Conference paper

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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 (Aβo) and ability to disrupt Aβo-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 (Aβo)-induced neurodegeneration, we investigated whether the neuroprotective potential of nature-inspired flavonoids against Aβ-promoted toxicity would translate into the ability to disrupt PrP^C-Aβo interactions. Hence, we synthesized a small library of flavones and studied their binding affinity towards Aβo 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 physicochemical properties and optimal water solubility even without the sugar moiety, and a high interaction with Aβo involving the whole flavone core. Its *C*-glucosyl derivative, was, however, the best compound to inhibit PrP^C-Aβo interactions in a dose-dependent manner, with 41 % of inhibition capacity at 10 μM. The potential of *C*-glucosyl flavones and their aglycones as protein-protein interaction inhibitors able to tackle PrP^C-Aβo 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: Aβo-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.

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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 (Aβ) 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 Aβ fibril networks that constitute amyloid plaques, the past decade has led the scientific community to agree that much smaller Aβ oligomers (Aβo) are actually the most neurotoxic amyloid species of Aβ. With a spherical shape ranging from 3 nm up to 10 nm in size [6], soluble Aβo 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 Aβo in AD started to emerge. In 2009, Laurén and co-workers described PrP^C as a high affinity cell-surface receptor for soluble Aβo, being able to mediate synaptic impairment caused by Aβo [9]. Accordingly, *Prnp*$^{(-/-)}$ mice (genetically engineered prion protein-deficient mice) are resistant to the neurotoxic effects of Aβo *in vivo* and *in vitro* [10]. With the subsequent discovery of Fyn kinase as a key mediator in Aβo-PrP^C-induced neuronal damage [11], it is currently believed that the Aβo-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 3β (GSK-3β) and protein tyrosine kinase 2 (Pyk2), which are ultimately involved in tau hyperphosphorylation [12, 13]. These data strongly suggest that tackling Aβo-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 Aβ and PrP^C [14]. Given the potential of Aβo-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 Aβ and/or exert neuroprotective effects could, in fact, act by disrupting Aβo-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 Aβ and inhibition of small Aβo 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 Aβ-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 $A\beta_{1\text{-}42}$-induced toxicity in SHSY-5Y human neuroblastoma cells, with the ability to restore cell viability at 50 μM (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 Aβo-PrP^C interactions. Figure 2 summarizes

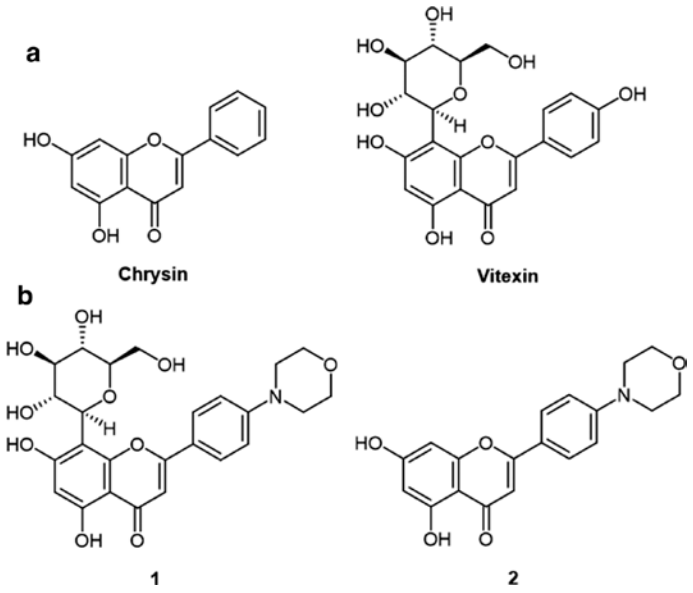


Fig. 1: (a) Chemical structure of chrysin and vitexin, two natural products with the ability to interact with Aβ; (b) lead scaffolds for the present study, compounds **1** and **2**.

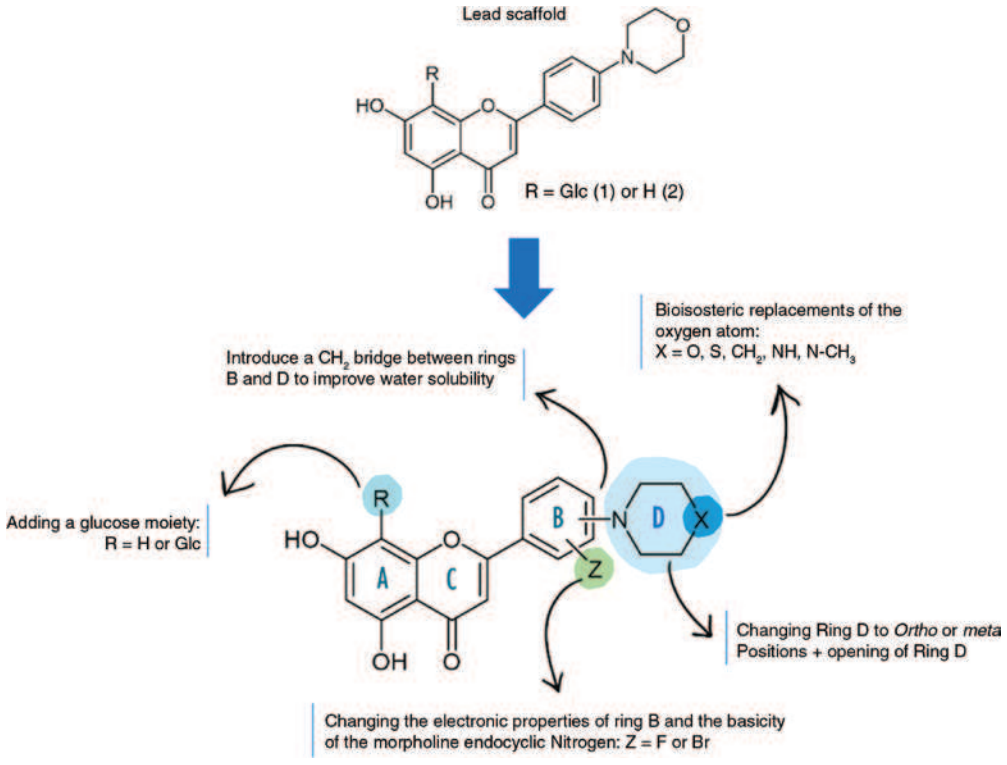


Fig. 2: Strategy for chemical modifications towards the optimization of the lead scaffold.

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 Aβo, while developing a brief

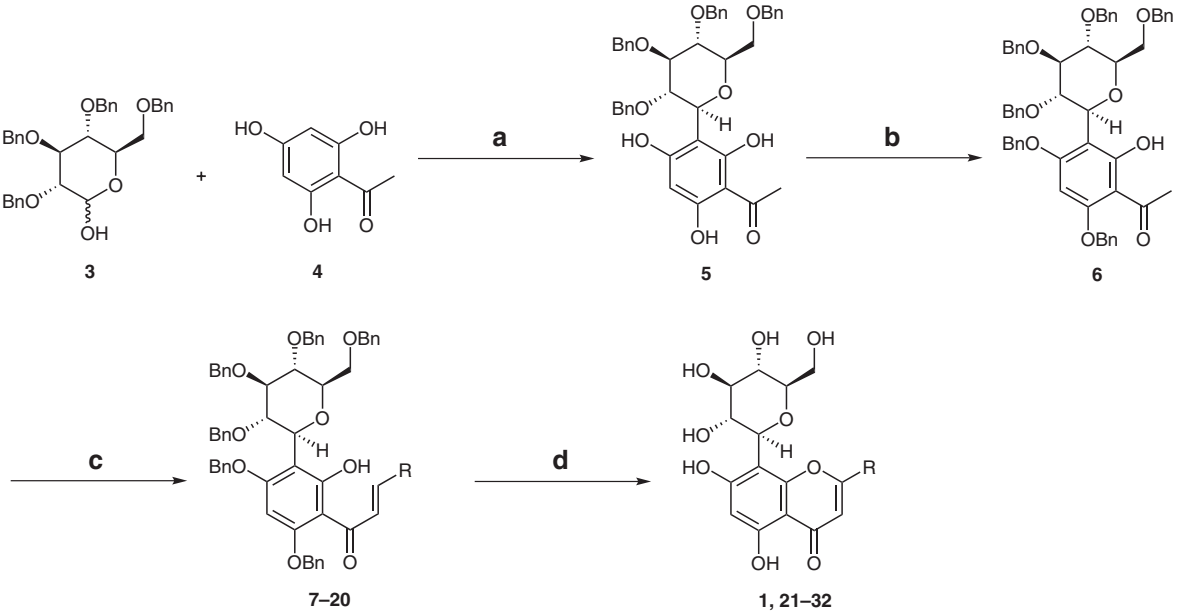
structure-activity relationship study. We subsequently present a functional assay aimed at exploring if some of the most relevant interactions with Aβo could translate into the highest potential to disrupt Aβo-PrP^C interactions.

Chemistry

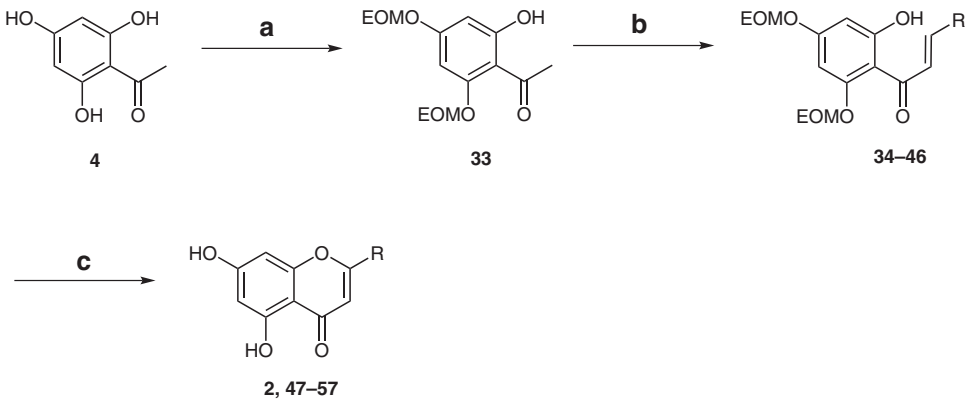
The synthetic route for *C*-glucosyl flavone derivatives started with a TMSOTf-promoted reaction between glycosyl donor **3** and 2,4,6-trihydroxyacetophenone **4**, to give intermediate **5** (Scheme 1). Then, after selective benzylation of two hydroxy groups, chalcone intermediates **7–20** were generated 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 BBr_3 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 K_2CO_3-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 chrysin **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, K_2CO_3, 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) I_2, pyridine, reflux, 48 h–72 h; then, (2) BBr_3, DCM, –78 °C, 2 h–4 h, 24 %–95 % over two steps.



4 33 34–46 2, 47–57

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 Aβo in a screening STD NMR, an assay previously adopted for the study of other polyphenol interactions with Aβo [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 $A\beta_{1\text{-}42}$ 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 Aβo.

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 chrysin **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 Aβo, 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 Aβo. 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*-glycosyl 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.

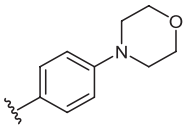
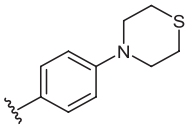
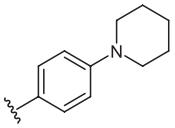
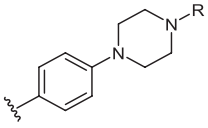
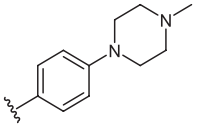
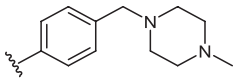
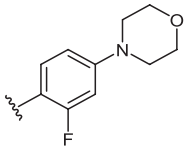
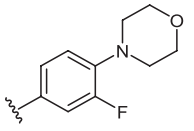
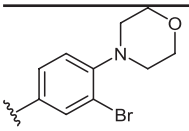
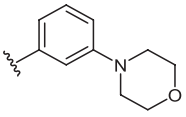
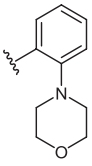
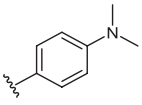
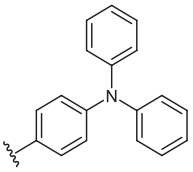
R	Yield			
	C-glucosyl flavones		Non-glucosylated flavones	
	Aldol condensation[a]	Cyclization and deprotection[b]	Aldol condensation[b]	Cyclization and deprotection[b]
	(**7**) 44 %	(**21**) 88 %	n.a.[c]	n.a.[c]
	(**8**) 67 %	(**1**) 74 %	(**34**) 76 %	(**2**) 99 %
	(**9**) 68 %	(**22**) 24 %	(**35**) 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.[d]	(**44**) 84 %	n.d.[d]
	(**19**) 55 %	(**31**) 91 %	(**45**) 99 %	(**56**) 96 %
	(**20**) 74 %	(**32**) 85 %	(**46**) 61 %	(**57**) 63 %

[a]Isolated yield; [b]reaction yields determined by LCMS; [c]not applicable; [d]not detected.

between rings B and D succeeded at further recovering interaction points with Aβo 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 Aβo 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 detivative **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 Aβo by STD NMR, with the exception of compound **51** (Fig. 4).

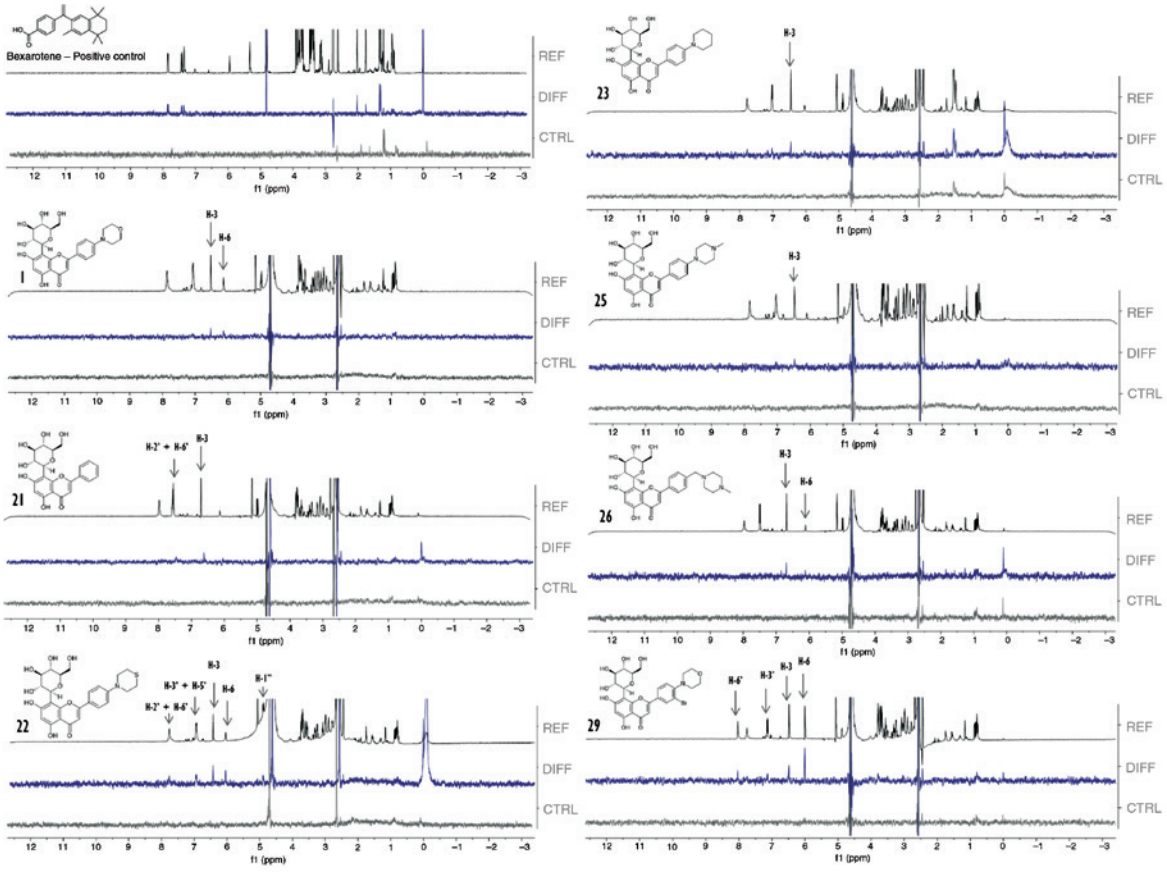


Fig. 3: STD-NMR screening assays to assess the binding affinity of flavones against Aβo. For reference (REF) and difference (DIFF) spectra, compounds were added in DMSO to a 2 μM solution of freshly prepared Aβo in a mixture of deutered phosphate buffer (pH 7.4) and neurobasal medium, to achieve a final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

The design of both compounds **26** and **51** was based on the introduction of a CH_2 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 Aβo and, overall, one of the most promising molecules of this study. Figure 4a shows that the binding mode of compound **51** towards Aβo 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 Aβo 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

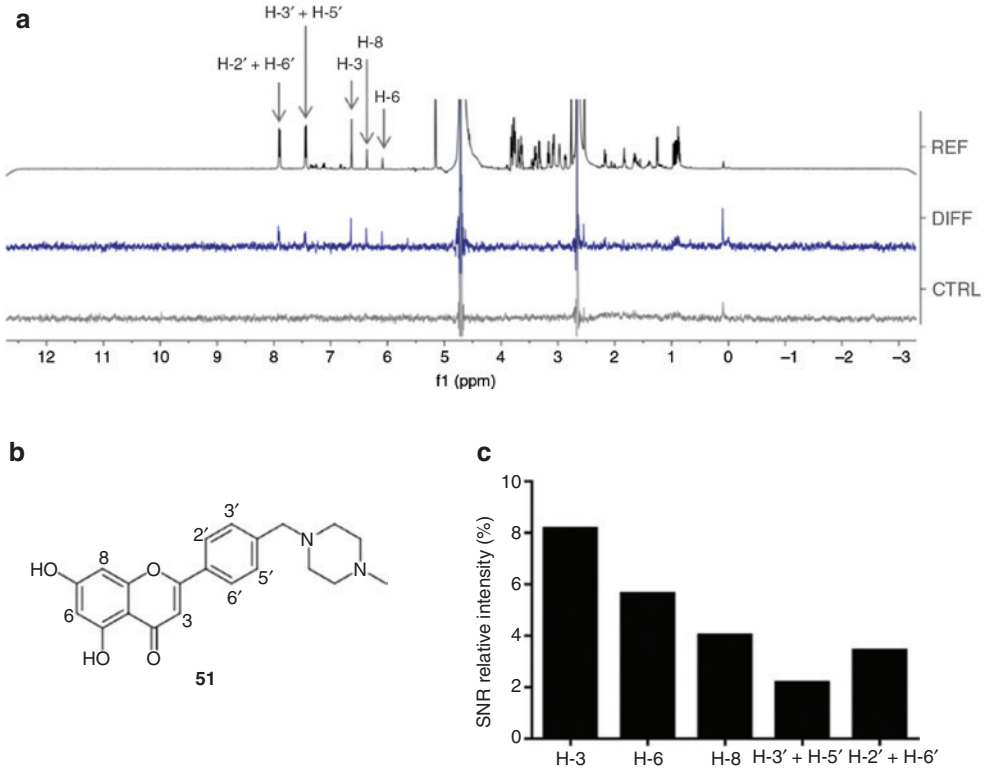


Fig. 4: Binding affinity of flavone **51** against Aβo, assessed by STD-NMR. (a) STD-NMR results: for the reference (REF) and difference (DIFF) spectra, **51** was added in DMSO to a 2 μM solution of freshly prepared Aβo in a mixture of deutered phosphate buffer (pH 7.4) and neurobasal medium, to achieve a final compound concentration of 200 μM (1:100 molar ratio); the control (CTRL) experiment was carried out in the same conditions, but in the absence of Aβo; (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 Aβ 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 Aβo. 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 Aβo, 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 Aβo at the same binding site as bexarotene. This compound binds to a linear motif at the *C*-terminal of $A\beta_{1\text{-}42}$ peptide, in a region encompassing residues 22–35, being therefore able not only to inhibit cholesterol-induced oligomerization of $A\beta_{1\text{-}42}$ into calcium-permeable ion channels formed by $A\beta_{1\text{-}42}$ in the neuronal lipid membrane, but also to counteract the disruption of actin cytoskeleton induced by Aβo [21, 22]. If **51** and bexarotene are both able to bind to the same binding site(s) in Aβo, 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.

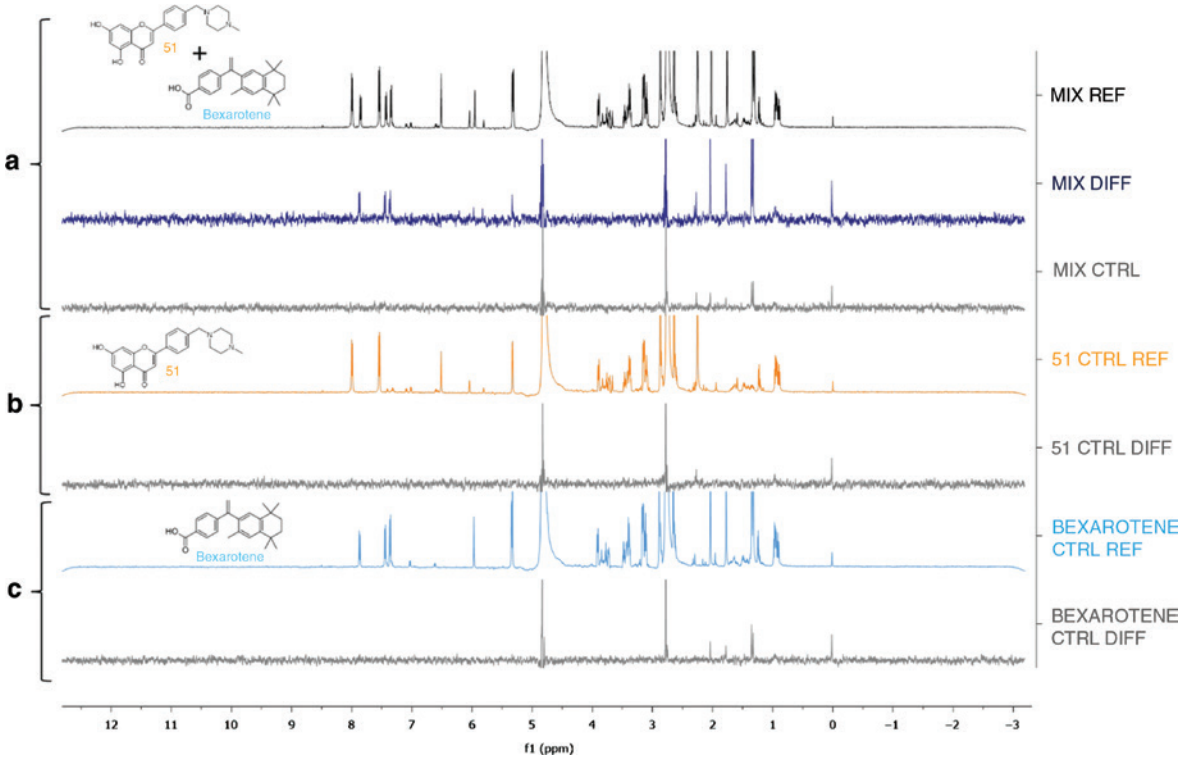


Fig. 5: STD-NMR competition assay between compound 51 and bexarotene towards Aβo. (a) Mixture between compound **51** and bexarotene, both at 200 μM; (b) compound **51** at 200 μM; (c) bexarotene at 200 μM. For reference (REF) and difference (DIFF) spectra, compounds were added in DMSO to a 2 μM solution of freshly prepared Aβo in a mixture of deutered 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 Aβo.

Assessment of Aβo-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 Aβo-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 do disrupt Aβo-PrP^C interactions (Fig. 6a), presenting an inhibition percentage of 41 % at 10 μM, with high statistical significance when compared to Aβo-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 Aβo 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 Aβo-PrP^C binding inhibition percentage; see Supporting Information, Fig. S3). These results seem to indicate: (1) that compound **51** has greater binding affinity for Aβo in a region that is not involved in Aβo-PrP^C interaction; and (2) that compounds **26** and **50**, which displayed only moderate or low affinity towards Aβo by STD NMR, are nonetheless able to bind to the Aβo-PrP^C interaction surface, thus competing with Aβo 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 Aβo-PrP^C. What's more, Fig. 6b shows that the effect of **26** is dose-dependent, with statistical significance achieved from 10 μM to 20 μM of compound when compared to the Aβo-PrP^C control.

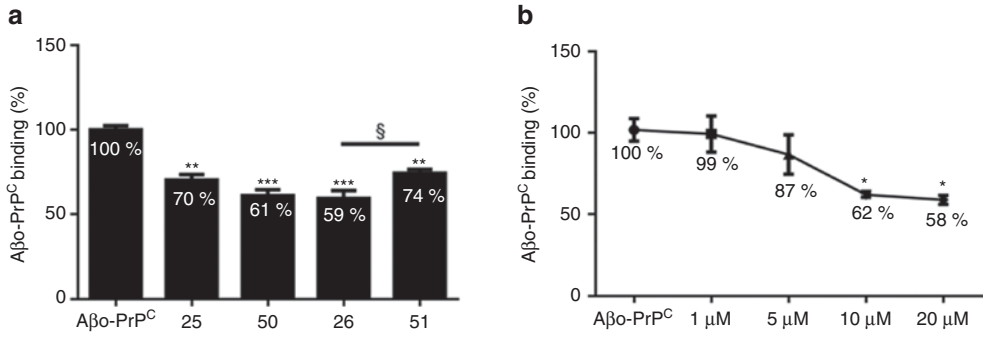


Fig. 6: Ability of *N*-methylpiperazinyl flavones to disrupt binding interactions between Aβo-PrP^C in HEK cells. (a) screening assay with *C*-glycosides and their aglycones at 10 μM; (b) dose-response effects of compound **26**. Results are presented as means ± standard deviation of two experiments 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. Aβo-PrP^C control; $^{§}P < 0.05$ 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 μM, with average cell survival rates above 77 % in all cases. However, cells treated with 50 μM of aglycones **50** and **51** only displayed 56 % ± 3 % and 36 % ± 6 % of cell viability, respectively, whereas their *C*-glycosides displayed 91 % ± 4 % and 79 % ± 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 Aβo-PrP^C units when compared to the non-treated control (Fig. 6a) and, thus, the use of these types of compounds as blockers of Aβo-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 Aβo-PrP^C complexes as valuable therapeutic targets for the development of PPIIs with neuroprotective activity, our goal was to study the synthesized compounds as Aβo-binders, and to assess whether the best molecules would also behave as promising Aβo-PrP^C interaction blockers.

C-glucosyl derivatives displayed optimal water solubility and were generally able to interact with Aβo 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 Aβo mediated by the whole flavone core. Our results additionally indicate that compound **51** competes with the bexarotene for the same binding site(s) in Aβo. To this point, mostly due to the low stability and high size variability of Aβo, 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 Aβo, 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 Aβo and PrP^C at 10 μM. Compound **26**, the *C*-glucosyl

derivative of **51**, presented the highest ability to block Aβo-PrP^C interactions in a dose-dependent manner, with 41 % of inhibition capacity at 10 μM. 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 Aβo-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 Aβo-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 2.1×50 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 μm 30×100 mm or Phenomenex gemini NX, C18, 10 μm 50×150 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 ^{19}F 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 (N_2) pressure was set to 1.4 bar, and the drying gas (N_2) 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 ≥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**.

(2*E*)-1-[4,6-dibenzyloxy-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}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_{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}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 *C*H-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 (*C*H_2-Ph)*, 72.9, 72.6 (C-1‴)*, 71.4, 71.3, 70.8, 70.6 (*C*H_2-Ph)*, 69.6, 69.5 (C-6‴)*. *Two peaks were observed due to the presence of rotamers.

(2*E*)-1-[4,6-dibenzyloxy-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}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, NCH_2CH_2O), 3.26–3.13 (m, 4H, NCH_2CH_2O)*. **^{13}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 *C*H-aromatics, C-1″, C-2″ and C-6″), 123.4, 123.2 (C-2)*, 119.3 (benzyl *C*H-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 (*C*H_2-Ph)*, 71.9, 71.5 (C-1‴)*, 70.3, 70.1, 69.9, 69.1, 68.4 (*C*H_2-Ph)*, 65.2 (NCH_2-*C*H_2O), 65.6, 65.5 (C-6‴)*, 47.1, 46.3 (NCH_2CH_2O). *Two peaks were observed due to the presence of rotamers.

(2*E*)-1-[4,6-dibenzyloxy-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}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, NCH_2CH_2S), 3.66–3.63 (m, 4H, NCH_2CH_2S). **^{13}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 *C*H-aromatics, C-1″, C-2″ and C-6″), 123.2, 122.9 (C-2)*, 119.3 (benzyl *C*H-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 (*C*H_2-Ph)*, 71.9, 71.5 (C-1‴)*, 71.0, 70.8, 70.2 (*C*H_2-Ph)*, 69.7, 69.2 (C-6‴)*, 49.8, 49.2 (NCH_2CH_2S)*, 25.9 (NCH_2CH_2S). *Two peaks were observed due to the presence of rotamers.

(2*E*)-1-[4,6-dibenzyloxy-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; $[\alpha]_D^{20} = -11°$ (*c* 0.1 $CHCl_3$); **^{1}H NMR** ($CDCl_3$) δ (ppm) 14.68 (s, 1H, OH-2′), 7.73–7.57 (olefinic AB system, 2H, J_{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, $NCH_2CH_2CH_2$). **^{13}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 *C*H-aromatics, C-1″, C-2″ and C-6″), 123.2, 122.9 (C-2)*, 119.3 (benzyl *C*H-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 (*C*H_2-Ph)*, 71.9, 71.5 (C-1‴)*, 70.5, 70.2, 69.5, 69.4 (*C*H_2-Ph)*, 69.3, 69.2 (C-6‴)*, 48.3 (N*C*H_2)*, 24.4 (NCH$_2$*C*H$_2$CH$_2$), 23.4 (NCH$_2$CH$_2$*C*H$_2$). *Two peaks were observed due to the presence of rotamers.

(2*E*)-1-[4,6-dibenzyloxy-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. **^{1}H NMR** ($CDCl_3$) 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-C*H*$_2$, H-1‴, H-2‴ and H-4‴), 3.89–3.46 (m, 8H, H-3‴, H-5‴, H-6‴a, H-6‴b and NC*H*$_2$CH$_2$N-Boc), 3.32–3.30, 3.18–3.15 (m, 4H, NCH$_2$C*H*$_2$N-Boc)*, 1.43 [s, 9H, C(C*H*$_3$)$_3$]. **^{13}C NMR** ($CDCl_3$) 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 *C*H-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*(CH$_3$)$_3$], 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 (*C*H_2-Ph)*, 71.9, 71.5 (C-1‴)*, 70.2 (*C*H_2-Ph)*, 69.7, 69.2 (C-6‴), 47.0, 46.0 (N*C*H$_2$*C*H$_2$N-Boc)*, 27.4 [C(*C*H$_3$)$_3$]. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{75}H_{75}NO_{11}$ 1143.5365, found 1143.5368; $[M+Na]^+$ calcd for $C_{75}H_{74}NNaO_{11}$ 1165.6184, found 1165.6178.

(2*E*)-1-[4,6-dibenzyloxy-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; $[\alpha]_D^{20} = -14°$ (*c* 0.1 $CHCl_3$); **^{1}H NMR** ($CDCl_3$) 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-C*H*$_2$, 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, NC*H*$_2$CH$_2$NCH$_3$)*, 2.51–2.46 (m, 4H, NCH$_2$C*H*$_2$NCH$_3$)*, 2.28 (s, 3H, NC*H*$_3$). **^{13}C NMR** ($CDCl_3$) 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 *C*H-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 (*C*H_2-Ph)*, 71.9, 71.5 (C-1‴)*, 70.2 (*C*H_2-Ph), 69.6, 69.2 (C-6‴), 53.8, 53.7 (NCH$_2$*C*H$_2$NCH$_3$)*, 46.8, 46.1 (N*C*H$_2$CH$_2$NCH$_3$)*, 45.2, 45.1(N*C*H3)*. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{68}H_{69}N_2O_9$ 1057.4998, found 1057.5002; $[M+Na]^+$ calcd for $C_{68}H_{68}N_2NaO_9$ 1079.4817, found 1079.4825.

(2*E*)-1-[4,6-dibenzyloxy-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. **^{1}H NMR** ($CDCl_3$) 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-C*H*$_2$, H-1‴), 4.70–4.45 (m, 5H, Ph-C*H*$_2$; part A of AB system, H-4‴), 4.36–4.24 (m, 2H, Ph-C*H*$_2$; 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, PhC*H*$_2$N), 2.46 (br s, 8H, N*C*H$_2$*C*H$_2$NCH$_3$), 2.27 (s, 3H, NC*H*$_3$). **^{13}C NMR** ($CDCl_3$) 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 *C*H-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 (*C*H_2-Ph)*, 72.8, 72.5 (C-1‴)*, 71.4, 71.3, 70.7, 70.2 (*C*H_2-Ph)*, 69.3 (C-6‴), 62.7, 62.6 (Ph*C*H_2N)*, 55.1 (N*C*H_2*C*H_2N)*, 46.0 (N*C*H_3)*. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{69}H_{71}N_2O_9$ 1071.5154, found 1071.5152; $[M+Na]^+$ calcd for $C_{69}H_{70}N_2NaO_9$ 1093.4974, found 1093.4965.

(2*E*)-1-[4,6-dibenzyloxy-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; $[\alpha]_D^{20} = -10°$ (*c* 0.1 $CHCl_3$); **^{1}H NMR** ($CDCl_3$) δ (ppm) 14.87, 14.55 (s, 1H, OH-2′)*, 7.93–7.68 (olefinic AB system, 2H, J_{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-C*H*$_2$, H-1‴), 4.70–4.48 (m, 5H, Ph-C*H*$_2$; part A of AB system, H-4‴), 4.35–4.24 (m, 2H, Ph-C*H*$_2$; part B of AB system, H-2‴), 3.86–3.57 (m, 8H, H-3‴, H-5‴, H-6‴a, H-6‴b, NCH$_2$C*H*$_2$O), 3.23–3.20, 3.15–3.13 (m, 4H, NC*H*$_2$CH$_2$O)*. **^{13}C NMR** ($CDCl_3$) 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, $J_{C\text{-}F}$ = 252.7 Hz, C-2″)*, 161.9, 161.7 (C-6′)*, 153.7, 153.6 (d, $J_{C\text{-}F}$ = 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 *C*H-aromatics, C-6″), 126.0, 125.9 (d, $J_{C\text{-}F}$ = 4.3 Hz C-2)*, 114.0 (d, $J_{C\text{-}F}$ = 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, $J_{C\text{-}F}$ = 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 (*C*H_2-Ph)*, 72.9, 72.5 (C-1‴)*, 71.2, 71.1, 70.7, 70.1 (*C*H_2-Ph)*, 69.4 (C-6‴), 66.7 (NCH$_2$*C*H$_2$O), 48.8, 47.8 (N*C*H$_2$CH$_2$O). **^{19}F NMR** ($CDCl_3$) δ (ppm) –112.57, –112.77 (dd, $J_{F\text{-}H\text{-}5''}$ = 13.7 Hz, $J_{F\text{-}H\text{-}2''}$ = 8.2 Hz).**Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{67}H_{65}FNO_{10}$ 1062.4584, found 1062.4594; $[M+Na]^+$ calcd for $C_{67}H_{64}FNNaO_{10}$ 1084.4406, found 1084.4406.

(2*E*)-1-[4,6-dibenzyloxy-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; $[\alpha]_D^{20} = -9°$ (*c* 0.1 $CHCl_3$); **^{1}H NMR** ($CDCl_3$) δ (ppm) 14.85, 14.52 (s, 1H, OH-2′)*, 7.74–7.53 (olefinic AB system, 2H, J_{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-C*H*$_2$, H-1‴), 4.73–4.45 (m, 5H, Ph-C*H*$_2$; part A of AB system, H-4‴), 4.36–4.24 (m, 2H, Ph-C*H*$_2$; part B of AB system, H-2‴), 3.89–3.57 (m, 8H, H-3‴, H-5‴, H-6‴a, H-6‴b, NCH$_2$C*H*$_2$O), 3.27–3.24, 3.15–3.13 (m, 4H, NC*H*$_2$CH$_2$O)*. **^{13}C NMR** ($CDCl_3$) 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, $J_{C\text{-}F}$ = 253.3 Hz, C-3″)*, 162.1, 161.7 (C-6′)*, 151.1, 150.9 (d, $J_{C\text{-}F}$ = 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 *C*H-aromatics and, C-1″ and C-6″), 126.4, 126.2 (d, $J_{C\text{-}F}$ = 4.5 Hz C-2)*, 118.1 (C-5″), 115.0, 114.9 (d, $J_{C\text{-}F}$ = 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 (*C*H_2-Ph)*, 72.8, 72.5 (C-1‴)*, 71.4, 71.1, 70.2, 69.4 (*C*H_2-Ph)*, 69.4 (C-6‴), 66.9 (NCH$_2$*C*H$_2$O), 50.7, 50.5 (N*C*H$_2$CH$_2$O)*. **^{19}F NMR** ($CDCl_3$) δ (ppm) –123.20 (dd, $J_{F\text{-}H\text{-}6''}$ = 13.8 Hz, $J_{F\text{-}H\text{-}3''}$ = 8.1 Hz). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{67}H_{65}FNO_{10}$ 1062.4584, found 1062.4593; $[M+Na]^+$ calcd for $C_{67}H_{64}FNNaO_{10}$ 1084.4406, found 1084.4414.

(2*E*)-1-[4,6-dibenzyloxy-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; $[\alpha]_D^{20} = -6°$ (*c* 0.1 $CHCl_3$); **^{1}H NMR** ($CDCl_3$) δ (ppm) 14.67, 14.34 (s, 1H, OH-2′)*, 7.75–7.56 (olefinic AB system, 2H, J_{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-C*H*$_2$, H-1‴), 4.71–4.48 (m, 5H, Ph-C*H*$_2$; part A of AB system, H-4‴), 4.35–4.23 (m, 2H, Ph-C*H*$_2$; part B of AB system, H-2‴), 3.91–3.57 (m, 8H, H-3‴, H-5‴, H-6‴a, H-6‴b, NCH$_2$C*H*$_2$O), 3.11–3.08 (m, 4H, NC*H*$_2$CH$_2$O). **^{13}C NMR** ($CDCl_3$) 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 *C*H-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, (*C*H_2-Ph)*, 72.8, 72.5 (C-1‴)*, 71.4, 71.2, 70.7, 70.3 (*C*H_2-Ph)*, 69.4 (C-6‴), 67.0 (NCH_2CH_2O), 51.8 (NCH_2CH_2O)*. *Two peaks were observed due to the presence of rotamers.

(2*E*)-1-[4,6-dibenzyloxy-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; $[\alpha]_D^{20} = -8°$ (*c* 0.1 $CHCl_3$); **^{1}H NMR** ($CDCl_3$) 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-C*H*$_2$, H-1‴), 4.69–4.48 (m, 5H, Ph-C*H*$_2$; part A of AB system, H-4‴), 4.35–4.23 (m, 2H, Ph-C*H*$_2$; part B of AB system, H-2‴), 3.83–3.57 (m, 8H, H-3‴, H-5‴, H-6‴a, H-6‴b, NCH_2CH_2O), 3.13–3.10, 3.06–3.03 (m, 4H, NCH_2CH_2O)*. **^{13}C NMR** ($CDCl_3$) 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 *C*H-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 (*C*H_2-Ph)*, 72.9, 72.5 (C-1‴)*, 71.2 (*C*H_2-Ph)*, 70.8, 70.2 (C-6‴)*, 69.4 (*C*H_2-Ph), 66.9 (NCH_2CH_2O), 49.1 (NCH_2CH_2O). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{67}H_{66}NO_{10}$ 1044.4681, found 1044.4681; $[M+Na]^+$ calcd for $C_{67}H_{65}NNaO_{11}$ 1066.4501, found 1066.4500.

(2*E*)-1-[4,6-dibenzyloxy-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; $[\alpha]_D^{20} = -7°$ (*c* 0.1 $CHCl_3$); **^{1}H NMR** ($CDCl_3$) 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-C*H*$_2$, H-1‴), 4.64–4.40 (m, 5H, Ph-C*H*$_2$; part A of AB system, H-4‴), 4.30–4.18 (m, 2H, Ph-C*H*$_2$; part B of AB system, H-2‴), 3.84–3.50 (m, 8H, H-3‴, H-5‴, H-6‴a, H-6‴b, NCH_2CH_2O), 2.93–2.90, 2.85–2.79 (m, 4H, NCH_2CH_2O)*. **^{13}C NMR** ($CDCl_3$) 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 *C*H-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 (*C*H_2-Ph)*, 71.9, 71.5 (C-1‴)*, 70.3, 70.2 (*C*H_2-Ph)*, 69.7, 69.2 (C-6‴)*, 68.4 (*C*H_2-Ph), 66.2, 66.1 (NCH_2CH_2O)*, 52.2 (NCH_2CH_2O). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{67}H_{66}NO_{10}$ 1044.4681, found 1044.4677; $[M+Na]^+$ calcd for $C_{67}H_{65}NNaO_{11}$ 1066.4501, found 1066.4497.

(2*E*)-1-[4,6-dibenzyloxy-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; $[\alpha]_D^{20} = -15°$ (*c* 0.1 $CHCl_3$); **^{1}H NMR** ($CDCl_3$) 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-C*H*$_2$, H-1‴), 4.74–4.46 (m, 5H, Ph-C*H*$_2$; part A of AB system, H-4‴), 4.36–4.25 (m, 2H, Ph-C*H*$_2$; 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 [$N(CH_3)_2$]*. **^{13}C NMR** ($CDCl_3$) 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 *C*H-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 (CH_2-Ph)*, 73.0, 72.6 (C-1‴)*, 73.1, 71.2, 70.7, 70.2 (CH_2-Ph)*, 69.4 (C-6‴), 40.2, 40.1 [N(CH_2)$_3$]. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{65}H_{64}NO_9$ 1002.4576, found 1002.4579; $[M+Na]^+$ calcd for $C_{65}H_{63}NNaO_9$ 1024.4395, found 1024.4399.

(2*E*)-1-[4,6-dibenzyloxy-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; $[\alpha]_D^{20} = -5°$ (*c* 0.1 $CHCl_3$); **^{1}H NMR** ($CDCl_3$) 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, NPh_2, 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-CH_2, H-1‴), 4.73–4.45 (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‴), 3.83–3.56 (m, 4H, H-3‴, H-5‴, H-6‴a, H-6‴b). **^{13}C NMR** ($CDCl_3$) 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 *C*H-aromatics, C-1″, C-2″ and C-6″), 125.3 (C-2)*, 124.0, 123.9 (benzyl *C*H-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 (CH_2-Ph)*, 73.1, 72.9 (C-1‴)*, 71.3, 71.2, 70.7, 70.2 (CH_2-Ph)*, 69.4 (C-6‴). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{75}H_{68}NO_9$ 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 I_2 (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 $MgSO_4$, 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 N_2 saturated atmosphere. A 1 M solution of BBr_3 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; $[\alpha]_D^{20} = +10°$ (*c* 0.5 MeOH); **^{1}H 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, $J_{1''\text{-}2''}$ = 9.9 Hz, H-1″)*, 4.14 (t, 1H, $J_{2''\text{-}1''\sim2''\text{-}3''}$ = 9.5 Hz, H-2″), 3.98–3.69 (m, 6H, H-6″a, H-6″b and NCH_2CH_2O), 3.70 (t, 1H, $J_{4''\text{-}3''\sim4''\text{-}5''}$ = 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 (NCH_2CH_2O, superimposed with the MeOD peak). **^{13}C 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 (NCH_2CH_2O), 63.0 (C-6″), 47.1 (NCH_2CH_2O). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{28}NO_{10}$ 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. **^{1}H NMR** (MeOD) δ (ppm) 8.12, 8.02 (d, 2H, J_{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, $J_{1''\text{-}2''}$ = 9.9 Hz, H-1″)*, 4.13 (t, 1H, $J_{2''\text{-}1''\text{–}2''\text{-}3''}$ = 9.3 Hz, H-2″), 3.99, 3.93 (br d, 1H, $J_{6''a\text{-}6''b}$ = 12.1 Hz, H-6″a)*, 3.83 (dd, 1H, $J_{6''b\text{-}6''a}$ = 12.1 Hz, $J_{6''b\text{-}5''}$ = 5.3 Hz, H-6″a), 3.69 (t, 1H, $J_{4''\text{-}3''\text{–}4''\text{-}5''}$ = 9.2 Hz, H-4″), 3.57–3.48 (m, 2H, H-3″ and H-5″). **^{13}C 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-thiazinnan-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; **^{1}H NMR** (MeOD) δ (ppm) 7.84, 7.74 (d, 2H, J_{ortho} = 8.5 Hz, H-2′ and H-6′)*, 6.99, 6.88 (d, 2H, J_{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, $J_{1''\text{-}2''}$ = 9.9 Hz, H-1″)*, 4.04 (t, 1H, $J_{2''\text{-}1''\text{–}2''\text{-}3''}$ = 9.6 Hz, H-2″), 3.84 (t, 1H, $J_{6''a\text{-}6''b}$ = 11.8 Hz, H-6″a), 3.74–3.68 (m, 5H, H-6″b and NCH_2CH_2S), 3.61 (t, 1H, $J_{4''\text{-}3''\text{–}4''\text{-}5''}$ = 9.4 Hz, H-4″), 3.44 (t, 1H, $J_{3''\text{-}2''\text{–}3''\text{-}4''}$ = 8.9 Hz, H-3″), 3.40–3.35 (m, 1H, H-5″), 2.59–2.57 (m, 4H, NCH_2CH_2S). **^{13}C 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 (NCH_2CH_2S), 25.3 (NCH_2CH_2S). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{28}NO_9S$ 518.1479, found 518.1478; $[M+Na]^+$ calcd for $C_{25}H_{27}NNaO_9S$ 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° (*c* 0.2 MeOH); **^{1}H NMR** (MeOD) δ (ppm) 7.93, 7.85 (d, 2H, J_{ortho} = 8.0 Hz, H-2′ and H-6′)*, 7.01 (d, 2H, J_{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, $J_{1''\text{-}2''}$ = 9.8 Hz, H-1″)*, 4.16 (t, 1H, $J_{2''\text{-}1''\text{–}2''\text{-}3''}$ = 9.2 Hz, H-2″), 3.97 (br d, 1H, $J_{6''a\text{-}6''b}$ = 12.1 Hz, H-6″a), 3.82 (dd, 1H, $J_{6''b\text{-}6''a}$ = 12.1 Hz, $J_{6''b\text{-}5''}$ = 5.7 Hz, H-6″a), 3.72 (t, 1H, $J_{4''\text{-}3''\text{–}4''\text{-}5''}$ = 9.2 Hz, H-4″), 3.56 (t, 1H, $J_{3''\text{-}2''\text{–}3''\text{-}4''}$ = 8.8 Hz, H-3″), 3.51–3.47 (m, 1H, H-5″), 3.41–3.38 (m, 4H, $NCH_2CH_2CH_2$), 1.70 (s, 6H, $NCH_2CH_2CH_2$). **^{13}C 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 ($NCH_2CH_2CH_2$), 25.2 ($NCH_2CH_2CH_2$), 24.1 ($NCH_2CH_2CH_2$). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{30}NO_9$ 500.1915, found 500.1914; $[M+Na]^+$ calcd for $C_{26}H_{29}NNaO_9$ 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° (*c* 0.3 MeOH); **^{1}H NMR** $[(CD_3)_2SO]$ δ (ppm) 8.01, 7.92 (d, 2H, J_{ortho} = 8.5 Hz, H-2′ and H-6′)*, 7.07, 7.01 (d, 2H, J_{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, $J_{1''\text{-}2''}$ = 9.9 Hz, H-1″)*, 3.85 (t, 1H, $J_{2''\text{-}1''\text{–}2''\text{-}3''}$ = 9.2 Hz, H-2″), 3.77 (br d, 1H, $J_{6''a\text{-}6''b}$ = 11.7 Hz, H-6″a), 3.56–3.22 (m, 8H, H-3″, H-4″, H-5″, H-6″b and NCH_2CH_2NH), 2.91–2.89 (m, 4H, NCH_2CH_2N). **^{13}C NMR** $[(CD_3)_2SO]$ 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 (NCH_2CH_2NH), 45.5 (NCH_2CH_2NH). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{29}N_2O_9$ 501.1868, found 501.1873; $[M+Na]^+$ calcd for $C_{25}H_{28}N_2NaO_9$ 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° (*c* 0.4 MeOH); **^{1}H NMR** [$(CD_3)_2SO$] δ (ppm) 13.25 (s, 1H, OH-5), 8.09 (d, 2H, J_{ortho} = 8.4 Hz, H-2′ and H-6′), 7.02 (d, 2H, J_{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, $J_{1''\text{-}2''}$ = 9.8 Hz, H-1″), 4.62–4.58 (m, 1H, OH), 3.85 (t, 1H, $J_{2''\text{-}1''\sim2''\text{-}3''}$ = 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 $NCH_2CH_2NCH_3$), 2.46–2.43 ($NCH_2CH_2NCH_3$), 2.23 (s, 3H, NCH_3). **^{13}C NMR** [$(CD_3)_2SO$] 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 ($NCH_2CH_2NCH_3$), 46.0 ($NCH_2CH_2NCH_3$), 45.1 (NCH_3). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{28}NO_{10}$ 502.1708, found 502.1700; $[M+Na]^+$ calcd for $C_{25}H_{27}NNaO_{10}$ 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° (*c* 0.1 MeOH); **^{1}H NMR** [MeOD] δ (ppm) 8.11, 8.01 (d, 2H, J_{ortho} = 8.1 Hz, H-2′ and H-6′)*, 7.56 (d, 2H, J_{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, $J_{1''\text{-}2''}$ = 9.9 Hz, H-1″), 4.12 (t, 1H, $J_{2''\text{-}1''\sim2''\text{-}3''}$ = 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 $PhCH_2N$), 2.66–2.51 ($NCH_2CH_2NCH_3$), 2.35 (s, 3H, NCH_3). **^{13}C 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 ($PhCH_2N$), 61.6 (C-6″), 54.3 ($NCH_2CH_2NCH_3$), 52.0 ($NCH_2CH_2NCH_3$), 44.4 (NCH_3). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{33}N_2O_9$ 529.2181, found 529.2184; $[M+Na]^+$ calcd for $C_{27}H_{32}N_2NaO_9$ 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° (*c* 0.1 MeOH); **^{1}H NMR** (MeOD) δ (ppm) 8.23, 7.91 (t, 1H, $J_{ortho\text{–}H\text{-}F}$ = 8.8 Hz, H-6′)*, 6.93 (d, 1H, J_{ortho} = 8.7 Hz, H-5′), 6.80 (d, 1H, $J_{H\text{-}F}$ = 16.1 Hz, H-3′), 6.65 (s, 1H, H-3), 6.29 (s, 1H, H-6), 4.99 (d, 1H, $J_{1''\text{-}2''}$ = 9.7 Hz, H-1″), 4.09 (t, 1H, $J_{2''\text{-}1''\sim2''\text{-}3''}$ = 9.3 Hz, H-2″), 3.98–3.79 (m, 6H, H-6″a, H-6″b and NCH_2CH_2O), 3.60 (t, 1H, $J_{4''\text{-}3''\sim4''\text{-}5''}$ = 9.6 Hz, H-4″), 3.53 (t, 1H, $J_{3''\text{-}2''\sim3''\text{-}4''}$ = 8.5 Hz, H-3″), 3.49–3.44 (m, 1H, H-5″), 3.39–3.29 (NCH_2CH_2O, superimposed with the MeOD peak). **^{13}C NMR** (MeOD) δ (ppm) 182.6 (C-4), 163.2 (C-7), 162.5 (d, $J_{C\text{-}F}$ = 250.0 Hz, C-2′), 161.4 (C-5), 160.3 (C-2), 156.6 (C-8a), 155.1 (d, $J_{C\text{-}F}$ = 11.1 Hz, C-4′), 129.9 (C-6′), 109.8 (C-5′), 108.2 (d, $J_{C\text{-}F}$ = 10.3 Hz, C-1′), 106.4 (C-3), 104.5 (C-4a), 103.7 (C-8), 100.8 (d, $J_{C\text{-}F}$ = 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 (NCH_2CH_2O), 61.4 (C-6″), 47.0 (NCH_2CH_2O). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{27}FNO_{10}$ 520.1614, found 520.1613; $[M+Na]^+$ calcd for $C_{25}H_{26}FNNaO_{10}$ 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° (*c* 0.5 MeOH); **^{1}H NMR** (MeOD) δ (ppm) 7.88–7.69 (m, 2H, H-2′ and H-6′)*, 7.12 (t, 1H, $J_{ortho\text{–}H\text{-}F}$ = 8.7 Hz, 1H, H-5′), 6.65 (s, 1H, H-3), 6.28 (s, 1H, H-6), 5.06, 5.01 (d, 1H, $J_{1''\text{-}2''}$ = 9.9 Hz, H-1″)*, 4.09 (t, 1H, $J_{2''\text{-}1''\sim2''\text{-}3''}$ = 9.6 Hz, H-2″), 4.01 (br d, 1H, $J_{6''a\text{-}6''b}$ = 12.4 Hz, H-6″a), 3.89–3.82 (m, 5H, H-6″b and NCH_2CH_2O), 3.71 (t, 1H, $J_{4''\text{-}3''\sim4''\text{-}5''}$ = 9.2 Hz, H-4″), 3.56 (t, 1H, $J_{3''\text{-}2''\sim3''\text{-}4''}$ = 8.6 Hz, H-3″), 3.53–3.49 (m, 1H, H-5″), 3.28–3.19 (m, 4H, NCH_2CH_2O). **^{13}C 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, $J_{C\text{-}F}$ = 245.7 Hz, C-3′), 143.0 (d, $J_{C\text{-}F}$ = 8.2 Hz, C-4′), 124.5 (d, $J_{C\text{-}F}$ = 7.8 Hz, C-1′), 123.6 (C-6′), 118.4 (C-5′), 114.2 (d, $J_{C\text{-}F}$ = 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 (NCH_2CH_2O), 61.8 (C-6″), 50.0 (NCH_2CH_2O). **^{19}F NMR** (MeOD) δ (ppm) –122.45, –122.62 (dd, $J_{F\text{-}H\text{-}5''}$ = 14.2 Hz,

$J_{F\text{-}H\text{-}2''}$ = 8.9 Hz). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{27}FNO_{10}$ 520.1614, found 520.1615; $[M+Na]^+$ calcd for $C_{25}H_{26}FNNaO_{10}$ 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° (*c* 0.2 MeOH); **^{1}H NMR** (MeOD) δ (ppm) 8.24, 8.17 (s, 1H, H-2′)*, 7.97, 7.92 (d, 1H, J_{ortho} = 8.6 Hz, H-6′)*, 7.20 (d, 1H, J_{ortho} = 8.5 Hz, H-5′), 6.64 (s, 1H, H-3), 6.26 (s, 1H, H-6), 5.04, 5.01 (d, 1H, $J_{1''\text{-}2''}$ = 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 NCH_2CH_2O), 3.60–3.53 (m, 2H, H-3″ and H-5″), 3.17–3.10 (m, 4H, NCH_2CH_2O). **^{13}C 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 (NCH_2CH_2O), 62.3 (C-6″), 51.5 (NCH_2CH_2O). *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{27}BrNO_{10}$ 580.0813, found 580.0811; $[M+Na]^+$ calcd for $C_{25}H_{26}BrNNaO_{10}$ 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° (*c* 0.1 MeOH); **^{1}H 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_{ortho} = 7.8 Hz, H-6′), 6.73 (s, 1H, H-3), 6.30 (s, 1H, H-6), 5.10, 5.01 (d, 1H, $J_{1''\text{-}2''}$ = 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 NCH_2CH_2O), 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, NCH_2CH_2O)*. **^{13}C 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 (NCH_2CH_2O), 61.9, 61.1 (C-6″)*, 49.2, 48.9 (NCH_2CH_2O)*. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{28}NO_{10}$ 502.1708, found 502.1709; $[M+Na]^+$ calcd for $C_{25}H_{27}NNaO_{10}$ 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° (*c* 0.5 MeOH); **^{1}H NMR** (MeOD) δ (ppm) 7.94, 7.84 (d, 2H, J_{ortho} = 8.4 Hz, H-2′ and H-6′)*, 6.81 (d, 2H, J_{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, $J_{1''\text{-}2''}$ = 9.6 Hz, H-1″)*, 4.18 (t, 1H, $J_{2''\text{-}1''\sim2''\text{-}3''}$ = 9.6 Hz, H-2″), 3.99, 3.94 (d, 1H, $J_{6''a\text{-}6''b}$ = 12.3 Hz, H-6″a)*, 3.82 (dd, 1H, $J_{6''b\text{-}6''a}$ = 12.2 Hz, $J_{6''b\text{-}5''}$ = 6.1 Hz, H-6″b), 3.73 (t, 1H, $J_{4''\text{-}3''\sim4''\text{-}5''}$ = 9.4 Hz, H-4″), 3.57 (t, 1H, $J_{3''\text{-}2''\sim3''\text{-}4''}$ = 9.1 Hz, H-3″), 3.52–3.47 (m, 1H, H-5″), 3.01 [s, 6H, $N(CH_3)_2$]. **^{13}C 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 [$N(CH_3)_2$]. *Two peaks were observed due to the presence of rotamers. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{23}H_{26}NO_9$ 460.1602, found 460.1605; $[M+Na]^+$ calcd for $C_{23}H_{25}NNaO_9$ 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° (*c* 0.8 MeOH); **^{1}H NMR** (MeOD) δ (ppm) 7.95–7.86 (d, 2H, J_{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_{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, $J_{1''\text{-}2''}$ = 9.9 Hz, H-1″)*, 4.13 (t, 1H, $J_{2''\text{-}1''\sim2''\text{-}3''}$ = 9.3 Hz, H-2″), 3.89, 3.83 (d, 1H, $J_{6''a\text{-}6''b}$ = 12.1 Hz, H-6″a)*, 3.78–3.41 (m, 4H, H-3″, H-4″, H-5″ and H-6″b)*. **^{13}C 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 (C_q-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 $C_{33}H_{30}NO_9$ 584.1915, found 584.1912; $[M+Na]^+$ calcd for $C_{33}H_{30}NNaO_9$ 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 $MgSO_4$, 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). **^{1}H NMR** ($CDCl_3$) 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, OCH_2O), 5.13 (s, 2H, OCH_2O), 3.70–3.60 (m, 4H, OCH_2CH$_3$), 2.55 (s, 3H, CH_3-Ac), 1.21–1.10 (m, 6H, OCH$_2$CH_3). **^{13}C NMR** ($CDCl_3$) 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 (OCH$_2$O), 64.0, 63.8 (OCH$_2$CH$_3$), 32.0 (CH$_3$-Ac), 14.0 (OCH$_2$CH$_3$). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{21}O_6$ 285.2333, found 285.1324; $[M+Na]^+$ calcd for $C_{14}H_{20}NaO_6$ 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 $MgSO_4$, filtered and concentrated under vacuum. The residue was purified using the most adequate purification method(s) to afford compounds **34–46**.

(2*E*)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-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; **^{1}H NMR** ($CDCl_3$) 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, OCH_2O), 5.16 (s, 2H, OCH_2O), 3.79 (t, *J* = 5.0 Hz, 4H, NCH$_2$CH_2O), 3.73–3.63 (m, 4H, OCH_2CH$_3$), 3.19 (t, *J* = 5.0 Hz, 4H, NCH_2CH$_2$O), 1.19–1.14 (m, 6H, OCH$_2$CH_3). **^{13}C NMR** ($CDCl_3$) 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, (OCH$_2$O), 65.6 (NCH$_2$CH$_2$O), 64.1, 63.8 (OCH$_2$CH$_3$), 47.0 (NCH$_2$CH$_2$O), 14.1 (OCH$_2$CH$_3$). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{32}NO_7$ 458.2173, found 458.2177; $[M+Na]^+$ calcd for $C_{25}H_{31}NNaO_7$ 480.1993, found 480.1998.

(2*E*)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-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; **^{1}H NMR** ($CDCl_3$) 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$_2$S), 2.66–2.63 (m, 4H, NCH$_2$CH_2S), 1.20–1.14 (m, 6H, OCH$_2$CH_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$_2$S), 25.0 (NCH$_2$$CH_2$S), 14.1 (OCH$_2$$CH_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.

(2*E*)-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; **^{1}H 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$_2$CH_2CH_2), 1.20–1.14 (m, 6H, OCH$_2$CH_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$_2$$CH_2$), 23.3 (NCH$_2CH_2$$CH_2$), 14.1 (OCH$_2$$CH_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.

(2*E*)-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. **^{1}H 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$_2$CH_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(*C*H$_3$)$_3$], 15.1 (OCH$_2$$CH_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.

(2*E*)-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; **^{1}H 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$_2$CH_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$_2$$CH_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.

(2*E*)-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; **^{1}H 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$_2$CH_3). **^{13}C NMR** [(CD$_3$)$_2$CO] 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$_2$O), 65.0, 64.4 (OCH$_2$CH$_3$), 61.7 (PhCH$_2$N), 54.0, 51.6 (NCH$_2$), 44.0 (NCH$_3$), 14.6 (OCH$_2$CH$_3$). HRMS-ESI (*m/z*): [M + H]$^+$ calcd for $C_{27}H_{37}N_2O_6$ 485.2646, found 485.2651; [M + Na]$^+$ calcd for $C_{27}H_{36}N_2NaO_6$ 507.2466, found 507.2469.

(2*E*)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-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; **^{1}H NMR** (CDCl$_3$) 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$_2$CH_2O), 3.73–3.63 (m, 4H, OCH_2CH$_3$), 3.19 (t, J = 5.0 Hz, 4H, NCH_2CH$_2$O), 1.19–1.15 (m, 6H, OCH$_2$CH_3). **^{13}C NMR** (CDCl$_3$) 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$_2$O), 65.5 (NCH$_2$CH$_2$O), 64.1, 63.8 (OCH$_2$CH$_3$), 46.7 (NCH$_2$CH$_2$O), 14.0 (OCH$_2$CH$_3$). **^{19}F NMR** (CDCl$_3$) 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 $C_{25}H_{31}FNO_7$ 476.2079, found 476.2085; [M + Na]$^+$ calcd for $C_{25}H_{30}FNNaO_7$ 498.1899, found 498.1904.

(2*E*)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-3-[3-fluoro-4-(morpholin-4-yl)phenyl]prop-2-en-1-one (41): Purified by column chromatography (cyclohexane/tetrahydrofurane 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; **^{1}H NMR** (CDCl$_3$) 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$_2$CH_2O), 3.81–3.69 (m, 4H, OCH_2CH$_3$), 3.17 (t, J = 5.0 Hz, 4H, NCH_2CH$_2$O), 1.28–1.20 (m, 6H, OCH$_2$CH_3). **^{13}C NMR** (CDCl$_3$) 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$_2$O), 66.8 (NCH$_2$CH$_2$O), 65.4, 64.9 (OCH$_2$CH$_3$), 50.5, 50.4 (NCH$_2$CH$_2$O), 15.1, 15.0 (OCH$_2$CH$_3$). **^{19}F NMR** (CDCl$_3$) 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 $C_{25}H_{31}FNO_7$ 476.2079, found 476.2081; [M + Na]$^+$ calcd for $C_{25}H_{30}FNNaO_7$ 498.1899, found 498.1897.

(2*E*)-1-[2,4-bis(ethoxymethoxy)-6-hydroxy]phenyl-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; **^{1}H NMR** (CDCl$_3$) 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$_2$CH_2O), 3.82–3.70 (m, 4H, OCH_2CH$_3$), 3.11 (t, J = 5.0 Hz, 4H, NCH_2CH$_2$O), 1.29–1.22 (m, 6H, OCH$_2$CH_3). **^{13}C NMR** (CDCl$_3$) 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$_2$O), 67.0 (NCH$_2$CH$_2$O), 65.4, 64.9 (OCH$_2$CH$_3$), 51.8 (NCH$_2$CH$_2$O), 15.1 (OCH$_2$CH$_3$). HRMS-ESI (*m/z*): [M + H]$^+$ calcd for $C_{25}H_{31}BrNO_7$ 536.1278, found 536.1282; [M + Na]$^+$ calcd for $C_{25}H_{30}BrNNaO_7$ 558.1098, found 558.1102.

(2*E*)-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/tetrahydrofurane 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. **^{1}H NMR** ($CDCl_3$) 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, OCH_2O), 5.24 (s, 2H, OCH_2O), 3.89–3.87 (m, 4H, NCH_2CH_2O), 3.79–3.70 (m, 4H, OCH_2CH_3), 3.21–3.18 (m, 4H, NCH_2CH_2O), 1.23 (t, 6H, *J* = 7.1 Hz, OCH_2CH_3). **^{13}C NMR** ($CDCl_3$) 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, (OCH_2O), 66.9 (NCH_2CH_2O), 65.3, 64.8 (OCH_2CH_3), 49.2 (NCH_2CH_2O), 15.1 (OCH_2CH_3). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{32}NO_7$ 458.2173, found 458.2176; $[M+Na]^+$ calcd for $C_{25}H_{31}NNaO_7$ 480.1993, found 480.1990.

(2*E*)-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; **^{1}H NMR** ($CDCl_3$) 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, OCH_2O), 5.16 (s, 2H, OCH_2O), 3.86–3.83 (m, 4H, NCH_2CH_2O), 3.72–3.63 (m, 4H, OCH_2CH_3), 2.93–2.91 (m, 4H, NCH_2CH_2O), 1.16 (t, 6H, *J* = 7.1 Hz, OCH_2CH_3). **^{13}C NMR** ($CDCl_3$) 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, (OCH_2O), 66.2 (NCH_2CH_2O), 64.2, 63.8 (OCH_2CH_3), 52.2 (NCH_2CH_2O), 14.1, 14.0 (OCH_2CH_3). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{25}H_{32}NO_7$ 458.2173, found 458.2177; $[M+Na]^+$ calcd for $C_{25}H_{31}NNaO_7$ 480.1993, found 480.1998.

(2*E*)-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; **^{1}H NMR** ($CDCl_3$) 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, OCH_2O), 5.23 (s, 2H, OCH_2O), 3.79 (q, 2H, *J* = 7.1 Hz, OCH_2CH_3), 3.73 (q, 2H, *J* = 7.1 Hz, OCH_2CH_3), 3.04 [s, 6H, $N(CH_3)_2$], 1.27–1.22 (m, 6H, OCH_2CH_3). **^{13}C NMR** ($CDCl_3$) 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, (OCH_2O), 65.2, 64.7 (OCH_2CH_3), 40.2 ($N(CH_2)_3$), 15.1 (OCH_2CH_3). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{23}H_{30}NO_6$ 416.2068, found 416.2074; $[M+Na]^+$ calcd for $C_{23}H_{29}NNaO_6$ 438.1887, found 438.1892.

(2*E*)-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. **^{1}H NMR** ($CDCl_3$) 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, NPh_2), 7.15–7.08 (m, 6H, NPh_2), 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, OCH_2O), 5.23 (s, 2H, OCH_2O), 3.78–3.69 (m, 4H, OCH_2CH_3), 1.23 (t, 6H, *J* = 7.1 Hz, OCH_2CH_3). **^{13}C NMR** ($CDCl_3$) 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, (OCH_2O), 65.2, 64.8 (OCH_2CH_3), 15.1 (OCH_2CH_3). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{33}H_{34}NO_6$ 540.2381, found 540.2387; $[M+Na]^+$ calcd for $C_{33}H_{33}NNaO_6$ 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 I_2 (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 $MgSO_4$, 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. **^{1}H NMR** [$(CD_3)OD$] 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, NCH_2CH_2O), 3.35–3.35 (NCH_2CH_2O, overlapped with metanol-d_4 peak). **^{13}C NMR** [$(CD_3)OD$] 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 (NCH_2CH_2O), 49.2 (NCH_2CH_2O). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{18}NO_5$ 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; **^{1}H NMR** [$(CD_3)_2CO$] 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, NCH_2CH_2O), 2.58–2.56 (m, 4H, NCH_2CH_2O). **^{13}C NMR** [$(CD_3)_2CO$] 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 (NCH_2CH_2O), 47.4 (NCH_2CH_2O). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{18}NO_4S$ 356.0951, found 356.0949; $[M+Na]^+$ calcd for $C_{19}H_{17}NNaO_4S$ 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; **^{1}H NMR** [$CO(CD_3)_2$] 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, NCH_2), 1.66 (br s, 6H, $NCH_2CH_2CH_2$). **^{13}C NMR** [$CO(CD_3)_2$] 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 (NCH_2), 25.2 ($NCH_2CH_2CH_2$), 24.2 ($NCH_2CH_2CH_2$). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{20}NO_4$ 338.1387, found 338.1385; $[M+Na]^+$ calcd for $C_{20}H_{20}NNaO_4$ 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; **^{1}H NMR** [$(CD_3)_2SO$] 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, NCH_2CH_2NH), 3.13–3.07 (m, 4H, NCH_2CH_2NH). **^{13}C NMR** [$(CD_3)_2SO$] 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 (NCH_2CH_2NH), 45.4 (NCH_2CH_2NH). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{19}N_2O_4$ 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; **^{1}H NMR** [MeOD/$CO(CD_3)_2$ 10:1] δ (ppm) 7.77 (d, 2H, J_{ortho} = 8.6 Hz, H-2′ and H-6′), 6.96 (d, 2H, J_{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, $NCH_2CH_2NCH_3$), 2.44–2.42 (m, 4H, $NCH_2CH_2NCH_3$), 2.18 (s, 3H, NCH_3). **^{13}C NMR** [MeOD/$CO(CD_3)_2$ 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 ($NCH_2CH_2NCH_3$), 46.9 ($NCH_2CH_2NCH_3$), 45.2 (NCH_3). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{21}N_2O_4$ 353.1496, found 353.1495; $[M+Na]^+$ calcd for $C_{20}H_{20}N_2NaO_4$ 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. **^{1}H NMR** $[CO(CD_3)_2]$ δ (ppm) 7.83 (d, 2H, J_{ortho} = 8.1 Hz, H-2′ and H-6′), 7.38 (d, 2H, J_{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, $PhCH_2N$), 3.39–3.21 (m, 8H, $NCH_2CH_2NCH_3$), 2.07 (s, 3H, NCH_3). **^{13}C NMR** $[CO(CD_3)_2]$ 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 ($PhCH_2N$), 54.9 ($NCH_2CH_2NCH_3$), 52.8 ($NCH_2CH_2NCH_3$), 45.3 (NCH_3). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{23}N_2O_4$ 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; **^{1}H NMR** $[(CD_3)_2CO]$ δ (ppm) 12.98 (s, 1H, OH-5), 7.90 (t, 1H, $J_{ortho-H-F}$ = 9.0 Hz, H-6′), 6.95 (d, 1H, J_{ortho} = 8.8 Hz, H-5′), 6.85 (d, 1H, J_{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, NCH_2CH_2O), 3.40–3.37 (m, 4H, NCH_2CH_2O). **^{13}C NMR** $[(CD_3)_2CO]$ δ (ppm) 181.9 (C-4), 164.0 (C-7), 162.2 (d, J_{C-F} = 250.8 Hz, C-2′), 162.1 (C-5), 159.9 (C-2), 157.9 (C-8a), 155.1 (d, J_{C-F} = 11.9 Hz, C-4′), 129.6 (d, J_{C-F} = 3.70 Hz, C-6′), 110.0 (d, J_{C-F} = 1.8 Hz, C-5′), 108.1 (d, J_{C-F} = 10.2 Hz, C-1′), 107.2 (d, J_{C-F} = 13.3 Hz, C-3), 101.2 (d, J_{C-F} = 27.5 Hz, C-3′), 104.4 (C-4a), 98.7 (C-6), 93.8 (C-8), 66.1 (NCH_2CH_2O), 47.1 (NCH_2CH_2O). **^{19}F NMR** ($CDCl_3$) δ (ppm) –110.42 (dd, $J_{F-H-5''}$ = 15.7 Hz, $J_{F-H-2''}$ = 8.8 Hz). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{17}FNO_5$ 358.1085, found 358.1081; $[M+Na]^+$ calcd for $C_{19}H_{16}FNNaO_5$ 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; **^{1}H NMR** $[(CD_3)_2CO]$ δ (ppm) 7.84–7.77 (m, 2H, H-2′ and H-6′), 7.18 (t, 1H, $J_{ortho-H-F}$ = 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, NCH_2CH_2O), 3.24–3.22 (m, 4H, NCH_2CH_2O). **^{13}C NMR** $[(CD_3)_2CO]$ δ (ppm) 182.0 (C-4), 164.1 (C-7), 162.6 (d, J_{C-F} = 2.5 Hz, C-2), 162.2 (C-5), 157.9 (C-8a), 154.8 (d, J_{C-F} = 246.5 Hz, C-3′), 143.0 (d, J_{C-F} = 8.0 Hz, C-4′), 124.4 (d, J_{C-F} = 8.2 Hz, C-1′), 123.3 (d, J_{C-F} = 2.9 Hz, C-6′), 118.7 (d, J_{C-F} = 3.7 Hz, C-5′), 114.1 (d, J_{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 (NCH_2CH_2O), 50.2 (d, J_{C-F} = 4.4 Hz, NCH_2CH_2O). **^{19}F NMR** ($CDCl_3$) δ (ppm) –122.29 (dd, $J_{F-H-6''}$ = 14.2 Hz, $J_{F-H-3''}$ = 8.8 Hz). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{17}FNO_5$ 358.1085, found 358.1083; $[M+Na]^+$ calcd for $C_{19}H_{16}FNNaO_5$ 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. **^{1}H NMR** $[(CD_3)_2CO]$ δ (ppm) 12.90 (s, 1H, OH-7), 8.27 (s, 1H, H-2′), 8.05 (d, 1H, J_{ortho} = 8.6 Hz, H-6′), 7.32 (d, 1H, J_{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, NCH_2CH_2O), 3.17–3.15 (m, 4H, NCH_2CH_2O). **^{13}C NMR** $[(CD_3)_2CO]$ 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 (NCH_2CH_2O), 51.7 (NCH_2CH_2O). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{17}BrNO_5$ 418.0285, found 418.0280; $[M+Na]^+$ calcd for $C_{19}H_{16}BrNNaO_5$ 440.0104, found 440.0102.

5,7-Dihydroxy-3′-(morpholin-4-yl)flavone (55): Purified by Isolute SCX-2 column chromatography (Biotage) 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; **^{1}H NMR** $[(CD_3)_2CO]$ 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, NCH_2CH_2O), 3.30–3.28 (m, 4H, NCH_2CH_2O). **^{13}C NMR** $[(CD_3)_2CO]$ 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 (NCH_2CH_2O), 48.7 (NCH_2CH_2O). HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{18}NO_5$ 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. **^{1}H NMR** $[(CD_3)_2SO]$ 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, $N(CH_3)_2$]. **^{13}C NMR** $[(CD_3)_2SO]$ 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 [$N(CH_3)_2$]. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{17}H_{16}NO_4$ 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. **^{1}H NMR** $[(CD_3)_2SO]$ δ (ppm) 7.86 (d, 2H, J_{ortho} = 8.8 Hz, H-2′ and H-6′), 7.39–7.34 (m, 4H, N-Ph_2), 7.18–7.15 (m, 8H, N-Ph_2, 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). **^{13}C NMR** $[(CD_3)_2SO]$ 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 $C_{27}H_{20}NO_4$ 422.1387, found 422.1384; $[M+Na]^+$ calcd for $C_{27}H_{19}NNaO_4$ 444.1206, found 444.1213.

Preparation of Aβo

ADDLs were prepared as described previously [29, 30] with some modifications. Briefly, 16 mg of lyophilized human Aβ1-42 peptide was equilibrated to room temperature for 30 min prior to resuspension in hexafluoropropan-2-ol (HFIP) to produce a 4.5 mg/mL Aβ 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 N_2 to remove HFIP for 1 h. The dried peptide films were stored at –20 °C in a desiccator jar. Aβ1-42 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 µL of DMSO to yield a final Aβ 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 µg/mL (50 µM) 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 Aβ1-42 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 Aβo were stored at –80 °C until needed for STD NMR experiments.

STD NMR screening assay against Aβo

All samples were prepared in 550 μL of deutered 10 mM phosphate buffer. Aβo and test compounds were added to reach final concentrations of 2 and 200 μM, 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 Aβo, 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 lien 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 % CO_2 atmosphere.

Immunocytochemistry analysis of compound-mediated Aβo-PrP^C disruption

Ninety six-well plates were incubated with 50 μL of poly-L-ornithine hydrobromide 100 μg/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 μL in each well. The cells were incubated at 37 °C with 5 % CO_2 overnight. Then, the medium was removed and 50 μL of conditioning medium with recombinant Aβo 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 μL 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 μM in the screening assay or 1 μM–20 μM 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 μL of PBS containing Mg^{2+} and Ca^{2+}, after which 100 μL 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 μL of PBS containing Mg^{2+} and Ca^{2+}. One hundred μL 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-Aβo 6E10 antibody prepared in 50 μL 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 μL 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 μL 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 μL 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× 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 % CO_2 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 % CO_2, 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.

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

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 Aβo and STD-NMR experiments were conducted by Ana Marta de Matos and James Grayson under the supervision of Gary Sharman. PrP^C-Aβo 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.

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