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Original article

CCK_2 receptor antagonists containing the conformationally constrained phenylalanine derivatives, including the new amino acid Xic

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Abstract

The conformationally constrained analogues of phenylalanine, tetrahydroisoquinoline-3-carboxylic acid (Tic), Sic, Hic and Nic, and the new amino acid Xic have been incorporated into a potent and highly selective cholecystokinin-2 (CCK_2) receptor antagonist (**2**) in place of the phenylalanine residue, producing compounds **15a**–**e**. High selectivities for CCK_2 over CCK_1 were observed for compounds **15a**–**e**. The in vitro profile of the analogue containing the Nic residue (**15d**) was identical to that of compound **2**, whereas the alternative conformational constraints resulted in a significant loss of affinity. The apparent advantage of Nic in the context of these CCK_2 ligands was subsequently demonstrated to be statistically significant. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: CCK_2; Gastrin; Conformational constrained amino acids; Tic; Phenylalanine; Antagonist

1. Introduction

The use of conformationally constrained amino acids to probe the binding mode of bioactive molecules to their receptors is a commonly used approach to the design of highly selective and active compounds [1]. Reducing the conformational freedom of a ligand may alter (a) the binding affinity of the ligand at a given receptor, (b) the selectivity of the ligand between different receptors, and (c) the stability of the ligand with respect to enzymatic degradation. Examination of the effect of restricting the conformational freedom of a given ligand on these properties may lead to increased insight into the bioactive conformation of the ligand and hence ultimately to the generation of more potent and selective molecules.

Many conformationally constrained analogues of amino acids have been synthesised and some have been used in biological studies. Focussing on phenylalanine, typical examples of conformationally constrained analogues include 3-phenylproline [2], β,β-diphenylalanine [3], α,β-dimethylphenylalanine [4], and β-methylphenylalanine [5]. Of particular relevance to the work reported herein is the phenylalanine analogue 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) **1a** (Fig. 1). Tic has been used to considerable effect in medicinal chemistry, its presence in ligands having been shown to influence both affinity and selectivity, such that it is now a commercially product. In one example, replacement of D-Phe[1] with D-Tic in the somatostatin-derived cyclic peptide CTP led to a significant increase in selectivity and affinity for the μ-opioid receptor producing one of the most potent and selective μ-opioid receptor antagonists described to date [6]. In a second example, replacement of Phe[2] in the dermorphin-derived δ-opioid receptor antagonist Tyr-Phe-Phe-Phe-NH_2 led to a dramatic improvement in its activity profile and stability [7]. Further modification has led to

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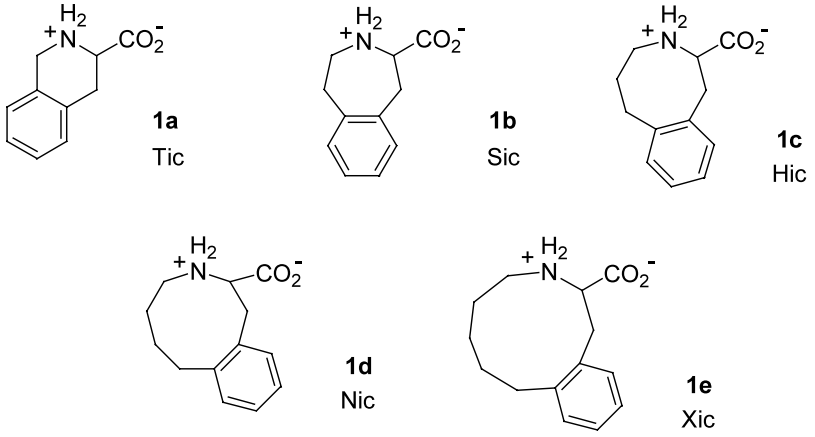

Fig. 1. Conformationally constrained analogues of Phe.

dipeptides, which display some of the best δ-opioid receptor selectivities known [8]. The continuing level of interest in Tic is reflected by the fact that several analogues of Tic, such as α-methyl Tic [9], α,β-dimethyl-Tic [4], β-phenyl-Tic [10], benzo[f]Tic, benzo[g]Tic, and benzo[h]Tic [11] have been the targets of synthetic Studies.

We synthesised the novel seven-, eight- and nine-membered analogues of Tic, i.e. Sic **1b**, Hic **1c** and Nic **1d** [12], in the belief that the incorporation of a series of compounds with varying degrees of conformational constraint into biologically active peptides or non-peptides would lead to greater insight into the conformational preferences of the ligand under investigation and the nature of its interaction with the active site. Our first test of this hypothesis was carried out on the non-peptide cholecystokinin-2 (CCK_2) receptor antagonist **2** (Fig. 2) [13], in which Phe was a key component. It seemed from the preliminary results, using Tic, Sic, Hic and Nic, that the size of the ring in the phenylalanine replacement is a determining factor for CCK_2 receptor affinity [14]. The full results and interpretation of this study, and its extension to include the, previously unreported, ten-membered analogue of Tic, i.e. Xic **1e**, are described herein. The study represents the first application of the conformationally constrained amino acids Sic, Hic, Nic and Xic.

2. Chemistry

The hydrochloride salts of the amino acids Sic, Hic, Nic and Xic were synthesised using the recently reported Heck cyclisation route designed and developed in our laboratories [12]. Our approach is summarised in Fig. 3. Reductive amination of aldehydes **3** [12] ($m = 1$–4) with (±)-serine methyl ester followed by N-protection with di-*tert*-butyl dicarbonate and introduction of a carbon=carbon double bond using tosyl chloride-triethylamine gave the Heck substrates **4**. Cyclisation of **4** with catalytic amounts of palladium acetate proceeded smoothly to generate products **5** containing seven-, eight-, nine- and ten-membered rings in 55, 73, 86, and 69% yield, respectively. Hydrogenation and hydrolysis of **5** provided the required hydrochloride salts **6b**–**e**.

The commercially available hydrochloride salt of Tic (**6a**) and the hydrochloride salts of Sic, Hic, Nic and Xic (**6b**–**e**) were converted into analogues of the compound **2** by the route depicted in Fig. 4. N-Protection of the hydrochloride salts **6a**–**e** with di-*tert*-butyl dicarbonate ($(BOC)_2O$)gave acids **7a**–**e**, which were coupled with 3,5-bis(benzyloxycarbonyl)aniline (**8**) using bromotris(pyrrolidine)phosphonium hexafluorophosphate (PyBroP®) to give, after deprotection of the intermediates **9a**–**e** with trifluoroacetic acid, amines **10a**–**e**. Reaction of amines **10a**–**e** with the anhydride **11** [13] gave acids **12a**–**e**, which were subsequently coupled with 1-adamantanemethylamine (**13**) using either PyBroP® or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC)–1-hydroxybenzotriazole (HOBt) to give amides **14a**–**e**. Subsequent hydrogenolysis revealed the diacids **15a**–**e**, which were characterised by ^{1}H-NMR and mass spectroscopy, and by microanalysis of their bis(*N*-methyl-D-glucamine) salts.

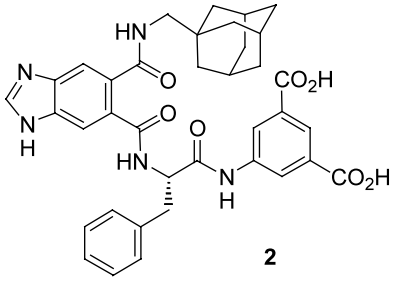

Fig. 2. A potent and selective CCK_2 antagonist containing Phe.

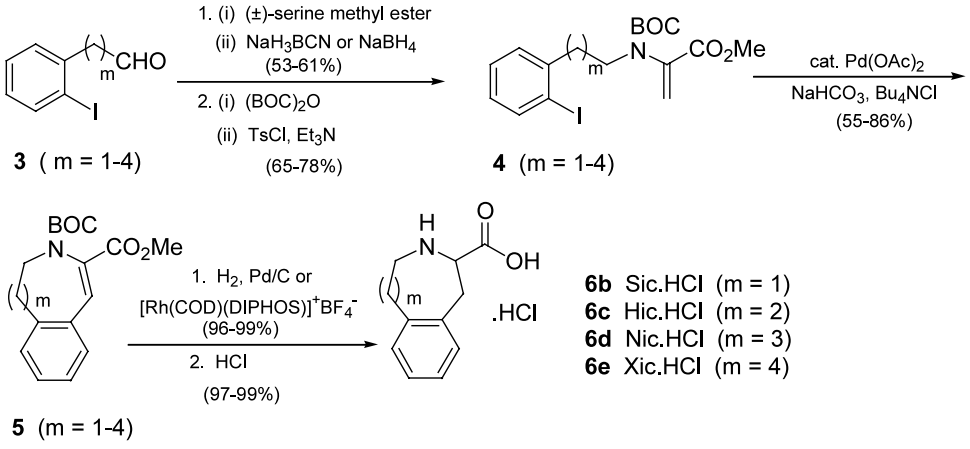

Fig. 3. Synthesis of the higher homologues of Tic.

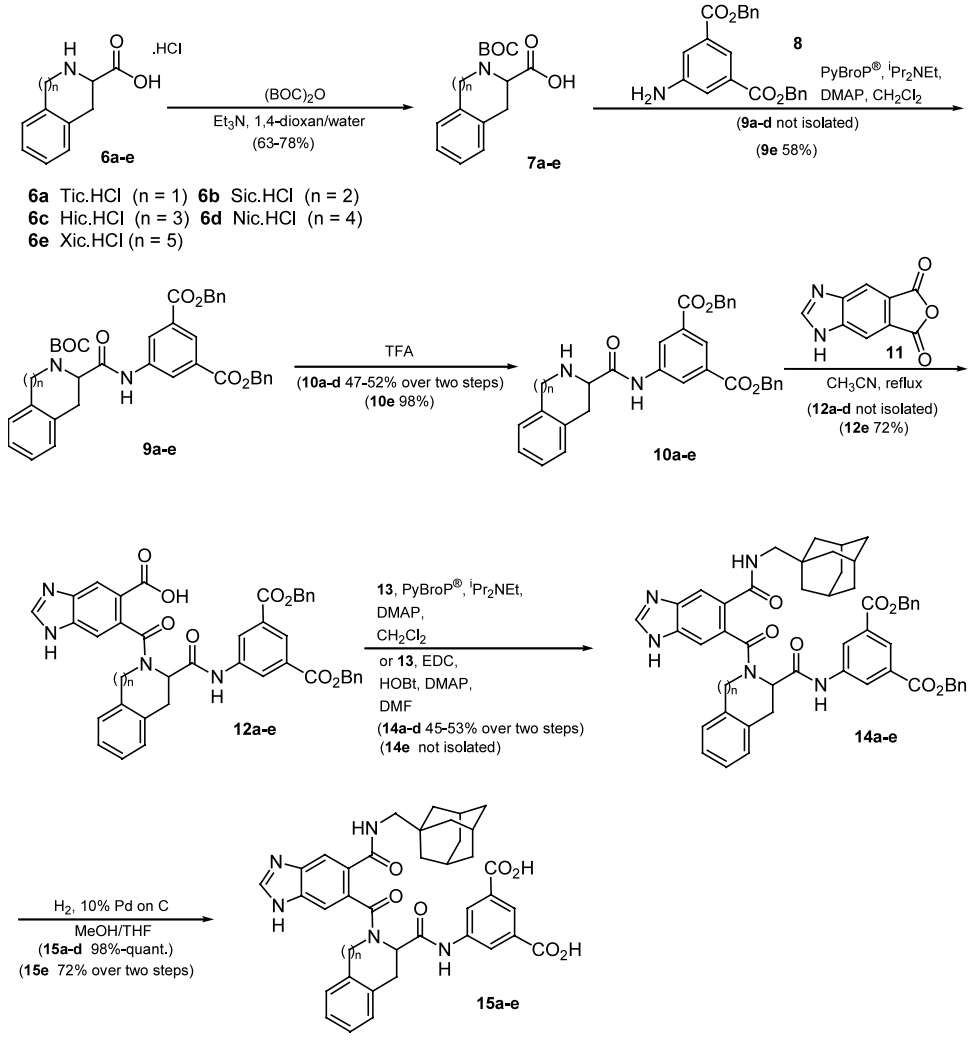

Fig. 4. Synthesis of CCK_2 antagonists containing Tic, Sic, Hic, Nic and Xic.

3. Pharmacology

Affinity estimates for these compounds at CCK_2 receptors were determined in two assays. The first was a functional assay based on the pentagastrin stimulated, lumen-perfused, isolated, immature rat stomach [15]. The second was a radioligand binding assay, which used [^{125}I]BH-CCK-8S to label CCK_2 receptors in

mouse cortical membranes [16]. This choice was made, because, the CCK_2 receptor types have been shown to be homogeneous within each of these assays, and because, L-365260, a well established reference competitive antagonist, displayed different affinity estimates (rat stomach $pK_B = 7.54 \pm 0.03$ [17]; mouse cortex $pK_i = 8.42 \pm 0.03$ [16]) between the two assays, suggesting that there are different CCK_2 receptor sub-types [16,17]. The data from the immature rat stomach assay were examined to determine whether the affinity estimates for each compound were significantly different from each other. These pK_B estimates were compared using one-way analysis of variance (ANOVA) followed by a Bonferroni modified *t*-test [21]. In order to assess the selectivity of the new ligands for the CCK_2 over the CCK_1 receptor, affinity estimates at CCK_1 receptors were determined using guinea-pig pancreatic membranes in a radioligand binding assay [18]. The isolated mouse stomach assay [15] is analogous to the rat model, but it has been shown to be particularly sensitive for the detection of partial agonists at the CCK_2 receptor [19]. Compounds **15a**–**e** were also tested in this assay to ascertain whether the inclusion of the new amino acids altered the behaviour of the ligands.

Table 1
Comparison of CCK_2 and CCK_1 receptor affinity estimates for compounds **2**, **15a**–**e** and **16**

Compound number [a]	*n*	Rat stomach [b]	Mouse cortex [c]	G.P. pancreas [f]
2	–	9.08 ± 0.10 [d]	8.28 ± 0.13 [e,g]	5.83 ± 0.27
16	–	8.72 ± 0.38	8.39 ± 0.09	5.2
15a	1	7.84 ± 0.13	6.75 ± 0.10	4.73 ± 0.04 [g]
15b	2	7.50 ± 0.10	$6.63 \pm$ ± 0.14 [g]	4.83 ± 0.08 [g]
15c	3	7.03 ± 0.17	6.65 ± 0.10	4.29 ± 0.08
15d	4	9.01 ± 0.21	8.30 ± 0.20 [g]	4.74 ± 0.04 [g]
15e	5	8.35 ± 0.11	7.95 ± 0.07	5.14 ± 0.12

[a] Compounds **2** and **15b**–**e** were tested as their bis(*N*-methyl-D-glucamine) salts.

[b] $pK_B \pm$ standard error of mean (S.E.M.) values were estimated from single shifts of pentagastrin concentration–effect curves in the isolated lumen–perfused immature rat stomach, in which the compounds behaved as simple competitive antagonists. The number of experiments and test concentrations for each compound were as follows: **15a** (12, 1 μM), **15b** (12, 1 μM), **15c** (15, 1 μM), **15d** (12, 0.03 μM), and **15e** (11, 0.1 μM).

[c] $pIC_{50} \pm$ S.E.M. values were estimated from three separate competition experiments in which 20 pM [^{125}I]BH-CCK-8S for CCK_2 was used to CCK_2 label binding sites in the mouse cortical homogenates. The $pIC_{50} \approx pK_i$ when $(1+[L]/K_L)$ approximated to unity.

[d] Value was taken from reference [26].

[e] Value is taken from reference [13].

[f] $pIC_{50} \pm$ S.E.M. competition with 20 pM [^{125}I]BH-CCK-8S at CCK_1 guinea pig pancreatic cells from three (one for compound **16**) separate experiments.

[g] The slope of these competition curves was significantly different from unity. Therefore, a strict comparison of the pIC_{50} values is potentially unreliable.

4. Results and discussion

An important characteristic of the parent ligand **2** is its reversed selectivity with respect to L-365260 in the two CCK_2 assays (Table 1). The same sense and degree of selectivity was maintained by the conformationally constrained analogues **15a**–**e**. In comparing compound **2** with compounds **15a**–**e** it is noted that the former is a single enantiomer, while the latter are racemates. The biological data for the antipode of compound **2**, compound **16** (derived from D-Phe), are also presented in Table 1 and it can be seen that chirality relatively little effect on affinity and selectivity. The differences between the affinities of compounds **2** and **16** are in the order of 0.5 log unit or less, which is within the limits of statistical error. From the data for compounds **2** and **15a**–**e**, it may be concluded that the selectivity ratio is not dependent on the ring-size of the conformationally restricted amino acids. In contrast, examination of the data from both CCK_2 assays reveals broadly parallel trends that relate affinity to ring-size. Thus, considering the two sets of data together, the affinity estimates for compounds **15a**–**c** appeared to be least an order of magnitude lower than those of compound **2**. The loss of affinity for the ten-membered ring analogue **15e** was less pronounced. However, the data for the nine-membered analogue **15d** were indistinguishable from those of compound **2**. The two sets of data provide compelling evidence for the pre-eminence of compound **15d**. However, a more rigorous statistical analysis was required to determine the significance of the differences between the compounds.

Compounds **15a**–**e** behaved as competitive antagonists to pentagastrin in the immature rat stomach assay. Each produced a significant rightward displacement of the pentagastrin dose–response curves, with no significant change in either maxima or mid-point slope parameter. With such consistent behaviour across the series of compounds, data were collected for a high number of iterations of the assay and analysed to determine whether the affinity estimates (pK_B) for each compound were significantly different from each other. Firstly, individual agonist (A) concentration-effect (E) curves were fitted to the Hill equation (Eq. (1)), to provide estimates of midpoint slope (n_H), midpoint location ($\log[A]_{50}$) and upper asymptote (α) as described previously [20].

$$E = \alpha[A]_H^n / [A]_{50}^n + [A]_H^n \quad (1)$$

Secondly, using Eq. (2), pK_B values were estimated by comparing the location of the pentagastrin $E/[A]$ curves in the presence of each antagonist (B) with the corresponding control curve (Table 1).

Table 2
Results of Bonferroni modified *t*-test to compare mean pK_B values

	15b	**15c**	**15d**	**15a**	**15e**
15b	–	2.29	6.97	−1.57	3.83
15c	2.29	–	9.63	-3.93	6.26
15d	6.97	9.63	–	5.39	2.99
15a	−1.57	−3.93	5.39	–	2.30
15e	3.83	6.26	2.99	2.30	–

t-Values were calculated, with those indicated in bold being statistically significantly different from each other at $P = 0.05$ ($t_{crit} = 2.85$).

$$pK_B = \log(r - 1) - \log B \quad (2)$$

where $r = \log[A]_{50B}/\log[A]_{50}$ and $[A]_{50B}$ is the concentration of agonist that produces 50% response in the presence of antagonist B. Comparison of the individual pK_B estimates, using one-way ANOVA, indicated a significant difference in mean values between groups ($F = 26.78$; total d.f. = 61). Subsequent Bonferroni modified *t*-tests [21], indicated significant differences in mean pK_B estimates as shown in Table 2. Therefore, it can be seen that the nine-membered ring compound (**15d**) expressed significantly higher affinity than all of the other cyclic analogues. Furthermore, the ten-membered analogue (**15e**) was significantly more potent than the seven- (**15b**) and eight-membered (**15c**) ring analogues. It is important to note from these results that statistical significance could not be ascribed to differences in potency of 0.5 log unit or less.

Similar statistical analysis of the data from the mouse cortex assay was not performed, because, the Hill slopes obtained for several of the compounds were greater than unity (Table 1). Although the reason for this variation from unity has not been found, the possibility that it could be attributed to residual efficacy was explored. The mouse stomach assay has been shown to be more sensitive than the rat stomach assay to the expression of agonism. In the mouse stomach assay partial agonism was observed for PD-134308, a reference CCK_2 receptor antagonist [22], which was found to be a competitive antagonist in the rat stomach assay [19]. Compounds **15a**–**e** were examined in the mouse stomach assay and no evidence for agonism was found at the concentration tested (10 μM).

An important property of compound **2** was the 3000-fold selectivity for the CCK_2 receptor (rat stomach) over the CCK_1 receptor (guinea pig pancreas) [13]. Compounds **15a**–**e** were similarly found to be highly selective for the CCK_2 receptor (Table 1). The CCK_2/CCK_1 selectivities for compound **15d** were in the order of 18 000-fold (rat stomach) and 3000-fold (mouse cortex).

5. Conclusions

We have investigated the effect of replacing the phenylalanine residue of the CCK_2 antagonist **2** with a series of conformationally constrained phenylalanine analogues, which has been expanded by the addition of the ten-membered ring analogue, Xic. From the work presented here it is evident that the size of the conformational constraint directly impinges upon the in vitro profile of the constrained ligands. The most pronounced effect was the drop in CCK_2 affinity for all but the Nic-containing analogue, the biological behaviour of which closely resembled that of compound **2**. It is possible, therefore, that information on the bioactive conformation of compound **2** may be gained from further studies of compound **15d**. The exact role of the conformational constraint remains to be determined. This could involve the precise positioning of either the phenylalanine side-chain itself or the other functional groups on the two arms of these molecules. A molecular modelling-based investigation of the ligands discussed here is in hand. The absence of protein crystal structures for G-protein coupled receptors, such as the CCK_2 receptor, is a recurrent problem. The construction of theoretical receptor structures has been possible using the α-carbon template of the transmembrane helices of rhodopsin [23]. It is hoped that conformationally constrained ligands, such as **15a**–**e**, may eventually prove useful tools for both homology- and ligand-based modelling approaches. Tic has been widely used as a conformationally constrained analogue of phenylalanine. From this study it is evident that consideration of a broader palette, including Sic, Hic, Nic and Xic, may be of value.

6. Experimental

6.1. Chemistry

Anhydrous *N*,*N*-dimethylformamide (DMF) and acetonitrile were purchased from Aldrich Chemical Company. Dichloromethane (dichloromethane) was distilled from calcium hydride. Triethylamine and diisopropylethylamine were distilled from and stored over potassium hydroxide pellets. The hydrogen used in the hydrogenation was BOC Grade 0 (99.99% minimum purity). Bromo-*tris*-pyrrolidino-phosphonium hexafluorophosphate (PyBroP®) was purchased from Novabiochem. HOBt, 4-dimethylaminopyridine (DMAP), EDC, [$(BOC)_2O$], 1-adamantanemethylamine **13**, and Tic–HCl (**6a**) were purchased from Aldrich Chemical Company and were used without purification. 3,5-Bis(-benzyloxycarbonyl)aniline **8** [24], benzimidazole-5,6-dicarboxylic acid anhydride **11** [13], Sic. HCl (**6b**) [12], Hic–HCl (**6c**) [12] and Nic–HCl (**6d**) [12] were synthe-

sised according to published procedures. Nic–HCl (**6e**), an analogue of **6a**–**d**, was synthesised using a modification of the published procedures for **6a**–**d** [12]. Melting points were recorded on a Büchi 510 or a Gallenkamp capillary melting point apparatus and are uncorrected. NMR spectra were recorded in $CDCl_3$ at room temperature (r.t.) (unless noted otherwise) on Bruker DRX 300, Bruker DRX 400 and Bruker AM-500 spectrometers; J values are reported in Hertz. Infrared (IR) spectra were obtained on a Unicam Mattson 5000 FTIR spectrometer. Mass spectra were recorded on Micromass Platform 2 and Micromass Autospec instruments. Elemental analyses were performed by the Imperial College Microanalytical Service or the School of Pharmacy Microanalytical Service. Flash chromatography was performed using Merck Kieselgel 60 silica grade 9385 (230–400 mesh). Thin-layer chromatography (TLC) was performed on Merck 60 F_{254} silica gel, in a closed vessel, and visualisation was achieved by UV light (254 nm) and/or by dipping into a solution of phosphomolybdic acid in EtOH, followed by heating with a heat gun. The solvent conditions used for TLC analysis corresponded to those given for the purification by column chromatography.

*6.1.1. General procedure for the preparation of the Boc amino acids **7a**–**e***

*6.1.1.1. N-(tert-butoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**7a**).* Di-*tert*-butyl dicarbonate (1.26 g, 5.79 mmol) and triethylamine (1 mL, 10% v/v) were added to a suspension of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride (**6a**) (1.03 g, 4.83 mmol) in a 1:1 mixture of 1,4-dioxan–water (10 mL). The reaction mixture was stirred at r.t. for 48 h. The solution was diluted with dichloromethane (30 mL) and washed with 5% aqueous (aq.) potassium hydrogensulfate (40 mL), brine (20 mL) and dried ($MgSO_4$). Evaporation of the solvents in vacuo gave an oil. Purification by column chromatography (SiO_2; dichloromethane–EtOAc, 98:2) afforded the title compound as a white powder (0.98 g, 3.52 mmol, 73%) melting point (m.p.) 116–117 °C (lit. [25] 122–123.5 °C); ^{1}H-NMR (300 MHz, 3:2 mixture of rotamers) δ 1.42, 1.52 (2 × s, 9H), 3.16–3.30 (m, 2H), 4.50 (d, 1H, $J = 16.5$), 4.60–4.70 (m, 1H), 4.80, 5.10 (2 × br s, 1H), 7.13–7.21 (m, 4H); IR (KBr) ν_{max} 3400–3100 m (O–H), 3054 m (C–H aromatic), 2984, 2958 w (C–H alkane), 1723 vs and 1698 s cm^{-1} (C=O carbamate and carboxylic acid); mass spectroscopy (MS) (CI) m/z 295 (MNH_4^+, 4%), 278 (10), 239 (68), 222 (32), 178 (98), 132 (100).

*6.1.1.2. N-(tert-butoxycarbonyl)-2,3,4,5-tetrahydro-1H-3-benzazepine-2-carboxylic acid (**7b**).* Colourless oil (137 mg, 0.47 mmol, 78%); ^{1}H-NMR (300 MHz, 2:3 mixture of rotamers) δ 1.38, 1.44 (2 × s, 9H), 2.87–3.00 (m, 2H), 3.05–3.15 (m, 1H), 3.25–3.33 (m, 2H), 4.16–4.30 (m, 1H), 4.92, 5.22 (2 × br m, 1H), 7.09–7.13 (m, 4H), 10.03 (br s, 1H); IR (neat) ν_{max} 3500–3100 m (O–H), 3057 m (C–H aromatic), 2980 m, 2920 m (C–H alkane), 1692 and 1675 s cm^{-1} (C=O carbamate and carboxylic acid); MS (CI) m/z 309 (MNH_4^+, 6%), 292 (17), 253 (47), 236 (30), 192 (41), 146 (100); Anal. Calc. for $C_{16}H_{21}NO_4$: C, 65.98; H, 7.22; N, 4.81. Found: C, 65.70; H, 6.97; N, 4.53%.

*6.1.1.3. N-(tert-butoxycarbonyl)-1,2,3,4,5,6-hexahydro-3-benzazocine-2-carboxylic acid (**7c**).* An oil (220 mg, 0.72 mmol, 69%); ^{1}H-NMR (300 MHz, 2:3 mixture of rotamers) δ 1.21, 1.37 (2 × s, 9H), 1.75–1.85 (m, 1H), 2.10–2.20 (m, 1H), 2.76 (t, 2H, $J = 6$), 2.77–2.90 (m, 1H), 3.13–3.30 (m, 2H), 3.72, 3.89 (2 × br d, 1H, $J = 14.5$), 4.91–4.96 and 5.15–5.45 (2 × m, 1H), 7.12–7.32 (m, 4H), 11.62 (br s, 1H); IR (neat) ν_{max} 3300–3000 br m, 3097 m, 3041 m, 3015 m (C–H aromatic), 2974, 2938 m (C–H alkane), 1695 s and 1667 m cm^{-1} (C=O carbamate and carboxylic acid); MS (CI) m/z 323 (MNH_4^+, 4%), 306 (16), 267 (87), 250 (52), 206 (43), 160 (100). Anal. Calc. for $C_{17}H_{23}NO_4$: C, 66.89; H, 7.54; N, 4.59. Found: C, 66.78; H, 7.36; N, 4.33%.

*6.1.1.4. N-(tert-butoxycarbonyl)-2,3,4,5,6,7-hexahydro-1H-3-benzazonine-2-carboxylic acid (**7d**).* An oil (238 mg, 0.746 mmol, 76%); ^{1}H-NMR (300 MHz, 2:3 mixture of rotamers) δ 0.92–1.06 (m, 1H), 1.30–1.43 (m, 1H), 1.49, 1.51 (2 × s, 9H), 1.72–2.10 (m, 2H), 2.75–3.05 (m, 3H), 3.30–4.15 (m, 4H), 7.13–7.24 (m, 4H); IR (neat) ν_{max} 3400–3000 br m, 3099 m, 3061 m, 3012 m (C–H aromatic), 2973 m, 2867 m (C–H alkane), 1740 s, 1704 s cm^{-1} (C=O carbamate and carboxylic acid); MS (CI) m/z 320 (MH^+, 100%), 264 (92), 220 (40), 174 (56). Anal. Calc. for $C_{18}H_{25}NO_4$: C, 67.71; H, 7.84; N, 4.39. Found: C, 67.84; H, 7.57; N, 4.11%.

*6.1.1.5. N-(tert-butoxycarbonyl)-1,2,3,4,5,6,7,8-octahydro-3-benzazecine-2-carboxylic acid (**7e**).* An off-white solid (148 mg, 0.44 mmol, 63%) m.p. 59–61 °C; ^{1}H-NMR (300 MHz, 2:3 mixture of rotamers) δ 1.24–1.86 (m, 6H), 1.26, 1.39 (2 × s, 9H), 2.59–2.69 (m, 2H), 2.79–2.86 (m, 2H), 3.15–3.43 (m, 3H), 7.16–7.25 (m, 4H); MS (EI) m/z 333 [M^+, 3%], 289 (2), 277 (19), 232 (51), 188 (61), 57 (100). Anal. ($C_{19}H_{27}NO_4$) C, H, N.

*6.1.2. The preparation of compounds **10a**–**d***

*6.1.2.1. 3-[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]carbonyl]-1,2,3,4-tetrahydroisoquinoline (**10a**).* Dry diisopropylethylamine (0.126 mL, 0.72 mmol) and 3,5-bis(benzyloxycarbonyl)aniline (**8**) (130 mg, 0.36 mmol) were added to a solution of **7a** (100 mg, 0.36 mmol) in dry dichloromethane (2.6 mL) kept under

argon. After 5 min, PyBroP® (168 mg, 0.36 mmol) and DMAP (3–4 mg, 0.03 mmol) were successively added and the resulting mixture was stirred for 19 h at r.t. Dichloromethane (10 mL) was added and the organic layer washed with 5% aq. potassium hydrogensulphate solution (2 × 5 mL), saturated aq. sodium hydrogencarbonate solution (5 mL), brine (5 mL) and dried ($MgSO_4$). Column chromatography (SiO_2; hexane–ethyl acetate, 9:1) afforded a mixture of the coupling product and 3,5-bis(benzyloxycarbonyl)aniline which was then stirred in trifluoroacetic acid (1 mL) for 1 h at r.t. Trifluoroacetic acid was removed in vacuo and the resulting yellow oil was dissolved in dichloromethane (10 mL). The organic layer was washed with a 5% sodium carbonate solution (2 × 5 mL), brine (5 mL) and dried ($MgSO_4$). Column chromatography (SiO_2; hexane–ethyl acetate, 4:1 then 1:1) afforded compound **10a** as a white powder (86 mg, 0.166 mmol, 46%) m.p. 107–109 °C; ^{1}H-NMR (500 MHz) δ 2.91 (dd, 1H, $J = 16.3$, 10.2), 3.33 (dd, 1H, $J = 16.3$, 5.5), 3.70 (dd, 1H, $J = 10.2$, 5.5), 3.99 (d, 1H, $J = 16.1$), 4.05 (d, 1H, $J = 16.1$), 5.39 (s, 4H), 7.08–7.10 (m, 1H), 7.18–7.21 (m, 3H), 7.33–7.47 (m, 10H), 8.48–8.51 (m, 3H), 9.65 (s, 1H); IR (KBr) ν_{max} 3322 w, 3302 w and 3281 w (N–H), 1725 vs (C=O ester), 1687 s (C=O amide), 1531 s (NH bending), 1243 vs cm^{-1} (C–O); MS (FAB) m/z 521 (MH^+, 74%), 132 (100), 91 (46), 77 (19). Anal. Calc. for $C_{32}H_{28}N_2O_5$: C, 73.82; H, 5.42; N, 5.38. Found: C, 73.89; H, 5.36; N, 5.33%.

*6.1.2.2. 2-[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]-carbonyl]-2,3,4,5-tetrahydro-1H-3-benzazepine (**10b**).* A white powder (123 mg, 0.231 mmol, 49%) m.p. 126–128 °C; ^{1}H-NMR (400 MHz) δ 2.87–2.94 (m, 2H), 2.98–3.04 (m, 1H), 3.09 (dd, 1H, $J = 14.8$, 9.4), 3.26–3.32 (m, 1H), 3.49 (dd, 1H, $J = 9.4$, 2.6), 3.57 (dd, 1H, $J = 14.8$, 2.6), 5.38 (s, 4H), 7.09–7.12 (m, 1H), 7.14–7.18 (m, 2H), 7.23–7.25 (m, 1H), 7.33–7.47 (m, 10H), 8.46–8.48 (m, 3H), 9.54 (s, 1H); IR (KBr) ν_{max} 3256 br w (N–H), 1716 vs (C=O ester), 1663 s (C=O amide), 1543 s (NH bending), 1224 s cm^{-1} (C–O); MS (CI) m/z 535 (MH^+, 55%), 362 (39), 189 (20), 174 (28), 146 (100), 91 (68). Anal. Calc. for $C_{33}H_{30}N_2O_5$: C, 74.14; H, 5.66; N, 5.24. Found: C, 74.12; H, 5.61; N, 5.22%.

*6.1.2.3. 2-[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]-carbonyl]-1,2,3,4,5,6-hexahydro-3-benzazocine (**10c**).* A white powder (160 mg, 0.292 mmol, 52%) m.p. 125–129 °C; ^{1}H-NMR (400 MHz) δ 1.70–1.72 (m, 1H), 1.94–1.99 (m, 1H), 2.77–2.87 (m, 2H), 2.90 (m, 2H), 3.17 (dd, 1H, $J = 13.8$, 8.8), 3.40 (dd, 1H, $J = 13.8$, 4.5), 3.56 (dd, 1H, $J = 8.8$, 4.5), 5.39 (s, 4H), 7.13–7.21 (m, 4H), 7.33–7.47 (m, 10H), 8.43 (d, 2H, $J = 1.4$), 8.47 (t, 1H, $J = 1.4$) and 9.42 (s, 1H); IR (KBr) ν_{max} 3358 w, 3245 w (N–H), 1719 vs (C=O ester), 1675 s (C=O amide), 1532 s (NH bending), 1229 s cm^{-1} (C–O); MS (CI) m/z 549 (MH^+, 37%), 160 (100), 91 (56); HRMS (FAB) Calc. for $C_{34}H_{33}N_2O_5$ (MH^+) 549.2389, Found: 549.2418.

*6.1.2.4. 2-[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]-carbonyl]-2,3,4,5,6,7-hexahydro-1H-3-benzazonine (**10d**).* An oil (187 mg, 0.333 mmol, 47%); ^{1}H-NMR (400 MHz) δ 1.16–1.86 (m, 3H), 2.13–2.17 (m, 1H), 2.76–2.79 (m, 2H), 2.85–2.95 (m, 2H), 3.28–3.30 (m, 2H), 3.45–3.49 (m, 1H), 5.39 (s, 4H), 7.11–7.23 (m, 4H), 7.33–7.47 (m, 10H), 8.47–8.48 (t, 1H, $J = 1.5$), 8.49–8.50 (d, 2H, $J = 1.5$), 10.27 (s, 1H); IR (KBr) ν_{max} 3440 w, 3363 w and 3233 br w (N–H), 1722 vs (C=O ester), 1699 vs (C=O amide), 1521 s (NH bending), 1237 s cm^{-1} (C–O); m/z (CI) 563 (MH^+, 60%), 174 (100), 91 (64). HRMS (FAB) Calc. for $C_{35}H_{35}N_2O_5$ (MH^+) 563.2546, Found: 563.2553.

*6.1.3. 2-[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]carbonyl]-1,2,3,4,5,6,7,8-octahydro-3-benzazecine (**10e**)*

*6.1.3.1. 2-[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]-carbonyl]-N-(tert-butoxycarbonyl)-1,2,3,4,5,6,7,8-octahydro-3-benzazecine (**9e**).* Purified 3,5-bis(benzyloxycarbonyl) aniline (**8**) (169 mg, 0.47 mmol) and diisopropylethylamine (0.136 mL, 0.78 mmol) were added to a stirred solution of **7e** (130 mg, 0.39 mmol) in dry dichloromethane (2.6 mL, 0.15 M). After 10 min, PyBroP® (0.219 g, 0.47 mmol) and DMAP (3–4 mg, ≈ 10 mol%) were added in one portion and the resulting mixture stirred for 23 h at r.t. under argon. Dichloromethane (10 mL) was added and the organic solution washed successively with 5% aq. potassium hydrogensulphate (2 × 10 mL), saturated aq. sodium hydrogencarbonate (10 mL) and brine (10 mL). The combined organic extracts were dried ($MgSO_4$) and the solvents evaporated in vacuo to afford an orange oil. Purification by flash column chromatography (SiO_2; hexane–ethyl acetate, 2:1) afforded compound **9e** as a white solid (155 mg, 0.226 mmol, 58%) m.p. 67–70 °C; ^{1}H-NMR (300 MHz, 2:3 mixture of rotamers) δ 1.12–1.84 (m, 6H), 1.26, 1.35 (2 × s, 9H), 2.54–2.72 (m, 2H), 2.89–3.50 (m, 6H), 5.40 (s, 4H), 7.09–7.61 (m, 15H), 8.37–8.51 (m, 2H); MS (FAB) m/z 677 (MH^+, 18%), 577 (47), 362 (12), 188 (58), 91 (100). Anal. Calc. for $C_{41}H_{44}N_2O_7$: C, 72.76; H, 6.55; N, 4.14. Found: C, 72.48; H, 6.64; N, 4.08%.

*6.1.3.2. Compound **10e**.* Compound **9e** (155 mg, 0.23 mmol) was stirred in trifluoroacetic acid (2.3 mL, 0.1 M) at r.t. for 1 h. The solvent was evaporated in vacuo and the resulting orange oil dissolved in dichloromethane (10 mL). The organic solution was washed successively with 5% w/w aq. sodium hydrogencarbonate (2 × 10 mL) and brine (10 mL). The organic extracts were dried ($MgSO_4$) and the solvent evaporated

in vacuo to afford a yellow oil. Purification by flash column chromatography (SiO_2; hexane–ethyl acetate, 1:1) afforded the title compound **10e** as fluffy white crystals (129 mg, 0.22 mmol, 98%). m.p. 60–63 °C; ^{1}H-NMR (300 MHz) δ 1.24–1.99 (m, 7H), 2.74–2.78 (m, 2H), 2.83–2.99 (m, 3H), 3.41–3.50 (m, 1H), 3.61–3.71 (m, 2H), 5.39 (s, 4H), 7.14–7.48 (m, 15H), 8.45–8.54 (m, 2H); MS (FAB) m/z 577 (MH^+, 79%), 188 (75), 154 (100), 136 (72). Anal. Calc. for $C_{36}H_{36}N_2O_5$: C, 74.98; H, 6.29; N, 4.86. Found: C, 74.69; H, 6.35; N, 4.62%.

6.1.4. The preparation of compounds **14a**,**b**

*6.1.4.1. 5-[[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]carbonyl]-[3-(1,2,3,4-tetrahydroisoquinolino)carbonyl]]-6-[[(1-adamantanemethyl)amino]carbonyl]benzimidazole (**14a**).* Benzimidazole-5,6-dicarboxylic acid anhydride (**11**) (29 mg, 0.15 mmol) and compound **10a** (80 mg, 0.15 mmol) were suspended in dry acetonitrile (1.5 mL) and the mixture was heated to reflux. A precipitate formed within 15 min and the mixture was heated for a further 3 h. The mixture was allowed to cool and MeOH was added until all the precipitate was dissolved. Pre-adsorption on silica followed by column chromatography (SiO_2; dichloromethane–MeOH, 99:1–90:10) gave a pale yellow solid as the impure carboxylic acid intermediate compound **12a**. PyBrOP® (47 mg, 0.10 mmol), diisopropylethylamine (0.035 mL, 0.20 mmol) and DMAP (1–2 mg, 0.010 mmol) were added to the solution of the intermediate compound **12a** in dichloromethane (1 mL) under argon. The mixture was stirred for 5 min at r.t. under argon before addition of 1-adamantanemethylamine **13** (17 mg, 0.10 mmol) in dichloromethane (1 mL). The resulting solution was stirred for 24 h under argon at r.t. Dichloromethane (10 mL) was added and the organic layer was washed with 2 M hydrochloric acid solution (2 × 5 mL), saturated aq. hydrogencarbonate solution (5 mL), brine (5 mL) and dried ($MgSO_4$). Column chromatography (SiO_2; dichloromethane–MeOH; 98:2) gave the title compound **14a** as an oil which crystallised as white crystals from hexane–dichloromethane (60 mg, 0.070 mmol, 45%) m.p. 167–169 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.20–1.33 (m, 6H), 1.47–1.69 (m, 6H), 1.80–1.87 (m, 3H), 2.80–2.84 (m, 1H), 2.95–3.00 (m, 1H), 3.19–3.30 (m, 1H), 3.40–3.48 (m. 1H), 4.42 (d, 1H, $J = 15.8$), 4.49 (d, 1H, $J = 15.8$), 5.30–5.40 (m, 5H), 6.96 (d, 1H, $J = 7$), 7.06 (t, 1H, $J = 7$), 7.11–7.18 (t, 1H, $J = 7$), 7.22 (d, 1H, $J = 7$), 7.32–7.43 (m, 10H), 7.62 (brs 1H), 8.18 (s, 1H), 8.22 (s, 1H), 8.46 (s, 1H), 8.66 (s, 2H), 8.88 (br dt, 1H), 10.04 (s, 1H), 12.87 (br s, 1H); IR (KBr) ν_{max} 3086 m and 3033 m (C–H aromatic), 2926, 2903 and 2848 m (C–H alkane), 1725 vs (C=O ester), 1634 vs (C=O amide), 1548 s (NH bending), 1240 s cm^{-1} (C–O); MS (FAB) m/z 856 (MH^+, 7.5%), 691 (2), 521 (5), 336 (100), 91 (50). Anal. Calc. for $C_{52}H_{49}N_5O_7$: C, 72.96; H, 5.77; N, 8.18. Found: C, 72.69; H, 5.61; N, 7.95%.

*6.1.4.2. 5-[[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]carbonyl]-[2-(1,2,3,4,5-tetrahydro-1H-3-benzazepino)carbonyl]]-6-[[(1-adamantanemethyl)amino]carbonyl]benzimidazole (**14b**).* A white solid which crystallised from hexane–ethyl acetate (118 mg, 0.136 mmol, 47%) m.p. 166–170 °C; ^{1}H-NMR (400 MHz) δ 1.36–1.48 (m, 9H), 1.57–1.60 (m, 3H), 1.82 (m, 3H), 2.73–2.98 (m, 3H), 3.02–3.15 (m, 1H), 3.28–3.60 (m, 3H), 3.63–3.75 (m, 1H), 5.35 (s, 4H), 5.68 (t, 1H, $J = 6$), 7.02–7.22 (m, 5H), 7.32–7.57 (m, 10H), 7.73 (br s, 1H), 7.94 (br s, 1H), 8.47 (s, 1H), 8.82 (s, 2H), 10.08 (s, 1H), 11.26 (s, 1H); IR (KBr) ν_{max} 3078 m (C–H aromatic), 2901 m, 2846 m (C–H alkane), 1725 vs (C=O ester), 1638 vs (C=O amide), 1547 s (NH bending), 1239 s cm^{-1} (C–O); MS (FAB) m/z 870 (MH^+, 13%), 705 (4), 535 (8), 336 (100), 91 (66). Anal. Calc. for $C_{53}H_{52}N_5O_7 \cdot 1.27\ H_2O$: C, 71.21; H, 6.15; N, 7.83. Found: C, 71.42; H, 6.45; N, 7.78%.

6.1.5. The preparation of compounds **14c**,**d**

*6.1.5.1. 5-[[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]carbonyl]-[2-(1,2,3,4,5,6-hexahydro-3-ben.zazocino)carbonyl]]-6-[[(1-adamantanemethyl)amino]carbonyl]benzimidazole (**14c**).* The benzimidazole-5,6-dicarboxylic acid anhydride (**11**) (67 mg, 0.36 mmol) and **10c** (150 mg, 0.27 mmol) were suspended in dry acetonitrile (6 mL) and the mixture was heated to reflux for 3 h. Column chromatography (SiO_2; dichloromethane–MeOH, 99:1–90:10) gave impure carboxylic acid intermediate compound **12c**. EDC (72 mg, 0.38 mmol), HOBt (51 mg, 0.38 mmol) and DMAP (3–4 mg, 0.03 mmol) were added to the solution of the intermediate compound **12c** dissolved in DMF (6 mL). The mixture was stirred for 5 min at r.t. before addition of 1-adamantanemethylamine (**13**) (50 mg, 0.30 mmol) in DMF (1 mL) under argon. The resulting solution was stirred for 17 h at r.t. DMF was evaporated in vacuo and the resulting oil was dissolved in dichloromethane (10 mL) and extracted with 2 M hydrochloric acid solution (2 × 5 mL), saturated aq. hydrogencarbonate solution (5 mL), brine (5 mL) and dried ($MgSO_4$). Column chromatography (SiO_2; dichloromethane–MeOH; 98:2) gave compound **14c** as a white solid which crystallised as white crystals from hexane–ethyl acetate (128 mg, 0.145 mmol, 53%) m.p. 169–171 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.22–1.60 (m, 14H), 1.71 (br s, 3H), 1.80–1.90 (m, 1H), 1.90–2.00 (m, 1H), 2.61–2.89 (m, 3H), 3.05–3.22 (m, 1H), 4.09–4.13 and 4.41–4.43 (2 × br m, 1H), 5.20 (br s, 1H), 5.40–5.74 (m, 4H), 5.50 (br s, 1H), 7.13–7.24 (m, 4H), 7.28–7.46 (m, 10H), 7.89–8.77 (m, 6H), 10.11 (s, 1H), 11.39 (s,

1H); IR (KBr) ν_{max} 3078 m, 3029 m (C–H aromatic), 2902, 2847 m (C–H alkane), 1725 vs (C=O ester), 1632 vs (C=O amide), 1564 s (NH bending), 1233 s cm^{-1} (C–O); MS (FAB) m/z 884 (MH^+, 6%), 719 (11), 336 (100), 91 (66). Anal. Calc. for $C_{54}H_{54}N_5O_7$: C, 73.27; H, 6.15; N, 7.92. Found: C, 73.51; H, 5.93; N, 7.76%.

*6.1.5.2. 5-[[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]-carbonyl]-[2-(2,3,4,5,6,7-hexahydro-1H-3-benzazonino)-carbonyl]] - 6 - [[(1 - adamantanemethyl)amino]carbonyl]-benzimidazole (**14d**).* A white solid, which crystallised from hexane–dichloromethane (96 mg, 0.11 mmol, 50%) m.p. 168–71 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.95–1.97 (m, 21H), 2.78–3.00 (m, 4H), 3.25–3.28 (m, 1H), 3.70–3.80 (m, 1H), 4.11 (br s, 1H), 5.38–5.74 (m, 4H), 7.19–7.25 (m, 4H), 7.33–7.45 (m, 10H), 7.77–8.74 (m, 6H), 10.10 (s, 1H), 12.86 (d, 1H, $J = 17$); IR (KBr) ν_{max} 2932 m, 2903 m, 2848 m (C–H alkane), 1726 vs (C=O ester), 1629 vs (C=O amide), 1552 s (NH bending), 1231 s cm^{-1} (C–O); MS (FAB) m/z 898 (MH^+, 22%), 733 (5), 563 (22), 336 (82), 91 (100). Anal. Calc. for $C_{55}H_{56}N_5O_7$: C, 73.46; H, 6.28; N, 7.79. Found: C, 73.62; H, 6.33; N, 7.56%.

*6.1.6. The preparation of compounds **15a–d***

*6.1.6.1. 5–[[[3,5–(Dicarboxyphenyl)amino]carbonyl]-[2-(1,2,3,4 - tetrahydroisoquinolino)carbonyl]] - 6 - [[(1 - adamantanemethyl)amino]carbonyl]benzimidazole (**15a**).* Palladium-on-charcoal (10%) (25 mg) was added to a solution of **14a** (250 mg, 0.29 mmol) in a 1:1 mixture of THF and MeOH (9 mL). The reaction mixture was stirred for 12 h under an atmosphere of hydrogen and then filtered through Celite and evaporated in vacuo to afford compound **15a** as a white powder (192 mg, 0.284 mmol, 98%) m.p. 240–242 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.34–1.85 (m, 15H), 2.90–3.00 (m, 1H), 3.10–3.18 (m, 1H), 3.60–3.70 (m, 2H), 4.41 (d, 1H, $J = 15.8$), 4.48 (d, 1H, $J = 15.8$), 5.36–5.39 (m, 1H), 6.96–7.24 (m, 4H), 7.37–7.70 (m, 2H), 8.10–8.95 (m, 6H), 9.97 (s, 1H), 12.87 (br s, 2H); IR (KBr) ν_{max} 3300–2400 br (O–H), 2907 s, 2848 m (C–H alkane), 1712 s (C=O carboxylic acid), 1632 vs (C=O amide), 1553 s cm^{-1} (NH bending); MS (FAB) m/z 676 (MH^+, 10%), 495 (4), 467 (2), 336 (100), 55 (99). Anal. Calc. for $C_{38}H_{37}N_5O_7 \cdot 1.45H_2O$: C, 65.03; H, 5.73; N, 9.98. Found: C, 65.08; H, 5.81; N, 9.96%. The bis(*N*-methyl-D-glucamine) salt was prepared, as a white solid, by lyophylisation of a solution of **15a** (1 equiv.) and *N*-methyl-D-glucamine (2 equiv.) in 1,4-dioxan–water.

*6.1.6.2. 5-[[[3,5-(Dicarboxyphenyl)amino]carbonyl]-[2-(2,3,4,5-tetrahydro-1H-3-benzazepino)carbonyl]]-6-[[(1-adamantanemethyl)amino]carbonyl]benzimidazole (**15b**).* A white powder (69 mg, 0.10 mmol, quant.) m.p. 249–252 °C (decomp.); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.21–1.57 (m, 12H), 1.62–1.84 (m, 3H), 2.90–3.17 (m, 5H), 3.67–3.72 (m, 3H), 5.50–5.53 (m, 1H), 7.14–7.24 (m, 5H), 7.98–8.83 (m, 6H), 10.05 (s, 1H), 13.07 (br s, 2H); IR (KBr) ν_{max} 3500–2500 br (O–H), 3290 s (NH amide), 2907 s and 2848 m (C–H alkane), 1694 s (C=O carboxylic acid), 1630 vs and 1600 s (C=O amide), 1547 s cm^{-1} (NH bending); MS (FAB) m/z 690 (MH^+, 14%), 509 (22), 467 (3), 336 (100). The bis(*N*-methyl-D-glucamine) salt was prepared, as a white solid, by lyophylisation of a solution of **15b** (1 equiv.) and *N*-methyl-D-glucamine (2 equiv.) in 1,4-dioxan–water. Anal. Calc. for $C_{39}H_{39}N_5O_7 \cdot 2C_7H_{17}NO_5 \cdot 5.2H_2O$: C, 54.18; H, 7.24; N, 8.35. Found: C, 54.09; H, 7.28; N, 8.44%.

*6.1.6.3. 5-[[[3,5-(Dicarboxyphenyl)amino]carbonyl]-[2-(1,2,3,4,5,6 - hexahydro - 3 - benzazocinocarbonyl]] - 6-[[(1-adamantanemethyl)amino]carbonyl]benzimidazole (**15c**).* A white powder (69 mg, 0.098 mmol, 99%) m.p. 248–250 °C (decomp.); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.21–2.05 (m, 19H), 2.51–3.62 (m, 8H), 4.11–4.14 and 4.46–4.50 (2 × m, 1H), 5.22 and 5.54 (2 × br s, 1H), 7.19–7.45 (m, 4H), 7.92–8.73 (m, 6H), 11.46 (m, 2H); IR (KBr) ν_{max} 3500–2500 br (O–H), 2932 m, 2901 s, 2848 m (C–H alkane), 1707 s (C=O carboxylic acid), 1649 vs, 1631 vs (C=O amide), 1557 s cm^{-1} (NH bending); MS (FAB) m/z 704 (MH^+, 9%), 523 (28), 467 (3), 336 (100). The bis(*N*-methyl-D-glucamine) salt was prepared, as a white solid, by lyophylisation of a solution of **15c** (1 equiv.) and *N*-methyl-D-glucamine (2 equiv.) in 1,4-dioxan–water. Anal. Calc. for $C_{39}H_{39}N_5O_7 \cdot 2C_7H_{17}NO_5 \cdot 5.2H_2O$: C, 54.18; H, 7.24; N, 8.35. Found: C, 54.09; H, 7.28; N, 8.44%.

*6.1.6.4. 5-[[[3,5-(Dicarboxyphenyl)amino]carbonyl]-[2-(2,3,4,5,6,7-hexahydro-1H-3-benzazonino)carbonyl]]-6-[[(1 - adamantanemethyl)amino]carbonyl]benzimidazole (**15d**).* A white powder (31 mg, 0.043 mmol, 98%) m.p. 244–246 °C (decomp.); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 1.02–1.87 (m, 21H), 2.78–3.05 (m, 4H), 3.45–4.62 (m, 3H), 7.15–7.30 (m, 4H), 7.98–8.80 (m, 6H), 10.01 (m, 1H), 12.90 (m, 2H); IR (KBr) ν_{max} 3500–2400 br (O–H), 2929 m, 2906 s, 2847 m (C–H alkane), 1714 s (C=O carboxylic acid), 1638 vs, 1633 vs, 1622s (C=O amide), 1554 s cm^{-1} (NH bending); MS (FAB) m/z 718 (MH^+, 4%), 537 (17), 336 (21), 73 (100). The bis(*N*-methyl-D-glucamine) salt was prepared, as a white solid, by lyophylisation of a solution of **15d** (1 equiv.) and *N*-methyl-D-glucamine (2 equiv.) in 1,4-dioxan–water. Anal. Calc. for $C_{41}H_{43}N_5O_7 \cdot 2C_7H_{17}NO_5 \cdot 5.2H_2O$: C, 54.92; H, 7.40; N, 8.15. Found: C, 54.89; H, 7.50; N, 8.08%.

*6.1.7. The preparation of compounds **15e***

*6.1.7.1. 5-[[[[3,5-Bis(benzyloxycarbonyl)phenyl]amino]-carbonyl]-[2-(1,2,3,4,5,6,7,8-octahydro-3-benzazecino)-carbonyl]]-6-(carboxylic acid)benzimidazole (**12e**).* Compounds **10e** (129 mg, 0.22 mmol) and **11** (51 mg, 0.27 mmol) were suspended in dry acetonitrile (2.2 mL, 0.1 M) and heated to reflux. After 15 min, a yellow precipitate began to form. After 5 h the mixture was allowed to cool and the pale yellow precipitate filtered off. Purification by flash column chromatography (SiO_2; dichloromethane–methanol, 9:1) afforded the title compound **12e** as a white powder (124 mg, 0.162 mmol, 72%) m.p. 201–203 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.84–1.22 (m, 5H), 1.50–1.90 (m, 2H), 2.53–3.56 (m, 9H), 5.38–5.40 (m, 4H), 7.18–7.50 (m, 14H), 8.01–8.54 (m, 4H), 8.62–8.79 (m, 2H); MS (FAB) m/z 765 (MH^+, 12%), 154 (100), 136 (83), 91 (80). Anal. Calc. for $C_{45}H_{40}N_4O_8$: C, 70.67; H, 5.27; N, 7.32. Found: C, 70.33; H, 5.63; N, 7.22%.

*6.1.7.2. 5-[[[3,5-(dicarboxyphenyl)amino]carbonyl]-[2-(1,2,3,4,5,6,7,8-octahydro-3-benzazecino)carbonyl]]-6-[[(1-adamantanemethyl)amino]carbonyl]benzimidazole (**15e**).* Diisopropylethylamine (0.042 mL, 0.24 mmol), PyBroP® (67 mg, 0.14 mmol) and DMAP (2–3 mg, $\approx$15 mol%) were added to a stirred solution **12e** (92 mg, 0.12 mmol) in dichloromethane (0.6 mL). After 10 min stirring, a solution of 1-admantanemethylamine **13** (24 mg, 0.14 mmol) in dichloromethane (0.6 mL, 0.1 M in total) was added and the reaction mixture stirred for 18 h at r.t. under argon. Dichloromethane (8 mL) was added and the solution washed successively with aq. 2 M hydrochloric acid (2 × 10 mL), saturated aq. sodium hydrogencarbonate solution (2 × 10 mL) and brine (10 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo to afford an off-white solid. Purification by flash column chromatography (SiO_2; dichloromethane–methanol, 6:1) afforded a mixture of the desired coupling product and PyBroP® residues (0.114 g). The crude mixture was dissolved in a 1:1 mixture of THF and methanol (4.0 mL) and 10% palladium-on-carbon catalyst (12 mg) added. The reaction mixture was put under an inert atmosphere and then filled with hydrogen via balloon. The mixture was stirred at r.t. under a hydrogen atmosphere for 20 h. Filtration through Kieselguhr (MeOH) and evaporation of the solvents in vacuo afforded a white solid (108 mg). Purification by flash column chromatography (SiO_2; dichloromethane–MeOH–acetic acid, 40:4:1) afforded compound **15e** as a white powder (63 mg, 0.086 mmol, 72%) m.p. 278–280 °C (decomp.) ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.01–3.68 (m, 35H), 7.11–7.52 (m, 4H), 7.97–8.67 (m, 5H), 9.78–9.84 (m, 1H); MS (FAB) m/z 732 (MH^+, 10%), 551 (22), 495 (15), 336 (48), 185 (100), HRMS (FAB) Calc. for $C_{42}H_{45}N_5O_7K$ (M + K) 770.2956. Found: 770.2962. The bis(*N*-methyl-D-glucamine) salt was prepared, as a white solid, by lyophylisation of a solution of **15e** (1 equiv.) and *N*-methyl-D-glucamine (2 equiv.) in 1,4-dioxan–water. Anal. Calc. for $C_{42}H_{45}N_5O_7{\cdot}2C_7H_{17}NO_5{\cdot}0.5H_2O$: C, 59.46; H, 7.22; N, 8.67. Found: C, 59.39; H, 6.96; N, 8.46%.

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