCH₂CH₂O), 3.30–3.28 (m, 4H, NCH₂CH₂O). ¹³C NMR [(CD₃)₂CO] δ (ppm) 182.2 (C-4), 164.3 (C-2), 164.0 (C-7), 162.2 (C-5), 158.0 (C-8a), 152.1 (C-3'), 132.2 (C-1'), 129.8 (C-5'), 118.6 (C-6'), 117.2 (C-4'), 112.8 (C-2'), 105.4 (C-4a and C-3), 98.8 (C-6), 94.1 (C-8), 66.4 (NCH₂CH₂O), 48.7 (NCH₂CH₂O). HRMS-ESI (m/z): [M + H]⁺ calcd for C₁₉H₁₈NO₅ 340.1179, found 340.1177.

5,7-Dihydroxy-4'-dimethylaminoflavone (56): Purified by preparative HPLC. Reaction yield over two steps: 54 %; LCMS: r.t. = 1.12 min, m/z = 298.0 [$M + H$]⁺ (low pH method); physical appearance: orange solid; m.p. = 291.3–292.7 °C. ¹H NMR [(CD₃)₂SO] δ (ppm) 13.11 (s, 1H, OH-5), 7.89 (d, 2H, J_{ortho} = 9.0 Hz, H-2' and H-6'), 6.81 (d, 2H, J_{ortho} = 9.0 Hz, H-3' and H-5'), 6.72 (s, 1H, H-3), 6.46 (d, 1H, J_{meta} = 2.1 Hz, H-8), 6.16 (d, 1H, J_{meta} = 2.1 Hz, H-6), 3.04 (s, 6H, N(CH₃)₂). ¹³C NMR [(CD₃)₂SO] δ (ppm) 181.9 (C-4), 164.7 (C-2), 164.6 (C-7), 161.9 (C-5), 157.7 (C-8a), 153.1 (C-4'), 128.3 (C-2', C-6'), 117.0 (C-1'), 112.1 (C-3', C-5'), 104.0 (C-4a), 101.7 (C-3), 99.2 (C-6), 94.3 (C-8), 40.4 [N(CH₃)₂]. HRMS-ESI (m/z): [M + H]⁺ calcd for C₁₇H₁₆NO₄ 298.1074, found 298.101.

5,7-Dihydroxy-4'-(diphenylamino)flavone (57): Purified by preparative HPLC. Reaction yield over two steps: 63 %; LCMS: r.t. = 1.38 min, m/z = 422.0 [$M + H$]⁺ (high pH method); physical appearance: dark orange oil. ¹H NMR [(CD₃)₂SO] δ (ppm) 7.86 (d, 2H, J_{ortho} = 8.8 Hz, H-2' and H-6'), 7.39–7.34 (m, 4H, N-Ph₂), 7.18–7.15 (m, 8H, N-Ph₂, H-3' and H-5'), 6.89 (s, 1H, H-3), 6.49 (d, 1H, J_{meta} = 2.1 Hz, H-8), 6.19 (d, 1H, J_{meta} = 2.1 Hz, H-6). ¹³C NMR [(CD₃)₂SO] δ (ppm) 182.1 (C-4), 164.7 (C-7), 164.1 (C-2), 161.8 (C-5), 158.0 (C-8a), 151.4 (C-4'), 146.1 (C_q-Ph), 129.3 (CH-Ph), 128.9 (C-2' and C-6'), 125.3, 125.2 (CH-Ph), 124.1 (C-1'), 122.0 (C-3' and C-5'), 104.7 (C-4a), 103.8 (C-3), 99.3 (C-6), 94.9 (C-8). HRMS-ESI (m/z): [M + H]⁺ calcd for C₂₇H₂₀NO₄ 422.1387, found 422.1384; [M + Na]⁺ calcd for C₂₇H₁₉NNaO₄ 444.1206, found 444.1213.

Preparation of A β o

ADDLs were prepared as described previously [29, 30] with some modifications. Briefly, 16 mg of lyophilized human A β 1-42 peptide was equilibrated to room temperature for 30 min prior to resuspension in hexafluoropropan-2-ol (HFIP) to produce a 4.5 mg/mL A β solution. The peptide solution was incubated at room temperature for a further 30 min to ensure complete dissolution. The resulting peptide solution was then aliquotted into 0.5 mg aliquots in LoBind microcentrifuge tubes, centrifuged at maximum speed for 5 min, and dried under N₂ to remove HFIP for 1 h. The dried peptide films were stored at –20 °C in a desiccator jar. A β 1-42 peptide films were removed from the freezer 10 min prior to use to equilibrate to room temperature. HiTrap desalting columns (5 mL; GE Healthcare) were carefully equilibrated with 20 mL of Neurobasal Medium (Gibco by Life Technologies). Each peptide film was resuspended in 250 μ L of DMSO to yield a final A β concentration of 10 mM and were vortexed and pooled prior to adding to the column. DMSO was removed from the peptide by passing the solution through the HiTrap column, and the peptide was eluted into two volumes of 1 mL of Neurobasal Medium passed through the column. The eluted peptide was collected into LoBind tubes and protein concentration was assessed via a Bradford Protein Assay kit (Thermo Fischer Scientific). Each tube was normalized with Neurobasal Medium to a final concentration of 220 μ g/mL (50 μ M) and split equally into LoBind tubes to allow an equal ratio of liquid:air (e.g. 1 mL peptide in a 2 mL tube). The normalized A β 1-42 monomers were then oligomerized for 1 h at 25 °C using a plate shaker at 330 rpm. Finally, the oligomers were centrifuged for 10 min at 14 500 rpm, the supernatant was collected and analysed using a DynaPro Dynamic Light Scattering (DLS) Instrument (Wyatt Technology), and the results analysed using Dynamics V7 Software. The freshly prepared A β o were stored at –80 °C until needed for STD NMR experiments.

STD NMR screening assay against A β o

All samples were prepared in 550 μ L of deuterated 10 mM phosphate buffer. A β o and test compounds were added to reach final concentrations of 2 and 200 μ M, respectively (1:100 molar ratio). The final percentage of DMSO was 2% in screening experiments, and 4% in the competition assay between compound **51** and bexarotene. Controls were prepared in the absence of A β o, but maintaining the same relative volumes of buffer, Neurobasal Medium and DMSO. All experiments were performed on a Bruker Avance III or 600 MHz spectrometer equipped with a QCI cryoprobe. For the acquisition, a standard Bruker pulse sequence was used, with 2 s of saturation at 0 ppm and -40 ppm for on and off resonance. Double solvent suppression was achieved with a DPFGSE element incorporating a selective 180 pulse designed to flip both the water (from buffer) and DMSO (from ligand stock) peaks. Typically, 128 scans were acquired prior to processing with a 1 Hz line broadening function. Data were processed in topspin using the stdsplit AU program.

Cell culture

Human embryonic kidney (HEK) cells were grown in Dulbecco's modified essential medium (DMEM; D5796, Sigma) supplemented with 10% fetal bovine serum (FBS; 10695023, Fisher), 1% penicillin-streptomycin (10452882, Fisher) and 4 mM L-glutamine (G7513, Sigma), and maintained at 37 °C in a 5% CO₂ atmosphere.

Immunocytochemistry analysis of compound-mediated A β o-PrP^C disruption

Ninety six-well plates were incubated with 50 μ L of poly-L-ornithine hydrobromide 100 μ g/mL for 40 min and washed with PBS 3 times. HEK cells were seeded onto the plates diluted in Phenol red-free DMEM medium with high glucose (Gibco) to achieve 2×10^4 cells per 100 μ L in each well. The cells were incubated at 37 °C with 5% CO₂ overnight. Then, the medium was removed and 50 μ L of conditioning medium with recombinant A β o prepared by the Sheffield Institute for Translational Neuroscience (SiTraN) 1000 pc/mL was added to each well. These oligomers were derived from Chinese Hamster Ovary cells (7PA2 cells) stably transfected with cDNA encoding APP751, an amyloid precursor protein that contains the Val717Phe familial Alzheimer's disease mutation, as previously described by Kittelberger et al. [31]. After a 2 h-long incubation period, the conditioning medium was removed and the cells were washed once with PBS. Then, 100 μ L of fresh Phenol red-free DMEM medium with high glucose were added to each well and the cells were incubated with the flavone derivatives at the desired concentration (10 μ M in the screening assay or 1 μ M–20 μ M in the dose-response experiment with compound **26**) in 0.5% of DMSO for 1 h. The medium was subsequently removed. The cells were washed with 100 μ L of PBS containing Mg²⁺ and Ca²⁺, after which 100 μ L of 4% PFA were added to each well. Once incubated for 15 min at room temperature, the PFA was removed and the cells were washed once again with 100 μ L of PBS containing Mg²⁺ and Ca²⁺. One hundred μ L of PBS-T with 5% of Donkey serum were added and the cells were incubated for 1 h at room temperature. Then, the blocking solution was removed and the primary antibody (anti-A β o 6E10 antibody prepared in 50 μ L of PBS-T with 5% of Donkey serum, 1:250 dilution) was added prior to overnight incubation at 4 °C. After removal of the primary antibody, the cells were washed 3 times with 50 μ L of PBS-T for 5 min at room temperature. Fifty microliter of the secondary antibody (Alexa fluor 594 anti-mouse antibody, prepared in PBS-T, 1:500 dilution) were subsequently added to each well and the cells were incubated another hour at room temperature. The liquid was aspirated and the cells were washed twice with PBS-T and once with PBS (50 μ L each wash). Fifty microliter of 4',6-diamidino-2-phenylindole (DAPI, 100 ng/mL in PBS) were added to each well. Following 5 min of incubation, DAPI was removed and the cells washed with 100 μ L of PBS 3 times. One hundred microliter of PBS were finally added and the imaging was carried out by The Wolfson Light Microscopy Facility, using ImageXpress Micro Widefield High Content Screening System, 20 \times magnification, and 30 pictures taken per each well.

Data analysis was executed using MetaXpress Software Multi-Wavelength Translocation Application Module. Results are presented as the average of two experiments performed in triplicates.

MTT cytotoxicity assay

HEK cells were seeded onto a 96-well plate at a density of 1×10^4 cells per well and incubated at 37 °C with 5 % CO₂ overnight prior to the assay. Each compound was added in DMSO to reach final concentrations of 1 µM, 5 µM, 10 µM, 20 µM and 50 µM, in triplicates, adjusting the final DMSO concentration to 0.5 %. After 24 h of incubation at 37 °C, 5 % CO₂, 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 10 mg/mL in PBS) were added to each well, and cells were incubated for another 2 h. Then, the medium was removed and the blue formazan crystals dissolved in 60 µL of acidified isopropanol. The plates were shaken to promote full dissolution of the crystals, after which the optical density (OD) at 570 nm (with a 690 nm reference filter) was measured using a microplate reader. Cell survival rates were calculated using equation (1), and the results presented as the average of two experiments performed in triplicates.

$$\text{Cell Survival (\%)} = \left[\frac{OD_{\text{sample}} - OD_{\text{medium}}}{OD_{\text{cell control}} - OD_{\text{medium}}} \right] \times 100 \quad (1)$$

Acknowledgments: The European Union is gratefully acknowledged for the support of the project entitled “Diagnostic and Drug Discovery Initiative for Alzheimer’s Disease” (D3i4AD), FP7-PEOPLE-2013-IAPP, GA 612347. The financial support by Fundação da Ciência e da Tecnologia of the strategic project UID/Multi/00612/2013 and of the Ph.D. grant SFRH/BD/93170/2013 (AMM) is also acknowledged.

Author contributions: Compound design, synthesis and characterization were carried out by Ana Marta de Matos, under the supervision of Teresa Man, David Evans (Eli Lilly, Erl Wood Manor) and Amélia Pilar Rauter. HRMS of final compounds was accomplished by Maria Conceição Oliveira. Preparation of Aβ and STD-NMR experiments were conducted by Ana Marta de Matos and James Grayson under the supervision of Gary Sharman. PrP^C-Aβ disruption and cytotoxicity assays in neuronal cells were conducted by Imane Idrissi under the supervision of Beining Chen. This paper was written by Ana Marta de Matos and revised by David Evans, Gary Sharman, Beining Chen and Amélia Pilar Rauter.

References

- [1] Alzheimer’s World Report. 2016. Alzheimer’s Disease International, 2016.
- [2] D. M. Castro, C. Dillon, G. Machnicki, R. F. Allegri. *Dement. Neuropsychol.* **4**, 262 (2010).
- [3] N. T. Vagelatos, G. D. Eslick. *Epidemiol. Rev.* **35**, 152. (2013).
- [4] L. Blanco-Silvente, X. Castells, M. Saez, M. A. Barceló, J. Garre-Olmo, J. Vilalta-Franch, D. Capellà. *Int. J. Neuropsychopharmacol.* **20**, 519 (2017).
- [5] J. A. Hardy, G. A. Higgins. *Science* **256**, 184 (1992).
- [6] U. Sengupta, A. N. Nilson, R. Kayed. *EBioMedicine* **6**, 42 (2016).
- [7] G. Valincius, F. Heinrich, R. Budvytyte, D. J. Vanderah, D. J. McGillivray, Y. Sokolov, J. E. Hall, M. Lösche. *Biophys. J.* **95**, 4845 (2008).
- [8] A. J. Miñano-Molina, J. España, E. Martín, B. Barneda-Zahonero, R. Fadó, M. Solé, R. Trullás, C. A. Saura, J. Rodríguez-Alvarez. *J. Biol. Chem.* **286**, 27311 (2011).
- [9] J. Laurén, D. A. Gimbel, H. B. Nygaard, J. W. Gilbert, S. M. Strittmatter. *Nature* **457**, 1128 (2009).
- [10] W. Kudo, H. P. Lee, W. Q. Zou, X. Wang, G. Perry, X. Zhu, M. A. Smith, R. B. Petersen, H. G. *Hum. Mol. Genet.* **21**, 1138 (2012).
- [11] J. W. Um, S. M. Strittmatter. *Prion* **7**, 37 (2013).
- [12] P. K. Kamat, A. Kalani, S. Rai, S. Swarnkar, S. Tota, C. Nath, N. Tyagi. *Mol. Neurobiol.* **53**, 648 (2016).
- [13] H. B. Nygaard, C. H. van Dyck, S. M. Strittmatter. *Alzheimers Res. Ther.* **6**, 8 (2014).

- [14] E. Risse, A. J. Nicoll, W. A. Taylor, D. Wright, M. Badoni, X. Yang, M. A. Farrow, J. Collinge. *J. Biol. Chem.* **290**, 17020 (2015).
- [15] A. M. Matos, J. S. Cristóvão, D. Yashunsky, N. E. Nifantiev, A. S. Viana, C. M. Gomes, A. P. Rauter. *Pure Appl. Chem.* **89**, 1305 (2017).
- [16] C. C. Guimarães, D. D. Oliveira, M. Valdevite, A. L. Saltoratto, S. I. Pereira, S. de C. França, A. M. Pereira, P. S. Pereira. *Food Chem. Toxicol.* **86**, 88 (2015).
- [17] D. Sheeba Malar, R. Beema Shafreen, S. Karutha Pandian, K. Pandima Devi. *Pharm. Biol.* **55**, 381 (2017).
- [18] C. Airoldi, E. Sironi, C. Dias, F. Marcelo, A. Martins, A. P. Rauter, F. Nicotra, J. Jimenez-Barbero. *Chem. Asian J.* **8**, 596 (2013).
- [19] J. Habchi, P. Arosio, M. Perni, A. R. Costa, M. Yagi-Utsumi, P. Joshi, S. Chia, S. I. Cohen, M. B. Müller, S. Linse, E. A. Nollen, C. M. Dobson, T. P. Knowles, M. Vendruscolo. *Sci. Adv.* **2**, e1501244 (2016).
- [20] T. T. Wager, X. Hou, P. R. Verhoest, A. Villalobos. *ACS Chem. Neurosci.* **1**, 435 (2010).
- [21] J. Fantini, C. Di Scala, N. Yahi, J. D. Troadec, K. Sadelli, H. Chahinian, N. Garmy. *ACS Chem. Neurosci.* **5**, 216 (2014).
- [22] K. N. Nam, A. Mounier, N. F. Fitz, C. Wolfe, J. Schug, I. Lefterov, R. Koldamova. *Sci. Rep.* **6**, 24048 (2016).
- [23] C. Fernandez, O. Nieto, J. A. Fontenla, E. Rivas, M. L. de Ceballos, A. Fernandez-Mayoralas. *Org. Biomol. Chem.* **1**, 767 (2003).
- [24] X. Jiang, H. Xin, Q. Ren, J. Gu, L. Zhu, F. Du, C. Feng, Y. Xie, X. Sha, X. Fang. *Biomaterials* **35**, 518 (2014).
- [25] Y. Zhang, T. Zhang, F. Wang, J. Xie. *J. Chromatogr. Sci.* **53**, 97 (2015).
- [26] E. M. Janle, M. A. Lila, M. Grannan, L. Wood, A. Higgins, G. G. Yousef, R. B. Rogers, H. Kim, G. S. Jackson, L. Ho, C. M. Weaver. *J. Med. Food.* **13**, 926 (2010).
- [27] Y. Zhao, L. Zhang, Y. Peng, Q. Yue, L. Hai, L. Guo, Q. Wang, Y. Wu. *Chem. Biol. Drug Des.* **91**, 707 (2018).
- [28] A. R. Jesus, C. Dias, A. M. Matos, R. F. de Almeida, A. S. Viana, F. Marcelo, M. P. Macedo, A. Airoldi, F. Nicotra, A. Martins, E. J. Cabrita, J. Jiménez-Barbero, A. P. Rauter. *J. Med. Chem.* **57**, 9463 (2014).
- [29] K. N. Dahlgren, A. M. Manelli, W. B. Stine Jr, L. K. Baker, G. A. Krafft, M. J. LaDu. *J. Biol. Chem.* **277**, 32046 (2002).
- [30] I. Kuperstein, K. Broersen, I. Benilova, J. Rozensk, W. Jonckheere, M. Debulpaepe, A. Vandersteen, I. Segers-Nolten, K. Van Der Werf, V. Subramaniam, D. Braecken, G. Callewaert, C. Bartic, R. D'Hooge, Martins, F. Rousseau, J. Schymkowitz, B. De Strooper. *EMBO J.* **29**, 3408 (2010).
- [31] K. A. Kittelberger, F. Piazza, G. Tesco, L. G. Reijmers. *PLoS One* **7**, e29940 (2012).

Supplementary Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/pac-2019-0114>).