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2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-methyl (Pbfm) as an Alternative to the Trityl Group for the Side-Chain Protection of Cysteine and Asparagine/Glutamine

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The benzyl derivative of the Pbf group, which is the most commonly used side-chain protecting group for Arg, has been proposed for the protection of the side chains of Cys, Asp, and Glu. In the three cases, the new protecting group (Pbfm) was removed with a high concentration of TFA during the cleavage and global deprotection step. In addition, the Pbfm group can be removed from the Cys residue by using very dilute TFA solutions. Furthermore, when Cys is protected with the Pbfm group, it can be removed by oxidative treatment, thereby directly rendering the disulfide bridge on the solid phase.

Introduction

The use of the fluorenylmethoxycarbonyl (Fmoc)/*tert*-butyl (*t*Bu) groups is the strategy of choice for the solid-phase synthesis of peptides on both research and industrial scales.[1–4] The relevance of this so-called Fmoc/*t*Bu approach is that Fmoc is the temporal protecting group of the α-amino function and the side chains are permanently protected by TFA-labile protecting groups, such as the *t*Bu group for Ser/Thr, Tyr, and Asp/Glu or the *tert*-butyloxycarbonyl (Boc) group for Lys, Trp, or His.[1,5] However, other residues such as Cys, Asn/Gln, or His are also protected by the TFA-labile trityl (Trt) group and Arg by the (2,2,5,7,8-pentamethylchroman-6-sulfonyl) (Pmc) and [2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl (Pbf) groups.[1]

The Trt group has been applied successfully for Cys[6] and Asn/Gln protection;[7] however, its high hydrophobicity implies that the adduct between the scavenger used during final TFA cleavage and the Trt cation are not totally removed during workup and therefore remain on the peptide fraction. Furthermore, and due to the high UV absorbance of the Trt group, its presence can mask the quality of the crude peptide. Therefore, there is a need for other protecting groups that show similar lability to Trt but are less hydrophobic. Here we describe new protecting groups for Cys and Asn derived from the 2,2,5,7,8-pentamethylchroman and 2,3-dihydrobenzofuran, which also serve as the basis for the Pmc and Pbf groups used for Arg.[8,9] Thus, the benzyl derivatives of the bicyclic systems 2,2,5,7,8-pentamethylchroman-6-methyl (Pmcm) and 2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-methyl (Pbfm)[10] are proposed for the side-chain protection of Cys and Asn/Gln (Figure 1).

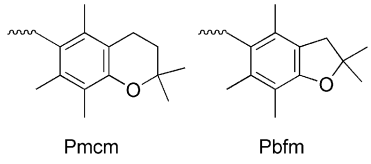

Figure 1. Structure of the Pmcm and Pbfm protecting groups.

Results and Discussion

Synthesis of the Protecting Group Precursors

The corresponding alcohols were prepared as protecting group precursors. Their syntheses were straightforward from the six- and five-membered ring cyclic ethers **1** (Scheme 1). Formylation was carried out by using a method previously described by our laboratory for the formylation of electron-rich aromatic rings,[11] which is based on the pioneering work of Gross et al.[12] and Landi and Ramig.[13] Excellent yields (Scheme 1) were achieved with dichloromethyl methyl ether and $TiCl_4$. The coordination of Ti with the oxygen atom of the dichloromethyl methyl ether increased the electrophilicity of this ether.

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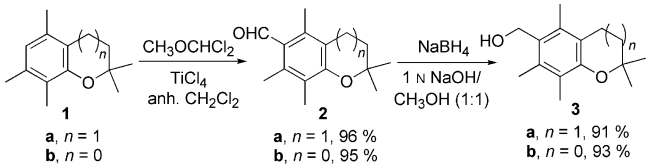


Scheme 1. Synthesis of the precursor alcohols of the protecting group.

Cys Derivatives

The thiol side chain of Cys was protected by the reaction of alcohols **3** with HCl·Cys in the presence of anhydrous TFA. The protected Cys residues, which were obtained in 82% (for **3a**) and 88% (for **3b**) yield, were further treated with Fmoc-succinimidyl (OSu) in the presence of triethylamine (TEA) in CH_3CN/H_2O (1:1) to give Fmoc-Cys(Pmcm)-OH (**4a**) and Fmoc-Cys(Pbfm)-OH (**4b**) (Figure 2) with a non-optimized yield of 70 and 75%, respectively.

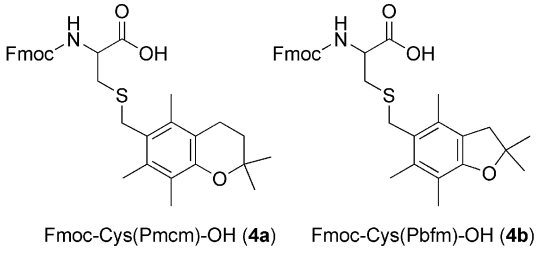


Figure 2. Structure of Fmoc-Cys(Pmcm)-OH and Fmoc-Cys(Pbfm)-OH.

The stability of the new derivatives was assayed with solutions with a low TFA concentration by using triethylsilane (TES) and triisopropylsilane (TIS) as scavengers.

The Pbfm group was more labile than the Pmcm group, which is consistent with the observation that Pbf is more labile than Pmc for Arg protection.[9] Moreover, TES was more effective for the push–pull effect that accompanies the scavenger purpose. In conclusion, Pbfm with 1% TFA in the presence of 5% TES is proposed as a convenient combination for the side-chain protection of Cys. Furthermore, the Pmcm and Pbfm groups should be totally removable with the use of highly concentrated solutions of TFA during the global deprotection step. Thus, the Pbfm group is similar to the Trt group for Cys protection purposes. Also, the former can be also removed by solutions of 1% TFA and is fully removed during global deprotection (Table 1).[1]

As model peptides, the pentapeptide H-Trp-Met-Asp-Phe-Cys-NH_2 (**5**), which incorporates the tetragastrine sequence, and oxytocin (**6**) were prepared. Compound **5**, which also contains two sensitive residues (Trp and Met), was synthesized with Fmoc chemistry on polyalkyl amide linker (PAL)-resin,[14] and Pbfm, *t*Bu, and Boc strategies were used for the side-chain protection of Cys, Asp, and Trp, respectively. The target peptide was obtained with an excellent purity after cleavage and global deprotection with reagent B (TFA–H_2O/phenol/TIS, 88:5:5:2), which is the most convenient cleavage cocktail for peptides containing Trp and Met.[15] No alkylation for Trp or Met was detected.

Table 1. Removal of the Pmcm and Pbfm groups from the thiol group of Cys.

	Removal cocktail	Time [min]	Removal yield [%] Pmcm	Pbfm
1.1	TFA/TES/CH_2Cl_2 (1:5:94)	5	59	71
1.2		15	83	91
1.3		30	100	100
2.1	TFA/TIS/CH_2Cl_2 (1:5:94)	5	37	71
2.2		30	87	100
2.3		60	100	100
3.1	TFA/TES/CH_2Cl_2 (0.5:5:94.5)	15	22	48
3.2		60	63	83
3.3		120	83	100
4.1	TFA/TIS/CH_2Cl_2 (0.5:5:94.5)	15	14	36
4.2		60	50	69
4.3		120	63	81

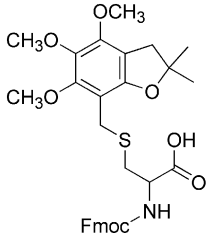
Oxytocin was synthesized by an Fmoc/PAL strategy by using Pbfm, Trt, and *t*Bu for the side-chain protection of Cys, Asn, and Tyr, respectively. Global deprotection with TFA/TES/CH_2Cl_2 (90:5:5) rendered the dihydrooxytocin in excellent yield. An additional advantage associated with the solid-phase approach is to carry out the maximum number of reactions while the peptide is still anchored to the resin. Thus, the oxidative deprotection of the Pbfm group was assayed on the solid phase, as described for other Cys protecting groups.[16] This reaction is particularly convenient for solid-phase strategies because the excess amounts of the oxidative reagents and the soluble side products can be removed effectively by filtration and washings. Several solvents were assayed by using 10 equiv. (5 for each Pbfm) of I_2 (Table 2).

Table 2. Oxidative removal of the Pbfm group.

	Solvent	Time [min]	Oxidation [%]
1.1	DMF/HOAc (4:1)	60	56
1.2		180	100
2.1	DMF/HOAc (1:1)	60	19
2.2		180	38
3.1	CH_2Cl_2/HFIP (1:1)	5	85
3.2		15	100
4.1	DMF/TFE/HOAc	5	73
4.2		15	100

The Pbfm group was also compatible with an oxidative removal process. Whereas the typical solid-phase conditions *N*,*N*-dimethylformamide (DMF)/acetic acid (HOAc) (4:1) completed the reaction in approximately 3 h, completion was achieved by fluorine solvents, such as 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and 2,2,2-trifluoroethanol (TFE), in only 15 min. The use of these solvents was proposed by Barlos and co-workers for rapid oxidation of free Cys-containing peptides in solution.[17]

To obtain a more labile protecting group, the Fmoc-Cys protected with a 4,5,6-trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-methyl moiety [Fmoc-Cys(Tmbm)-OH, **7**] (Figure 3) was prepared by using a similar route to that used for **4**.

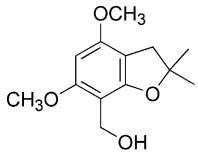


Fmoc-Cys(Tmbm)-OH (**7**)

Figure 3. Structure of Fmoc-Cys(Tmbm)-OH (**7**).

The Tmbm group, which should be more labile than the Pbfm group because it contains more electron-donating groups, was removed with high concentrations of TFA; however, TFA/TES/CH_2Cl_2 (1:5:94) (Table 1, Entries 1.1–1.3) achieved only 58% removal after 1 h and 75% after 2 h. This finding is attributed to the less stable carbocation formed compared to that formed when Pbfm is used. This may indicate a lack of planarity caused by the more hindered methoxy groups compared with the methyl groups.

Finally, with the objective to prepare a Cys derivative that overcomes the problems found for **7**, (4,6-dimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)methanol (Dmbm-OH, **8**; Figure 4) was prepared by using regioselective formylation based on the use of dichloromethyl methyl ether and $TiCl_4$.[11] After several attempts, the reaction of **8** with HCl·Cys in the presence of anhydrous TFA consistently rendered starting materials. These results could be explained by the extreme TFA lability of Dmbm; however, as the preparation of Dmbm is a reversible reaction, this was displaced to the starting materials.[18]



Dmbm-OH (**8**)

Figure 4. Structure of Dmbm-OH.

To explain the distinct behavior of the Dmbm, Pbfm, and Tmbm groups, we conducted a computational study. It was assumed that the mechanism of these reactions occurs through an intermediate carbocation generated from a protecting group. On the basis of this hypothesis, the protecting group that generates a stable carbocation will be more labile than that which generates a less stable one. Following the above reasoning, the scale of lability of the protecting groups can be obtained from calculation of the solvation Gibbs energy difference between a carbocation and its precursor protecting group. All geometries and energies presented in this study were calculated by using the B3LYP density functional theory,[19] as implemented in the Gaussian98 program package.[19d] Geometry optimizations were performed by using the 6-31G(d,p) basis set. The Hessian matrices were calculated at the same level of theory. The Hessian matrices provide a control that the stationary points localized are correct, with no imaginary frequencies for minima, and they also allow evaluation of the zero-point vibrational energy (ZPE) effects on the energy. Electrostatic solvent effects were modeled by the conductor-like solvation model COSMO at the B3LYP/6-31G(d,p) level. In this model, a cavity around the system is surrounded by a polarizable dielectric continuum.[20] The dielectric constant for the solvent was chosen to be $\varepsilon = 80$, a widely accepted standard for CH_2Cl_2. In Table 3 we report the results for the list of protecting groups. The ΔE term is the total electronic energy difference between the protecting group and its carbocation, $\Delta E = (E_{cabocation} + E_{H-}) - E_{protecting\ group}$. The ΔE + ZPE tern is their energy correction taking into account the ZPE, and ΔH and ΔG are the corresponding enthalpy and Gibbs energy respectively. Finally, $\Delta G_{solvation}$ is the Gibbs energy with the solvent effects. The values are given in kcal mol^{-1}.

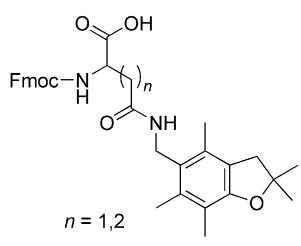
Table 3. Computed electronic energy differences, enthalpies, Gibbs energies, and solvation Gibbs energies between protecting groups and their carbocations. All values are in kcal mol^{-1}.

	ΔE	ΔE + ZPE	ΔH	ΔG	$\Delta G_{solvation}$
Dmbm	239.8	233.5	234.7	226.7	276.7
Pbfm	244.5	238.0	239.3	231.8	283.8
Tmbm	240.6	234.5	235.6	229.5	284.5

Our results show that the protecting groups Tmbm and Pbfm were the least labile. The low stability of the Tmbm cation was due to the effect of the methoxy group with respect to the methylene one. The cation originated from the Dmbm protecting group was the most stable in both electronic (ΔE) and solvent effects ($\Delta G_{solvation}$). In contrast, the cations generated from the Pbfm and Tmbm groups were the least stable and this instability was derived from the electronic structure and solvent effects. However, the solvent effects were greater for the Tmbm group. These energetic differences can be attributed to the presence of a methyl group and a methoxy group in the *meta* position of the Pbfm and Tmbf groups, respectively. In contrast, the Dmbm species does not have a group in this position.

Asn/Gln Derivatives

Given that the Pbfm group was more labile than the Pmcm group, it was used for the protection of the amide side chain of Asn and Gln. Fmoc-Asn/Gln(Pbfm)-OH (**9a**,**b**; Figure 5) were prepared by two routes. The first in-

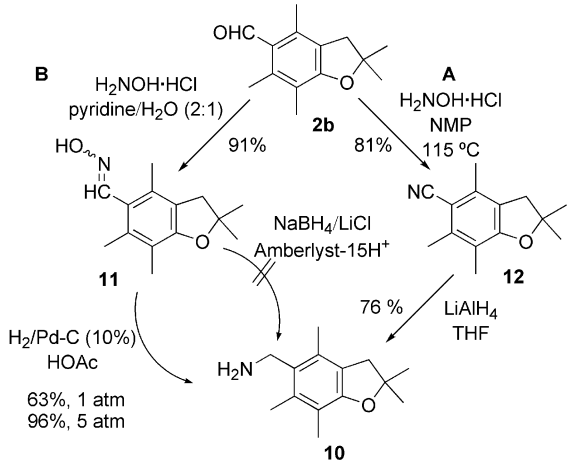


Fmoc-Asn/Gln(Pbfm)-OH (**9a**,**b**)

Figure 5. Structure of Fmoc-Asn/Gln(Pbfm)-OH.

volved only one reaction, Fmoc-Asn/Gln-OH with the alcohol Pbfm-OH in HOAc, in the presence of catalytic amounts of Ac_2O and H_2SO_4 (75% for **9a** and 65% for **9b**).[22] These conditions were not applicable for the direct introduction of the Trt group on Fmoc-Asn/Gln-OH.[7]

Alternatively, **9a**,**b** were obtained by reaction of the amine corresponding to the Pbfm (**10**) group with Asp/Glu derivatives. Scheme 2 shows the two synthetic routes tested for the preparation of the amine.



Scheme 2. Synthetic routes used for the preparation of Pbfm-NH_2.

Although both routes led to the desired amine, the route through the oxime and reduction at 5 atm (B) was more convenient than that through the nitrile (A).[21]

Fmoc-Asp/Glu(Pbfm)-OH were prepared by the following synthetic route: (i) Reaction of the Z-Asp/Glu-OBzl with Pbfm-NH_2, in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC), and 1-hydroxybenzotriazole (HOBt) in DMF/CH_2Cl_2 (1:1; 70% yield for Asn and 64% for Gln). (ii) Catalytic hydrogenation on C with HOAc/H_2O (87% yield for Asn and 85% for Gln). (iii) Reaction with Fmoc-OSu [53% yield for Asn (**9a**) and 59% for Gln (**9b**)].[22]

With the use of Fmoc-Asn/Gln(Pbfm)-OH as a substrate, the Pbfm group was removed by using TFA/CH_2Cl_2 (90:5) containing 5% of either TES or TIS as scavengers. As a model peptide, the so-called Riniker peptide, H-Lys-Gln-His-Asn-Pro-Tyr-Gln-Trp-Asn-Gly-NH_2, was synthesized through a Fmoc/PAL resin strategy by using Pbfm for the four Asn/Gln residues, Boc for Trp and Lys, *t*Bu for Trp and Tyr, and Trt for His. After global cleavage/deprotection with TFA–H_2O/phenol/TIS (88:5:5:2), the peptide was obtained in excellent yield. No alkylation of Trp was detected. Thus, the Pbfm group is also similar to Trt for Asn/Gln protection purposes.

Conclusions

The preparation and use of the Pmcm and Pbfm derivatives of Fmoc-Cys/Asn/Gln-OH in a solid-phase mode has revealed the Pbfm group is a feasible alternative to the Trt group. The Pbfm group, like its precursor Pbf, is less hydrophobic than the Trt group, and therefore, it can be more easily removed during workup.

In addition, several conclusions can be drawn from this study: (i) The benzofuran (Pbfm) moiety is more acid-labile than the chroman (Pmcm) one, which is in agreement with the Arg side-chain protecting groups. (ii) TES is more efficient than TIS as a push–pull additive for enhancing the cleavage/deprotection reaction. However, TES is not compatible with Trp-containing peptides because it favors Trp reduction. (iii) The use of fluorine solvents, such as HFIP and TFE, could enhance the oxidative removal of Cys protecting groups to render the disulfide bridge in the solid phase.

The Pbfm group, which is a benzyl-type protecting group, can be applied to other functional groups. Computational studies, which have rationalized the present results, should be used in the future design of new protecting groups, thereby rendering some experimental work unnecessary.

Experimental Section

General: All reagents were of reagent grade and were purchased from commercial sources and used without further purification. Protected amino acid derivatives and resins were obtained from Calbiochem-Novabiochem GmbH (Bad Sodem, Germany) or Bachem Feinchemikalien AG (Bubendorf, Switzerland). DCC, DMAP, DIPCDI, and HOBt were supplied by Fluka Chimie AG (Buchs, Switzerland). PyAOP and HATU were supplied by Perseptive Biosystems. $TiCl_4$, *n*-BuLi, dichloromethoxymethane, α-cyano-4-hydroxycinnamic acid, and 2,5-dihydroxybenzoic were purchased from Fluka or Aldrich. Commercial solutions of *n*-BuLi in hexanes were titrated prior to use following a procedure described in the literature. Solvents were of reagent grade and were distilled before use. THF, DCM (Normasolv grade), and Et_2O were obtained from Scharlau (Barcelona – Spain). DMF (peptide synthesis grade) was supplied by Scharlau or Panreac. (Barcelona – Spain). Dioxane was obtained from Merck (Darmstadt, Germany) and TFA (peptide synthesis grade) from Kali-Chemie AG (Hannover, Germany). THF was heated at reflux with sodium/benzophenone under a nitrogen atmosphere, distilled, and stored over sodium. DCM was stored over CaH_2 under an argon atmosphere. DMF was bubbled with nitrogen to remove volatile contaminants and stored over activated molecular sieves (4 Å, Merck). Et_2O was stored over sodium. THF, DCM, and dioxane were eluted prior to use through an Al_2O_3 column to remove trace amounts of peroxide and/or acid. All HPLC grade solvents were obtained from Panreac, Scharlau, or Merck. Deionized water for HPLC was previously filtered through a Milli-Q Plus system (Millipore, Bedford, USA) (18 $m\Omega cm^{-1}$).

Melting points were obtained with a Buchi apparatus, and rotatory powers were determined in a Perkin–Elmer 241 MC polarimeter (589 nm, 23 °C, 1 dm path length cell). IR spectra were registered with a Nicolet 510 FTIR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded at the NMR Facility at the University of Barcelona by using a Varian XL-200 (200 MHz) or a Varian Unity XL-300 (300 MHz). Chemical shifts for protons are given in δ relative to tetramethylsilane (TMS) and are referenced to residual protium in the NMR solvent ($CDCl_3$: δ = 7.26 ppm). Chemical shifts

for carbon are given in δ relative to tetramethylsilane (TMS) and are referenced to the carbon resonances in the solvent ($CDCl_3$: δ = 77.0 ppm). Mass spectroscopic data were obtained at the Mass Spectroscopy Facility at the University of Barcelona by using an HP-5988 spectrometer (Hewlett Packard) for electronic impact (70 eV impact mode) and chemical ionization, a VG QUATTRO instrument (Micromass) for electrospray (capillary voltage of 3.5 kV) and a Biflex™ III (Bruker) or a Voyager-DERP™ (Applied Biosystems) spectrometer for MALDI-TOF (nitrogen laser of 337 nm, α-cyano-4-hydroxycinnamic acid or 2,5-dihydroxybenzoic acid as matrices). Peptide samples were lyophilized in a 12 EL Freeze mobile or a QD 6 Freeze mobile (Virtis) lyophilizer. Amino acid analyses were carried out with a Beckman 6300 System after hydrolyzing peptide resins with 12 M HCl/propionic acid (1:1) at 155 °C for 1.5–3 h.

HPLC columns (Nucleosil C18 and C4 reversed-phase column, 250 × 40 mm, 10 μm) were obtained from Scharlau (Barcelona, Spain). Analytical HPLC was carried out with a Shimadzu instrument comprising two solvent delivery pumps (model LC-6A), an automatic injector (model SIL-6B), a variable wavelength detector (model SPD-6A), a system controller (model SCL-6B), and a plotter (model C-R6A). UV detection was performed at 220 nm and linear gradients of CH_3CN (0.036% TFA) into H_2O (0.045% TFA) were run at 1.0 mL min^{-1} flow rate. Flash chromatography was carried out by using Chromatogel 60 Å CC silica gel (230–400 mesh) from SDS (Peypin, France). For thin-layer chromatography (TLC), silica gel plates Merck 60_{F254} were used, and compounds were visualized by irradiation with UV light and/or by treatment with a solution of phosphomolybdic acid (7 g) in EtOH (100 mL) or a solution of ninhydrin (3% in EtOH), followed by heating.

2,2,5,7,8-Pentamethylchroman (1a): A mixture of 2,3,5-trimethylphenol (5 g, 36.7 mmol) and TFA (20 mL) was purged with N_2 and 2-methyl-3-buten-ol (4.2 mL, 40.4 mmol) was added dropwise. The mixture was stirred for 30 min at room temperature and volatiles were removed under vacuum. Then Et_2O (30 mL) was added, and the resulting solution was washed with saturated aqueous $NaHCO_3$ (3 × 25 mL) and brine (25 mL). The final organic solution was dried with anhydrous $MgSO_4$, and the solvent was removed under vacuum to afford an oil that was purified by chromatography (hexane/DCM, 9:1) to give the title compound as a white solid (3.4 g, 45%). M.p. 39–41 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 21.5 min. TLC (SiO_2): R_f = 0.41 (hexane/DCM, 9:1). IR (KBr): $\tilde{\nu}$ = 2975, 2929, 1457, 1407, 1262, 1163, 1098 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38 (s, 6 H, 2 CH_3), 1.85 (t, J = 6.8 Hz, 2 H, CH_2), 2.14 (s, 3 H, CH_3), 2.23 (s, 3 H, CH_3), 2.27 (s, 3 H, CH_3), 2.66 (t, J = 6.9 Hz, 2 H, CH_2), 6.61 (s, 1H aryl) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.3 (CH_3 aryl), 18.8 (CH_3 aryl), 19.7 (CH_3 aryl), 20.4 (CH_2 C4), 26.9 (2 CH_3 C2), 32.8 (CH_2 C3), 73.0 (C2), 116.6 (C8), 121.9 (C5′), 122.2 (CH aryl), 133.3 (C5), 134.6 (C7), 151.7 (C8′) ppm. MS (QI, NH_3): m/z (%) = 204 (100) $[M]^+$.

2,2,5,7,8-Pentamethylchroman-6-carbaldehyde (2a): A solution of **1a** (4.8 g, 23.5 mmol) in anhydrous DCM (70 mL) was purged with N_2 and dichloromethoxymethane (4.26 mL, 47.1 mmol) was added at room temperature. The resulting solution was left for 5 min. Then, $TiCl_4$ (6.32 mL, 58 mmol) was added, and the mixture was left for 30 min. The reaction mixture was left for 45 min and saturated aqueous NH_4Cl solution (100 mL) was carefully added. The mixture was stirred for 2 h, and the resulting two phases were separated; the aqueous layer was washed with DCM (3 × 50 mL). The combined organic layers were washed with 1 N HCl solution (2 × 30 mL), saturated aqueous $NaHCO_3$ solution (3 × 30 mL), and brine (30 mL). The resulting organic solution was dried with anhydrous $MgSO_4$, and the solvent was removed under vacuum to yield the desired product as a blue solid (5.46 g, 95%), which was used in the next step without further purification. M.p. 81–83 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 20.8 min. TLC (SiO_2): R_f = 0.75 (DCM). IR (KBr): $\tilde{\nu}$ = 2975, 1673, 1567, 1453, 1368, 1165, 1115 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.33 (s, 6 H, 2 CH_3), 1.84 (t, J = 6.8 Hz, 2 H, CH_2), 2.13 (s, 3 H, CH_3), 2.44 (s, 3 H, CH_3), 2.47 (s, 3 H, CH_3), 2.70 (t, J = 6.9 Hz, 2 H, CH_2), 10.6 (s, 1 H CHO) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.3 (CH_3 aryl), 14.8 (CH_3 aryl), 15.6 (CH_3 aryl), 20.6 (CH_2 C4), 26.7 (2 CH_3 C2), 32.6 (CH_2 C3), 74.1 (C2), 117.4 (C8), 123.4 (C5′), 126.1 (C6), 138.1 (C5), 138.4 (C7), 155.7 (C8′), 193.7 (CHO) ppm. MS (QI, NH_3): m/z (%) = 233 (100) $[M + 1]^+$.

(2,2,5,7,8-Pentamethylchroman-6-yl)methanol (3a): A 1:1 mixture of MeOH and 1 N NaOH solution (100 mL) was shaken under a N_2 atmosphere at room temperature for 15 min. Then, finely powdered 2,2,5,7,8-pentamethylchroman-6-carbaldehyde (2.5 g, 10.8 mmol) was added, and the reaction mixture was left for 10 min, after which $NaBH_4$ (0.6 g, 15.9 mmol) was added over 20 min. After 4 h, the mixture was concentrated under vacuum to half volume and washed with Et_2O (2 × 30 mL). The organic phases were combined and washed with 0.1 N HCl solution (2 × 30 mL) and brine (30 mL). The resulting organic solution was dried with anhydrous $MgSO_4$, and the solvent was removed under vacuum to afford **3a** (2.35 g, 93%) as a white solid, which was used in the next step without further purification. M.p. 142–145 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 16.2 min. TLC (SiO_2): R_f = 0.36 (DCM/MeOH, 99:1). IR (KBr): $\tilde{\nu}$ = 3190, 2915, 1572, 1449, 1380, 1162, 1108 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (s, 6 H, 2 CH_3), 1.80 (t, J = 6.9 Hz, 2 H, CH_2), 2.12 (s, 3 H, CH_3), 2.27 (s, 3 H, CH_3), 2.31 (s, 3 H, CH_3), 2.64 (t, J = 6.9 Hz, 2 H, CH_2), 4.73 (s, 2 H CH_2OH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.8 (CH_3 aryl), 16.6 (CH_3 aryl), 15.6 (CH_3 aryl), 21.9 (CH_2 C4), 26.7 (2 CH_3 C2), 32.9 (CH_2 C3), 59.7 (CH_2 CH_2OH), 73.0 (C2), 117.0 (C8), 122.6 (C5′), 128 (C6), 133.3 (C5), 134.6 (C7), 151.6 (C8′) ppm. MS (QI, NH_3): m/z (%) = 217 (100) $[M - OH]^+$.

2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran (1b): Isobutylaldehyde (12 mL, 132.2 mmol) and H_2SO_4 (0.451 g) were added to a stirred solution of 2,3,5-trimethylphenol (15 g, 110.1 mmol) in toluene (15 mL). The reaction mixture was heated at reflux with removal of water (Dean–Stark) for 4 h. Distillation under vacuum of the volatiles afforded the desired product (15.9 g, 76%) as a white solid, which was used in the next step without further purification. M.p. 44–46 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 20.9 min. TLC (SiO_2): R_f = 0.40 (hexane/DCM, 9:1). IR (KBr): $\tilde{\nu}$ = 2973, 2923, 1457, 1410, 1285, 1152, 1088 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 6 H, 2 CH_3), 2.07 (s, 3 H, CH_3), 2.14 (s, 3 H, CH_3), 2.19 (s, 3 H, CH_3), 2.89 (s, 2 H, CH_2), 6.47 (s, 1 H, aryl) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.5 (CH_3 aryl), 18.4 (CH_3 aryl), 19.2 (CH_3 aryl), 28.6 (2 CH_3 C2), 42.3 (CH_2 C3), 85.8 (C2), 115.2 (C7), 122.1 (CH, C5), 122.6 (C4′), 131.1 (C4), 136.3 (C6), 157.3 (C7′) ppm. MS (QI, NH_3): m/z (%) = 191 (100) $[M + 1]^+$.

2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-carbaldehyde (2b): This product was prepared following the procedure described for **2a**. Compound **1b** (12 g, 63.2 mmol) afforded **2b** (13.2 g, 96%) as a blue solid, which was used in the next step without further purification. M.p. 86–88 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 18.5 min. TLC (SiO_2): R_f = 0.78 (DCM). IR (KBr): $\tilde{\nu}$ = 2929, 1669, 1586, 1439, 1270, 1098, 969 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.49 (s, 6 H, 2 CH_3), 2.12 (s, 3 H, CH_3), 2.44 (s, 3 H, CH_3), 2.50 (s, 3 H, CH_3), 2.97 (s, 2 H, CH_2), 10.5 (s, 1 H CHO) ppm. ^{13}C

NMR (75 MHz, $CDCl_3$): δ = 11.5 (CH_3 aryl), 15.2 (CH_3 aryl), 17.0 (CH_3 aryl), 28.5 (2 CH_3 C2), 42.0 (CH_2 C3), 87.2 (C2), 116.9 (C7), 124.4 (C4′), 126.0 (C5), 136.1 (C4), 142.3 (C6), 161.1 (C7′), 199.3 (CHO) ppm. MS (QI, NH_3): *m/z* (%) = 219 (100) $[M + 1]^+$, 236 (20) $[M + 18]^+$.

(2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-yl)methanol (3b): This product was prepared following the procedure described for **3a**. Compound **2b** (5.60 g, 25.7 mmol) afforded **3b** (5.65 g, 91%) as a white solid, which was used in the next step without further purification. M.p. 124–27 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 13.2 min. TLC (SiO_2): R_f = 0.30 (DCM/MeOH, 99:1). IR (KBr): $\tilde{\nu}$ = 3261, 2927, 1594, 1458, 1381, 1158, 1094 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 6 H, 2 CH_3), 2.11 (s, 3 H, CH_3), 2.25 (s, 3 H, CH_3), 2.29 (s, 3 H, CH_3), 2.92 (s, 2 H, CH_2), 4.69 (s, 2 H, CH_2OH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.1 (CH_3 aryl), 15.2 (CH_3 aryl), 15.9 (CH_3 aryl), 28.5 (2 CH_3 C2), 42.8 (CH_2 C3), 59.4 (CH_2 CH_2OH), 85.6 (C2), 116.1 (C7), 123.1 (C4′), 128.4 (C5), 131.0 (C4), 136.3 (C6), 156.9 (C7′) ppm. MS (QI, NH_3): *m/z* (%) = 203 (100) $[M - OH]^+$.

(4,6-Dimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)methanol (8)

2-Hydroxy-4,6-dimethoxybenzaldehyde: A solution of 3,5-dimethoxyphenol (9 g, 58.4 mmol) in anhydrous DCM (100 mL) was purged with N_2 and cooled to 0 °C. Then, $TiCl_4$ (13.9 mL, 128.4 mmol) was added over 20 min, and the mixture was left for 1 h at room temperature, after which dichloromethoxymethane (5.89 mL, 64.2 mmol) was added over 15 min. The reaction mixture was left for 45 min and then saturated aqueous NH_4Cl solution (250 mL) was carefully added. The mixture was shaken for 2 h. The resulting two phases were separated, and the aqueous layer was washed with DCM (3 × 100 mL). The combined organic layers were washed with 1 N HCl solution (2 × 100 mL) and brine (100 mL). The resulting organic solution was dried with anhydrous $MgSO_4$, and the solvent was removed under vacuum to afford a reddish solid, which was chromatographed on SiO_2 (DCM) to yield the desired product (6.91 g, 65%) as a white solid. M.p. 63–66 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 13.2 min. TLC (SiO_2): R_f = 0.68 (DCM). IR (KBr): $\tilde{\nu}$ = 2977, 1615, 1505, 1458, 1225, 1159, 1115, 1048 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 3.81 (s, 3 H, OCH_3), 3.83 (s, 3 H, OCH_3), 5.88 (d, *J* = 2.3 Hz, 1 H, aryl), 5.99 (d, *J* = 2.3 Hz, 1 H, aryl), 10.1 (s, 1 H, CHO), 12.5 (s, 1 H, OH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 55.60 (OCH_3 aryl), 55.63 (OCH_3 aryl), 90.5 (CH aryl), 92.9 (CH aryl), 106.0 (C1), 163.5 (C6), 166.3 (C4), 168.1 (C2), 191.7 (CHO) ppm. MS (QI, NH_3): *m/z* (%) = 183 (100) $[M + 1]^+$.

2,4-Dimethoxy-6-(2-methylallyloxy)benzaldehyde: A mixture of 6-hydroxy-2,4-dimethoxybenzaldehyde (2.7 g, 14.8 mmol), K_2CO_3 (3.08 g, 21.9 mmol), KI (0.25 g, 1.48 mmol), 3-chloro-2-methylpropene (2.2 mL, 22.2 mmol), anhydrous acetone (15 mL), and anhydrous DMF (3 mL) was heated at reflux for 19 h. The mixture was cooled and filtered, and the insoluble salts were washed with acetone. The solvent was removed under vacuum to afford an oil, which was chromatographed on SiO_2 (Et_2O) to yield the title compound (4.2 g, 80%) as a reddish solid. M.p. 50–53 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 14.2 min. TLC (SiO_2): R_f = 0.71 (Et_2O). IR (KBr): $\tilde{\nu}$ = 2971, 1676, 1607, 1452, 1236, 1213, 1165, 1134, 1050 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.80 (s, 3 H, CH_3), 3.80 (s, 3 H, OCH_3), 3.83 (s, 3 H, OCH_3), 4.44 (s, 2 H, CH_2), 4.96 (s, 1 H, C=CH_2), 5.13 (s, 1 H, =CH_2), 6.03 (s, 2 H, aryl), 10.4 (s, 1 H, CHO) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 19.2 (CH_3), 55.4 (OCH_3 aryl), 55.9 (OCH_3 aryl), 72.2 (CH_2), 90.5 (CH aryl), 91.2 (CH aryl), 109.0 (C1), 112.9 (=CH_2), 139.9 (=C), 163.5/163.4 (C2 and C6), 166 (C4), 191.7 (CHO) ppm. MS (QI, NH_3): *m/z* (%) = 237 (100) $[M + 1]^+$.

4,6-Dimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-carbaldehyde: A solution of 2,4-dimethoxy-6-(2-methylallyloxy)benzaldehyde (1 g, 4.23 mmol) in anhydrous DCM (7 mL) was purged with N_2 and cooled to –70 °C. Then, $AlCl_3$ (1.12 g, 8.47 mmol) was added over 30 min, and the mixture was left for 30 min at –70 °C. It was then warmed to room temperature and H_2O (10 mL) was added. The aqueous suspension was washed with DCM (3 × 100 mL), and the organic layers were combined and washed with brine (10 mL). The solvent was removed under vacuum to afford an oil, which was chromatographed on SiO_2 (Et_2O) to yield the dihydrobenzofuran derivative (0.09 g, 15%) as an oil. HPLC (4:6 to 10:0 over 30 min): t_R = 9.1 min. TLC (SiO_2): R_f = 0.57 (Et_2O). IR (KBr): $\tilde{\nu}$ = 1677, 1423, 1298, 1120 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 6 H, 2 CH_3), 2.83 (s, 2 H, CH_2), 3.88 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 5.94 (s, 1 H, aryl), 10.2 (s, 1 H, CHO) ppm. MS (QI, NH_3): *m/z* (%) = 237 (100) $[M + 1]^+$.

(4,6-Dimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)methanol: This product was obtained from 4,6-dimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-carbaldehyde following the procedure described for **3b**. The aldehyde derivative (0.05 g, 0.21 mmol) yielded the alcohol derivative (0.035 g, 70%) as an oil, which was used in the next step without further purification. HPLC (2:8 to 10:0 over 30 min): t_R = 15.0 min. TLC (SiO_2): R_f = 0.34 (Et_2O). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 6 H, 2 CH_3), 2.89 (s, 2 H, CH_2), 3.82 (s, 3 H, OCH_3), 3.83 (s, 3 H, OCH_3), 4.64 (s, 2 H, CH_2O), 5.98 (s, 1 H, aryl) ppm. MS (QI, NH_3): *m/z* (%) = 221 (100) $[M - OH]^+$.

***N*-(9-Fluorenylmethoxycarbonyl)-(*S*)-(2,2,5,7,8-pentamethylchroman-6-yl)-L-cysteine [Fmoc-Cys(Pmcm)-OH, 4a]**

***S*-(2,2,5,7,8-Pentamethylchroman-6-yl)-L-cysteine, Trifluoroacetate Salt:** Compound **3a** (2.0 g, 8.57 mmol) was added to a solution of L-cysteine hydrochloride (1.35 g, 8.55 mmol) in TFA (20 mL) over 5 min. The mixture was stirred for 30 min at room temperature, and the solvent was removed under vacuum. MeO*t*Bu (45 mL) and 10% aqueous NaAcO (100 mL) were added to the resulting crude material, and the mixture was stirred for 20 min and solids were filtered. The insoluble material was washed with MeO*t*Bu and crushed with hot MeO*t*Bu to yield the trifluoroacetate salt of the protected amino acid (3.40 g, 82%) as a white solid. M.p. 149–156 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 11.4 min. TLC (SiO_2): R_f = 0.87 (MeOH/AcOH/H_2O, 8:1:1). IR (KBr): $\tilde{\nu}$ = 2975, 1576, 1412, 1351, 1272, 1167, 1107 cm^{-1}. ^1H NMR (300 MHz, CD_3OD/basic D_2O): δ = 1.23 (s, 6 H, 2 CH_3), 1.74 (t, *J* = 6.8 Hz, 2 H, CH_2), 2.02 (s, 3 H, CH_3), 2.22 (s, 3 H, CH_3), 2.25 (s, 3 H, CH_3), 2.58 (t, *J* = 6.9 Hz, 2 H, CH_2), 2.74 (dd, *J* = 8.7, 13.5 Hz, 1 H, CH_2-β), 3.04 (dd, *J* = 4.1, 13.5 Hz, 1 H, CH_2-β), 3.44 (dd, *J* = 8.7, 4.1 Hz, 1 H, CH-α), 3.84 (s, 2 H, CH_2S) ppm. ^{13}C NMR (75 MHz, CD_3OD/basic D_2O): δ = 12.2 (CH_3 aryl), 15.1 (CH_3 aryl), 16.1 (CH_3 aryl), 22.2 (CH_2 C4), 27.0 (2 CH_3 C2), 33.2 (CH_2 C3), 34.0 (CH_2 CH_2-β), 40.5 (CH_2 CH_2S), 56.5 (CH CH-α), 74.0 (C2), 118.1 (C8), 123.1 (C5′), 126.1 (C6), 133.9 (C5), 135.1 (C7), 151.1 (C8′), 181.0 (COOH) ppm. MS (ESI+): *m/z* (%) = 338 (100) $[M - CF_3COO]^+$, 218.3 (18) $[Pmcm + 1]^+$.

***N*-(9-Fluorenylmethoxycarbonyl)-*S*-(2,2,5,7,8-pentamethylchroman-6-yl)-L-cysteine:** A solution of the trifluoroacetate salt of the protected amino acid (0.4 g, 0.887 mmol) and Et_3N (312 μL, 2.25 mmol) in water/CH_3CN (1:1, 16 mL) was cooled to 0 °C, and a solution of Fmoc-OSu (0.329 g, 0.976 mmol) in CH_3CN (2 mL) was added over 30 min. The mixture was stirred for 2 h at room temperature, Et_3N (0.2 equiv.) was added to keep the pH at 9–10

and stirring was continued for a further 2 h. The mixture was cooled to 0 °C and a 1 N aqueous HCl solution was added to reach pH 2–3. The volatiles were then removed under vacuum to half volume. The resulting mixture was washed with AcOEt (3 × 15 mL), the organic layers were combined, and the final organic solution was washed with brine (20 mL) and dried with anhydrous $MgSO_4$. Evaporation of the solvent under vacuum afforded an oily foaming solid. This solid was chromatographed on SiO_2 (CH_3Cl/MeOH, 95:5) to yield **4a** (0.347 g, 70%) as a white solid. M.p. 115–118 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 25.5 min. TLC (SiO_2): R_f = 0.44 ($CHCl_3$MeOH 9:1). IR (KBr): $\tilde{\nu}$ = 2929, 1725, 1509, 1449, 1320, 1223, 1167, 1106 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.27 (s, 6 H, 2 CH_3), 1.76 (t, J = 9.2 Hz, 2 H, CH_2), 2.08 (s, 3 H, CH_3), 2.19 (s, 3 H, CH_3), 2.24 (s, 3 H, CH_3), 2.58 (t, J = 9.2 Hz, 2 H, CH_2), 3.10 (br. s, 2 H, CH_2-β), 3.82 (s, 2 H, CH_2S), 4.23 (t, J = 7.8 Hz, 1 H, Fmoc), 4.44 (d, J = 7.7 Hz, 2 H, Fmoc), 4.68 (m, 1 H, CH-α), 5.67 (d, J = 8.0 Hz, 1 H, NH), 7.31 (m, 2 H, Fmoc), 7.38 (m, 2 H, Fmoc), 7.58 (m, 2 H, Fmoc), 7.74 (m, 2 H, Fmoc) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.9 (CH_3 aryl), 14.8 (CH_3 aryl), 15.7 (CH_3 aryl), 21.2 (CH_2 C4), 26.7 (2 CH_3 C2), 32.9 (CH_2 CH_2-β), 33.1 (CH_2 C3), 34.9 (CH_2 CH_2S), 47.1 (CH Fmoc), 53.6 (CH CH-α), 67.3 (CH_2 Fmoc), 72.9 (C2), 116.9 (C8), 119.9 (CH Fmoc), 122.5 (C5′), 123.6 (C6), 125 (CH Fmoc), 127 (CH Fmoc), 127.7 (CH Fmoc), 132.8 (C5), 134.1 (C7), 141.2 (C Fmoc), 143.7 (C Fmoc), 151.1 (C8′), 155.9 (CO), 175.1 (COOH) ppm. MS (ESI+): m/z (%) = 560.7 (23) $[M + 1]^+$, 217.9 (100) $[Pmcm + 1]^+$. $[a]_D$ = +19.1 (c = 1.1, $CHCl_3$).

***N*-(9-Fluorenylmethoxycarbonyl)-*S*-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)-L-cysteine [Fmoc-Cys(Pbfm)-OH, 4b]:** This product was obtained from **3b** and L-cysteine hydrochloride following the procedure described for **4a**.

***S*-(2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-yl)-L-cysteine, Trifluoroacetate Salt:** L-Cysteine (1.43 g, 9.09 mmol) and **3b** (2 g, 9.09 mmol) yielded the trifluoroacetate salt (3.6 g, 88%) of the protected amino acid as a white solid. M.p. 144–147 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 8.9 min. TLC (SiO_2): R_f = 0.84 (MeOH/AcOH/H_2O, 8:1:1). IR (KBr): $\tilde{\nu}$ = 2973, 1701, 1578, 1414, 1288, 1207, 1086 cm^{-1}. ^1H NMR (300 MHz, CD_3OD/basic D_2O): δ = 1.40 (s, 6 H, 2 CH_3), 2.03 (s, 3 H, CH_3), 2.23 (s, 3 H, CH_3), 2.26 (s, 3 H, CH_3), 2.74 (dd, J = 8.3, 13.3 Hz, 1 H, CH_2-β), 2.91 (s, 2 H, CH_2), 3.05 (dd, J = 4.2, 13.3 Hz, 1 H, CH_2-β), 3.43 (dd, J = 8.3, 4.2 Hz, 1 H, CH-α), 3.83 (s, 2 H, CH_2S) ppm. ^{13}C NMR (75 MHz, CD_3OD/basic D_2O): δ = 12.4 (CH_3 aryl), 15.7 (CH_3 aryl), 16.3 (CH_3 aryl), 28.7 (2 CH_3 C2), 32.8 (CH_2 CH_2-β), 40.6 (CH_2 CH_2S), 43.7 (CH_2 C3), 56.5 (CH CH-α), 86.6 (C2), 116.5 (C7), 124.3 (C4′), 126.6 (C5), 131.8 (C4), 136.6 (C6), 157.2 (C7′), 180.8 (COOH) ppm. MS (ESI+): m/z (%) = 324.9 (100) $[M - CF_3COO]^+$, 204.2 (28) $[Pmbf + 1]^+$.

***N*-(9-Fluorenylmethoxycarbonyl)-*S*-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)-L-cysteine (4b):** The trifluoroacetate salt (1 g, 2.38 mmol) of the protected amino acid and FmocOSu (0.98 g, 2.9 mmol) afforded **4b** (0.97 g, 75%) as a white solid. M.p. 100–104 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 24.6 min. TLC (SiO_2): R_f = 0.44 ($CHCl_3$MeOH 9:1). IR (KBr): $\tilde{\nu}$ = 2971, 1698, 1530, 1451, 1368, 1254, 1084 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 6 H, 2 CH_3), 2.06 (s, 3 H, CH_3), 2.17 (s, 3 H, CH_3), 2.22 (s, 3 H, CH_3), 2.88 (s, 2 H, CH_2), 3.10 (br. s, 2 H, CH_2-β), 3.79 (s, 2 H, CH_2S), 4.22 (t, J = 7.1 Hz, 1 H, Fmoc), 4.43 (d, J = 7.1 Hz, 2 H, Fmoc), 4.67 (m, 1 H, CH-α), 5.70 (d, J = 7.8 Hz, 1 H, NH), 7.27 (m, 2 H, Fmoc), 7.35 (m, 2 H, Fmoc), 7.58 (m, 2 H, Fmoc), 7.76 (m, 2 H, Fmoc) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.3 (CH_3 aryl), 15.3 (CH_3 aryl), 16.0 (CH_3 aryl), 28.5 (2 CH_3 C2), 32.7 (CH_2 CH_2-β), 34.2 (CH_2 CH_2S), 42.9 (CH_2 C3), 47.1 (CH Fmoc), 52.7 (CH CH-α), 67.2 (CH_2 Fmoc), 85.5 (C2), 116 (C7), 119.9 (CH Fmoc), 122.7 (C4′), 125 (CH Fmoc), 127 (CH Fmoc), 127.7 (CH Fmoc), 131.1 (C4), 136.5 (C6), 156.2 (C7′), 141.2 (C Fmoc), 143.6 (C Fmoc), 155.3 (CO), 175.1 (COOH) ppm. MS (ESI+): m/z (%) = 546.6 (8) $[M + 1]^+$, 203.9 (100) $[Pmbf + 1]^+$. $[a]_D$ = +17.8 (c = 1.13, $CHCl_3$).

***N*-(9-Fluorenylmethoxycarbonyl)-*S*-(4,5,6-trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)-L-cysteine [Fmoc-Cys(Tmbm)-OH, 7]**

4,5,6-Trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran: This product was prepared following the procedure described for **1b**. 3,4,5-Trimethoxyphenol (15 g, 81.4 mmol) afforded the dihydrobenzofuran derivative (7.8 g, 40%) as a white solid, which was used in the next step without further purification. M.p. 37–39 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 13.7 min. TLC (SiO_2): R_f = 0.63 (petroleum ether/AcOEt, 8:2). IR (KBr): $\tilde{\nu}$ = 2971, 2936, 1615, 1474, 1416, 1300, 1198, 1121 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 6 H, 2 CH_3), 3.02 (s, 2 H, CH_2), 3.78 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 6.13 (s, 1 H, aryl) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.1 (2 CH_3 C2), 41.2 (CH_2 C3), 56.0 (OCH_3 aryl), 59.8 (OCH_3 aryl), 61.2 (OCH_3 aryl), 87.2 (C2), 90.0 (CH C7), 108.8 (C4′), 134.7 (C5), 150.1 (C6), 153.7 (C4), 155.3 (C7′) ppm. MS (QI, NH_3): m/z (%) = 239 (100) $[M + 1]^+$.

4,5,6-Trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-carbaldehyde: A solution of 4,5,6-trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran (0.5 g, 2.1 mmol) in anhydrous DCM (5 mL) was purged with N_2 and $TiCl_4$ (1.15 mL, 10.5 mmol) was added over 20 min. The mixture was left for 1 h at room temperature and dichloromethoxymethane (0.8 mL, 8.84 mmol) was added over 15 min. The reaction mixture was left for 1 h and saturated aqueous NH_4Cl solution (20 mL) was then carefully added. The mixture was shaken for 2 h, the resulting two phases were separated, and the aqueous layer was washed with DCM (3 × 15 mL). The combined organic layers were washed with 1 N HCl solution (2 × 25 mL), saturated aqueous $NaHCO_3$ solution (3 × 25 mL), and brine (25 mL). The resulting organic solution was dried with anhydrous $MgSO_4$, and the solvent was removed under vacuum to afford an oil, which was chromatographed on SiO_2 (hexanes/Et_2O, 2:8) to yield the desired compound (0.41 g, 73%) as a white solid. M.p. 64–65 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 11.8 min. TLC (SiO_2): R_f = 0.47 (hexanes/Et_2O, 2:8). IR (KBr): $\tilde{\nu}$ = 2975, 2939, 1684, 1594, 1457, 1416, 1358, 1200, 1047 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.52 (s, 6 H, 2 CH_3), 3.02 (s, 2 H, CH_2), 3.80 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 4.03 (s, 3 H, OCH_3), 10.2 (s, 1 H, CHO) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.2 (2 CH_3 C2), 40.3 (CH_2 C3), 59.7 (OCH_3 aryl), 61.3 (OCH_3 aryl), 62.1 (OCH_3 aryl), 89.4 (C2), 109.9 (C7), 112.8 (C4′), 138.1 (C5), 154.1 (C6), 155.8 (C4), 157.0 (C7′) ppm. MS (QI, NH_3): m/z (%) = 267 (100) $[M + 1]^+$.

(4,5,6-Trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)methanol: This product was obtained from 4,5,6-trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-carbaldehyde by following the procedure described for **3b**. The aldehyde derivative (1 g, 3.76 mmol) yielded the alcohol derivative (1 g, 91%) as an oily solid, which was used in the next step without further purification. HPLC (3:7 to 10:0 over 30 min): t_R = 9.6 min. TLC (SiO_2): R_f = 0.44 (hexanes/AcOEt, 1:1). IR (KBr): $\tilde{\nu}$ = 3504, 2962, 1613, 1457, 1420, 1368, 1268, 1104, 1055 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.41 (s, 6 H, 2 CH_3), 3.02 (s, 2 H, CH_2), 3.80 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 4.63 (s, 2 H, CH_2OH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.2 (2 CH_3 C2), 41.1 (CH_2 C3), 55.8 (CH_2 CH_2OH), 59.9 (OCH_3 aryl), 61.1 (OCH_3 aryl), 61.6 (OCH_3 aryl), 87.5 (C2), 111.4 (C7), 112.7 (C4′), 138.1 (C5), 149.8

(C6), 151.9 (C4), 153.5 (C7′) ppm. MS (QI, NH_3): *m*/*z* (%) = 251 (100) $[M - OH]^+$.

(L)-*S*-(4,5,6-Trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)-cysteine, Trifluoroacetate Salt: This product was obtained from (4,5,6-trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)-methanol and (L)-cysteine hydrochloride by following the procedure described for the trifluoroacetate salt of (L)-*S*-(2,2,5,7,8-pentamethylchroman-6-yl)cysteine. (L)-Cysteine (0.49 g, 3.10 mmol) and (4,5,6-trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)methanol (0.83 g, 3.10 mmol) yielded the trifluoroacetate salt (0.3 g, 20%) of the protected amino acid as a white solid. HPLC (3:7 to 10:0 over 30 min): t_R = 6.5 min. TLC (SiO_2): R_f = 0.81 (MeOH/AcOH/H_2O, 8:1:1). IR (KBr): $\tilde{\nu}$ = 3504, 2962, 1613, 1457, 1420, 1368, 1268, 1104, 1055 cm^{-1}. ^1H NMR (300 MHz, CD_3OD/basic D_2O): δ = 1.44 (s, 6 H, 2 CH_3), 2.68 (dd, J = 8.6, 13.3 Hz, 1 H, CH_2-β), 3.04 (s, 2 H, CH_2), 3.07 (br. dd, 1 H, CH_2-β), 3.38 (dd, J = 4.0, 8.6 Hz, 1 H, CH-α), 3.66 (s, 2 H, CH_2S), 3.76 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3) ppm. ^{13}C NMR (75 MHz, CD_3OD/basic D_2O): δ = 28.4 (2 CH_3 C2), 36.2 (CH_2 CH_2-β), 40.0 (CH_2 CH_2S), 42.1 (CH_2 C3), 56.8 (CH CH-α), 60.5 (OCH_3 aryl), 61.7 (OCH_3 aryl), 62.2 (OCH_3 aryl), 88.7 (C2), 110.9 (C7), 114 (C4′), 141 (C5), 150.5 (C6), 153 (C4), 155 (C7′), 180.7 (COOH) ppm. MS (ESI+): *m*/*z* (%) = 372 (100) $[M - CF_3COO]^+$.

(L)-*N*-(9-Fluorenylmethoxycarbonyl)-*S*-(4,5,6-trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)cysteine (7): The fully protected amino acid was obtained from the trifluoroacetate salt of (L)-*S*-(4,5,6-trimethoxy-2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)-cysteine by following the procedure described for **4a**. The salt of the amino acid (0.4 g, 0.83 mmol) and FmocOSu (0.3 g, 0.88 mmol) yielded **7** (0.34 g, 79%) as a white solid. HPLC (3:7 to 10:0 over 30 min): t_R = 21.5 min. TLC (SiO_2): R_f = 0.41 ($CHCl_3$MeOH 9:1). IR (KBr): $\tilde{\nu}$ = 2936, 1723, 1520, 1455, 1420, 1277, 1098 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 6 H, 2 CH_3), 2.90 (m, 1 H, CH-β), 3.02 (s, 2 H, CH_2), 3.03 (br. dd, 1 H, CH-β), 3.71 (s, 2 H, CH_2S), 3.80 (s, 6 H, 2 OCH_3), 3.90 (s, 3 H, OCH_3), 4.25 (t, J = 7.2 Hz, 1 H, Fmoc), 4.44 (d, J = 7.1 Hz, 2 H, Fmoc), 4.67 (m, 1 H, CH-α), 6.17 (d, J = 7.9 Hz, 1 H, NH), 7.27 (m, 2 H, Fmoc), 7.38 (m, 2 H, Fmoc), 7.62 (m, 2 H, Fmoc), 7.74 (m, 2 H, Fmoc) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.6 (CH_2 CH_2-β), 28.1 (2 CH_3 C2), 32.6 (CH_2 CH_2S), 41.3 (CH_2 C3), 47.1 (CH Fmoc), 54.8 (CH CH-α), 59.9 (OCH_3 aryl), 61.0 (OCH_3 aryl), 61.4 (OCH_3 aryl), 67.2 (CH_2 Fmoc), 87.8 (C2), 108.7 (C7), 112.5 (C4′), 119.9 (CH Fmoc), 125 (CH Fmoc), 127 (CH Fmoc), 127.6 (CH Fmoc), 139 (C5), 141.2 (C Fmoc), 143.6 (C Fmoc), 149.5 (C6), 151.7 (C4), 153.4 (C7′), 156.3 (CO), 174.7 (COOH) ppm. MS (ESI+): *m*/*z* (%) = 594.6 (60) $[M + 1]^+$, 251.8 (100) $[Pmbf + 1]^+$. $[\alpha]_D$ = +29.7 (*c* = 1, $CHCl_3$).

***N*$^{\alpha}$-(9-Fluorenylmethoxycarbonyl)-*N*$^{\omega}$(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)asparagine [Fmoc-Asn(Pbfm)-OH, 9a]**

2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-carbaldehyde Oxime (11): A mixture of hydroxylamine hydrochloride (0.702 g, 10.1 mmol) and pyridine/water (2:1, 12 mL) was magnetically stirred for 10 min at room temperature and finely powdered **2b** (2 g, 9.17 mmol) was added. The reaction mixture was left for 4 h. Volatiles were then removed under vacuum until dryness. The crude product thus obtained was stirred for 30 min in an aqueous solution of 10% AcOH (15 mL). AcOEt (20 mL) was then added, and the resulting two phases were separated. The aqueous layer was washed with AcOEt (2 × 20 mL), and the organic layers were combined and washed with brine (20 mL). The organic solution was dried with anhydrous $MgSO_4$, and the solvent was removed under vacuum to afford the desired oxime (1.94 g, 91%) as a white solid, which was used in the next step without further purification. M.p. 144–146 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 16.1 min. TLC (SiO_2): R_f = 0.52 (DCM/MeOH, 98:2). IR (KBr): $\tilde{\nu}$ = 3408, 2964, 1372, 1260, 1019 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.47 (s, 6 H, 2 CH_3), 2.11 (s, 3 H, CH_3), 2.22 (s, 3 H, CH_3), 2.25 (s, 3 H, CH_3), 2.95 (s, 2 H, CH_2), 8.38 (s, 1 H CH=N) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.9 (CH_3 aryl), 16.5 (CH_3 aryl), 17.3 (CH_3 aryl), 28.5 (2 CH_3 C2), 42.6 (CH_2 C3), 86.1 (C2), 116.2 (C7), 121.5 (C4′), 123.6 (C5), 131.2 (C4), 136.6 (C6), 150.5 (CH CH=N), 157.3 (C7′) ppm. MS (QI, NH_3): *m*/*z* (%) = 234 (100) $[M + 1]^+$.

2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-carbonitrile (12): A solution of **2b** (1 g, 4.6 mmol) in NMP (10 mL) was stirred for 2 min at room temperature. Hydroxylamine hydrochloride (0.382 g, 5.5 mmol) was then added, and the resulting mixture was heated at reflux at 115 °C for 3 h. The reaction mixture was warmed to room temperature, water (50 mL) was added, the resulting aqueous suspension was washed with AcOEt (3 × 25 mL), and the organic phases were combined and finally washed with brine (20 mL). The solvent was removed under vacuum to afford an oil, which was chromatographed on SiO_2 (DCM) to yield **12** (0.803 g, 81%) as a white solid. M.p. 133–134 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 20.4 min. TLC (SiO_2): R_f = 0.68 (DCM/MeOH, 98:2). IR (KBr): $\tilde{\nu}$ = 2967, 2205, 1596, 1418, 1331, 1262, 1096 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.48 (s, 6 H, 2 CH_3), 2.08 (s, 3 H, CH_3), 2.34 (s, 3 H, CH_3), 2.41 (s, 3 H, CH_3), 2.93 (s, 2 H, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.6 (CH_3 aryl), 17.6 (CH_3 aryl), 17.9 (CH_3 aryl), 28.4 (2 CH_3 C2), 41.9 (CH_2 C3), 87.4 (C2), 104.5 (C5), 116.7 (CN), 118.8 (C7), 123.8 (C4′), 135.7 (C4), 141.6 (C6), 160.3 (C7′) ppm. MS (QI, NH_3): *m*/*z* (%) = 216 (11) $[M]^+$, 233 (100) $[M + 17]^+$.

(2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-yl)methanamine (10, acetate salt) from 11: A mixture of **11** (2.85 g, 12.2 mmol), 10% Pd/C (1 g), and glacial AcOH (125 mL) was purged with N_2 and left at room temperature for 30 min at 5 atm in a Parr apparatus. The final suspension was filtered through Celite. Celite was washed with DCM, and the volatiles were removed under vacuum to afford the acetate salt of **10** (3.28 g, 96%) as a white solid.

(2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-yl)methanamine (10, acetate salt) from 12: Anhydrous $LiAlH_4$ (0.351 g, 9.24 mmol) was added to a solution of **12** (0.5 g, 2.31 mmol) in THF (15 mL), and the reaction mixture was stirred for 5 h at room temperature. Crushed ice (15 mL) was added very slowly, and the resulting mixture was stirred for 10 min and washed with AcOEt (3 × 15 mL). The organic layers were combined, washed with brine (25 mL), and dried with anhydrous $MgSO_4$. Evaporation of the solvent under vacuum afforded an oil, which was washed several times with glacial AcOH to yield the desired product (0.552 g, 76%) as a white solid. M.p. 95–97 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 7.8 min. TLC (SiO_2): R_f = 0.23 (DCM/MeOH/AcOH, 90:5:5). IR (KBr): $\tilde{\nu}$ = 2973, 2929, 1560, 1461, 1403, 1335, 1281, 1088, 1013 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.44 (s, 6 H, 2 CH_3), 1.90 (s, 3 H, acetate), 2.08 (s, 3 H, CH_3), 2.20 (s, 3 H, CH_3), 2.21 (s, 3 H, CH_3), 2.91 (s, 2 H, CH_2),4.03 (s, 2 H, CH_2N), 8.53 (br. s, NH_3^+) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.3 (CH_3 aryl), 15.4 (CH_3 aryl), 16.3 (CH_3 aryl), 22.5 (CH_3 acetate), 28.5 (2 CH_3 C2), 37.3 (CH_2 CH_2N), 42.8 (CH_2 C3), 85.8 (C2), 116.2 (C7), 122.0 (C4′), 123.6 (C5), 131.4 (C4), 136.5 (C6), 157.4 (C7′), 177.2 (CO) ppm. MS (QI, NH_3): *m*/*z* (%) = 203 (100) $[Pmbf]^+$, 220 (1) $[M - CH_3COO]^+$.

***N*$^{\alpha}$-Benzyloxycarbonyl-*N*$^{\omega}$-(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)asparagine Benzyl Ester:** A solution of Z-Asn·OBzl (0.5 g, 1.4 mmol) and HOBt (0.236 g, 1.54 mmol) in DMF/DCM

(1:1, 10 mL) was cooled to 0 °C and was stirred for 5 min. DCC (0.317 g, 1.54 mmol) was then added. The mixture was left for 1 h at 0 °C and 1 h at room temperature. This solution was then added to a solution of **10** (0.429 g, 1.54 mmol) and Et_3N (279 μL, 2 mmol) in DMF/DCM (1:1, 4 mL). The resulting reaction mixture was left for 4 h at room temperature, cooled to 0 °C, and filtered. Solids were washed with DCM, solvents were removed under vacuum, and the resulting crude product was dissolved with AcOEt (20 mL). The organic solution was washed with aqueous 1 N HCl solution (2 × 15 mL), saturated aqueous $NaHCO_3$ solution, and brine (15 mL), and dried with anhydrous $MgSO_4$. The solvent was then removed under vacuum. An oily solid was recovered, which was crystallized in Et_2O/hexanes to afford the title compound (0.547 g, 70%) as a white solid. M.p. 108–112 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 21.9 min. TLC (SiO_2): R_f = 0.54 (DCM/MeOH, 97:3). IR (KBr): $\tilde{\nu}$ = 3321, 3035, 2930, 1729, 1640, 1544, 1457, 1335, 1262, 1088 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.45 (s, 6 H, 2 CH_3), 2.08 (s, 3 H, CH_3), 2.11 (s, 3 H, CH_3), 2.14 (s, 3 H, CH_3), 2.66 (br. dd, 1 H, CH-β), 2.89 (br. dd, 1 H, CH-β), 2.91 (s, 2 H, CH_2), 4.36 (d, J = 4.6 Hz, 2 H, CH_2NH), 4.60 (m, 1 H, CH-α), 5.08 and 5.17 (2 s, 2 × 2 H, CH_2-Z and CH_2-Bzl), 7.32 (m, 10 H, aryl) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.2 (CH_3 aryl), 15.3 (CH_3 aryl), 16.1 (CH_3 aryl), 28.5 (2 CH_3 C2), 33.9 (CH_2 CH_2-β), 38.4 (CH_2 CH_2-NH), 42.8 (CH_2 C3), 51.0 (CH CH-α), 66.9 and 67.4 (2 CH_2 CH_2-Z and CH_2-Bzl), 85.6 (C2), 116.2 (C7), 123.4 (C4′), 124.7 (C5), 128.0 (10 CH aryl-Z and aryl-Bzl), 131.0 (C4), 135.3 (C6), 136.1 and 136.2 (C-aryl Z and C-aryl Bzl), 156.9 (C7′), 169.0 and 170.9 (CO) ppm. MS (ESI+): m/z (%) = 559.2 (8) [M + 1]$^+$, 449.4 (100) [M – 109]$^+$. $[\alpha]_D$ = –11.0 (c = 1.04, $CHCl_3$).

N^{ω}-(2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-yl)asparagine, Trifluoroacetate Salt: A mixture of Z-Asn(Pmbf)-OBzl (0.5 g, 0.9 mmol) and 10% of Pd/C (0.1 g) in AcOH/H_2O (4:1, 15 mL) was blown with a stream of hydrogen at room temperature for 2 h with vigorous stirring. The mixture was filtered through Celite. Celite was then washed with MeOH, and the volatiles were removed under vacuum. The resulting crude product was crushed and washed with hot MeO*t*Bu, yielding the desired product (0.355 g, 87%) as a white solid. M.p. 158–161 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 8.2 min. TLC (SiO_2): R_f = 0.71 (MeOH/AcOH/H_2O, 8:1:1). IR (KBr): $\tilde{\nu}$ = 2973, 1648, 1416, 1341, 1291, 1258, 1158, 1090, 1014 cm^{-1}. ^1H NMR (300 MHz, CD_3OD/basic D_2O): δ = 1.40 (s, 6 H, 2 CH_3), 1.90 (s, 3 H, CH_3COO^-), 2.04 and 2.17 (2 s, 2 × 3 H, CH_3), 2.34 (dd, J = 7.7, 14.9 Hz, 1 H, CH-β), 2.65 (dd, J = 14.8, 4.2 Hz, 1 H, CH-β), 2.92 (s, 2 H, CH_2), 3.54 (dd, J = 4.2, 7.7 Hz, 1 H, CH-α), 4.34 (s, 2 H, CH_2NH) ppm. ^{13}C NMR (75 MHz, CD_3OD/basic D_2O): δ = 11.0 (CH_3 aryl), 14.3 (CH_3 aryl), 14.9 (CH_3 aryl), 23.0 (CH_3 CH_3COO^-), 27.2 (2 CH_3 C2), 37.9 (CH_2 CH_2-β), 40.3 (CH_2 CH_2-NH), 42.3 (CH_2 C3), 53.7 (CH CH-α), 85.4 (C2), 115.3 (C7), 123.0 (C4′), 124.9 (C5), 130.9 and 135.7 (C4 and C6), 156.2 (C7′), 179.4 and 180.1 (CO) ppm. MS (ESI+): m/z (%) = 334.9 (18) [M + 1]$^+$, 203 (100) [Pmbf]$^+$.

N^{α}-(9-Fluorenylmethoxycarbonyl)-N^{ω}(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)asparagine (9a): A solution of the acetate salt of the protected amino acid (0.15 g, 0.38 mmol) in aqueous 6% Na_2CO_3 solution/CH_3CN (2:1, 15 mL) was cooled to 0 °C, and a solution of Fmoc-OSu (0.141 g, 0.42 mmol) in CH_3CN (2 mL) was added over 20 min. The mixture was stirred for 5 h at room temperature and water (20 mL) was added. The resulting mixture was cooled to 0 °C, a 1 N aqueous HCl solution was added to reach pH 2–3, and the mixture was washed with AcOEt (3 × 10 mL). The organic layers were combined, and the final organic solution was washed with brine (20 mL) and dried with anhydrous $MgSO_4$. Evaporation of the solvent under vacuum afforded an oily solid, which was crystallized in AcOEt/hexanes to yield **9a** (0.112 g, 53%) as a white solid. M.p. 135–138 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 21.1 min. TLC (SiO_2): R_f = 0.73 ($CHCl_3$/MeOH/AcOH, 9:0.5:0.5). IR (KBr): $\tilde{\nu}$ = 3315, 2979, 1640, 1446, 1250, 1078 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 1.37 (s, 6 H, 2 CH_3), 2.09, 2.12, and 2.14 (3 s, 3 × 3 H, CH_3), 2.66 (dd, J = 7.7, 14.8 Hz, 1 H, CH-β), 2.74 (dd, J = 14.8, 4.2 Hz, 1 H, CH-β), 2.87 (s, 2 H, CH_2), 4.19 (t, J = 7.1 Hz, CH-Fmoc), 4.29 (m, 4 H, CH_2-Fmoc and CH_2-NH), 4.56 (dd, J = 4.2, 7.7 Hz, 1 H, CH-α), 7.29 (m, 2 H, Fmoc), 7.38 (m, 2 H, Fmoc), 7.64 (m, 2 H, Fmoc), 7.78 (d, J = 7.3 Hz, 2 H Fmoc) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 10.8 (CH_3 aryl), 14.1 (CH_3 aryl), 14.8 (CH_3 aryl), 27.1 (2 CH_3 C2), 37.0 (CH_2 CH_2-NH), 38 (CH_2 CH_2-β), 42.2 (CH_2 C3), 47.3 (CH CH-Fmoc), 50.9 (CH CH-α), 66.7 (CH_2 CH_2-Fmoc), 85.1 (C2), 115.3 (C7), 119.4 (CH Fmoc), 123.0 (C4′), 124.6 (C5), 124.8 (CH Fmoc), 126.7 (CH Fmoc), 127.3 (CH Fmoc), 130.8 and 135.7 (C4 and C6), 141.1 and 143.7 (2 C C-Fmoc), 156.4 (C7′), 157.1 (CO carbamate),170.4 (CO amide), 173.1 (COOH) ppm. MS (ESI+): m/z (%) = 557.3 (19) [M + 1]$^+$, 203 (100) [Pmbf]$^+$. $[\alpha]_D$ = +11.7 (c = 1.01, MeOH.).

N^{α}-(9-Fluorenylmethoxycarbonyl)-N^{ω}(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)glutamine [Fmoc-Gln(Pbfm)-OH, 9b]: This product was obtained from **10** and Z-Gln-OBzl following the procedure described for **9a**.

N^{α}-Benzyloxycarbonyl-N^{ω}(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)glutamine Benzyl Ester: of Z-Gln-OBzl (0.5 g, 1.35 mmol) and **10** (0.412 g, 1.48 mmol) yielded the fully protected amino acid (0.494 g, 64%) as a white solid. M.p. 99–102 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 23.6 min. TLC (SiO_2): R_f = 0.39 (DCM/MeOH, 97:3). IR (KBr): $\tilde{\nu}$ = 3311, 3035, 2930, 1725, 1648, 1534, 1455, 1333, 1262, 1090 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.45 (s, 6 H, 2 CH_3), 1.90 (m, 2 H, CH_2-β), 2.02 (m, 2 H, CH_2-γ), 2.10 (s, 3 H, CH_3), 2.14 (s, 3 H, CH_3), 2.17 (s, 3 H, CH_3), 2.93 (s, 2 H, CH_2), 4.37 (br. s, 2 H, CH_2NH), 4.36 (m, 1 H, CH-α), 5.07 and 5.14 (2 s, 2 × 2 H, CH_2-Z and CH_2-Bzl), 7.31 (m, 10 H, aryl) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.2 (CH_3 aryl), 15.4 (CH_3 aryl), 16.1 (CH_3 aryl), 24.9 (CH_2 CH_2-β), 28.5 (2 CH_3 C2), 33.9 (CH_2 CH_2-γ), 38.5 (CH_2 CH_2-NH), 42.9 (CH_2 C3), 53.7 (CH CH-α), 66.9 and 67.4 (2 CH_2 CH_2-Z and CH_2-Bzl), 85.6 (C2), 116.1 (C7), 123.4 (C4′), 125 (C5), 128.1 (10 CH aryl-Z and aryl-Bzl), 131.0 and 135.2 (C4 and C6), 136.1 and 136.2 (C-aryl Z and C-aryl Bzl), 156.8 (C7′), 171.2 and 171.7 (CO) ppm. MS (ESI+): m/z (%) = 573.1 (62) [M + 1]$^+$, 449.3 (92) [M – 123]$^+$, 225.5 (100) [M – 347]$^+$. $[\alpha]_D$ = –11.2 (c = 1.04, $CHCl_3$).

N^{ω}(2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-yl)glutamine, Trifluoroacetate Salt: Z-Gln(Pmbf)-OBzl (0.5 g, 0.87 mmol) yielded the acetate salt (0.355 g, 85%) as a white solid. M.p. 149–152 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 8.7 min. TLC (SiO_2): R_f = 0.69 (MeOH/AcOH/H_2O, 8:1:1). IR (KBr): $\tilde{\nu}$ = 3464, 2975, 1629, 1455, 1418, 1331, 1158, 1088, 1015 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 1.41 (s, 6 H, 2 CH_3), 1.89 (s, 3 H, CH_3COO^-), 1.81 (m, 1 H, CH-β), 1.91 (m, 1 H, CH-β), 2.04, 2.15, and 2.16 (3 s, 3 × 3 H, CH_3), 2.23 (m, 2 H, CH_2-γ), 2.93 (s, 2 H, CH_2), 3.19 (m, 1 H, CH-α), 4.31 (s, 2 H, CH_2NH) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 10.9 (CH_3 aryl), 14.2 (CH_3 aryl), 14.8 (CH_3 aryl), 27.2 (2 CH_3 C2), 31.9 (CH_2 CH_2-γ), 32.4 (CH_2 CH_2-β), 37.9 (CH_2 CH_2-NH), 42.3 (CH_2 C3), 55.7 (CH CH-α), 85.4 (C2), 115.3 (C7), 123.1 (C4′), 124.9 (C5), 130.9 and 135.7 (C4 and C6), 156.2 (C7′), 174.3 and 180.9 (CO) ppm. MS (ESI+): m/z (%) = 348.8 (20) [M + 1]$^+$, 203 (100) [Pmbf]$^+$. $[\alpha]_D$ = +3.50 (c = 1.09, MeOH).

N^{α}-(9-Fluorenylmethoxycarbonyl)-N^{ω}(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)glutamine (9b): The amino acid salt (0.15 g, 0.37 mmol) yielded **9b** (0.124 g, 59%) as a white solid. M.p. 129–

132 °C. HPLC (3:7 to 10:0 over 30 min): t_R = 21.8 min. TLC (SiO_2): R_f = 0.71 ($CHCl_3$/MeOH/AcOH, 9:0.5:0.5). IR (KBr): $\tilde{\nu}$ = 3309, 2973, 1634, 1451, 1254, 1088 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ = 1.39 (s, 6 H, 2 CH_3), 1.85 (m, 1 H, CH-β), 1.93 (m, 1 H, CH-β), 2.03, 2.14, and 2.15 (3 s, 3 × 3 H, CH_3), 2.20 (m, 1 H, CH-γ), 2.90 (s, 2 H, CH_2), 4.10 (m, 1 H, CH-α), 4.18 (t, J = 7.0 Hz, CH-Fmoc), 4.30–4.31 (m, 4 H, CH_2-Fmoc and CH_2-NH), 7.28 (m, 2 H, Fmoc), 7.31 (m, 2 H, Fmoc), 7.64 (t, J = 7.9 Hz, 2 H, Fmoc), 7.75 (d, J = 7.2 Hz, 2 H Fmoc) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 10.8 (CH_3 aryl), 14.1 (CH_3 aryl), 14.8 (CH_3 aryl), 27.1 (2 CH_3 C2), 28.5 (CH_2 CH_2-β), 31.9 (CH_2 CH_2-γ), 37.9 (CH_2 CH_2-NH), 42.8 (CH_2 C3), 48.4 (CH CH-Fmoc), 54.7 (CH CH-α), 66.4 (CH_2 CH_2-Fmoc), 85.1 (C2), 115.3 (C7), 119.4 (CH Fmoc), 123.0 (C4′), 124.8 (C5), 124.9 (CH Fmoc), 126.7 (CH Fmoc), 127.3 (CH Fmoc), 130.8 and 135.7 (C4 and C6), 141.1 and 143.8 (2 C C-Fmoc), 156.3 (C7′), 156.9 (CO carbamate), 173.5 (CO amide), 175.5 (COOH) ppm. MS (ESI+): *m/z* (%) = 571 (80) $[M + 1]^+$, 203 (100) $[Pmbf]^+$. $[\alpha]_D$ = +6.90 (*c* = 1.14, MeOH).

Peptide Elongation: Protected amino acids (5 equiv.) were coupled with *N*,*N*′-diisopropylcarbodiimide (DIPCDI) and HOBt (5 equiv. each) in DMF for 1 h. The extension of the reaction was followed by the Kaiser test. In cases where neutral coupling was necessary, the coupling system DIPCDI/1-hydroxy-7-azabenzotriazole (HOAt) (5 equiv. each) was used.

Removal of Fmoc Group: The peptide resin was treated with piperidine/DMF (1:4; 2 × 1 min, 2 × 5 min) and washed with DMF (3 × 1 min) and CH_2Cl_2 (3 × 1 min).

Solid-Phase Dimerization: Formation of the intramolecular disulfide bridge was obtained by treatment of the resin-bound peptide with I_2 (10 equiv., 0.06 M) in the corresponding solvent for the time indicated in Table 2. The resin was then repeatedly washed with CH_2Cl_2 (10 × 1 min), DMF (10 × 1 min), and CH_2Cl_2 (10 × 1 min).

Cleavage from PAL Resin: The peptide was cleaved from the resin by treatment with the corresponding TFA cocktail; the filtrates were added to a cold diethyl ether. After centrifugation, the solid was washed with additional ether (3×), dissolved in HOAc/H_2O and lyophilized.

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