

Multicomponent synthesis of dihydrobenzoxazepinones, bearing four diversity points, as potential α -helix mimics

Luca Banfi · Andrea Basso · Valentina Cerulli ·
Giuseppe Guanti · Paulina Lecinska ·
Ilaria Monfardini · Renata Riva

Received: 14 June 2009 / Accepted: 24 October 2009 / Published online: 28 November 2009
© Springer Science+Business Media B.V. 2009

Abstract A very short convergent synthesis of dihydrobenzoxazepinones, bearing four diverse diversity points, based on coupling the Ugi reaction with a Mitsunobu cyclization, was developed. These compounds are potential α -helix mimics, where three of the four appendages are expected to imitate the residues in i , $i + 4$ and $i + 7$ positions. A library of 22 compounds bearing lipophilic substituents, designed to interact with the hydrophobic cleft of anti-apoptotic protein Bcl-xL, was synthesized. Preliminary biochemical tests, based on competitive binding, have already been carried out.

Keywords Ugi reaction · Multicomponent reactions · Mitsunobu reaction · Alpha-helix mimics

Although most of the drugs presently in clinical use are directed towards traditional biological targets such as receptors, enzymes, ion channels and nucleic acids, protein–protein interactions (PPIs) are becoming more and more important as targets for the discovery of innovative drugs due to the mounting awareness of their central role in many biological processes [1–4]. For example, interactions between pro-apoptotic and anti-apoptotic proteins are key regulatory events in the balance between cell survival (and hence proliferation) and apoptosis. Therefore, ligands (antagonists) of anti-apoptotic proteins hold promise to become innovative and selective anti-tumour drugs [5].

Antagonists of specific PPIs may also be very useful as chemical tools in proteomics to recognize the role of many proteins and elucidate the complex net of protein signalling.

The development of small molecules as PPI antagonists is, however, a formidable challenge because large surface contact areas are involved and the ‘active site’ is also relatively flat, not exposing well-defined pockets as in the case of enzymes and receptors. In most cases, PPIs take place in connection with α -helices and, thus, a successful strategy involves the use of large peptide or peptidomimetic oligomers (‘foldamers’) with a well-defined conformation that can reproduce this secondary structural element [6–8]. However, oligomers are not ideal from the point of view of pharmacokinetics and small molecule inhibitors, although more difficult to find, remain, without any doubt, more attractive.

Fortunately, it was shown that the majority of binding energy is often provided by few crucial contacts, involving in particular in the case of α -helices, the side chains of three specific amino acids (corresponding to the i , $i + 4$ and $i + 7$ positions; see Fig. 1). These residues project approximately from the same side of the helix with well-known distance and angular relationships. An artificial, rigid structure, able to display at least three substituents in the appropriate spatial orientation, may be the right solution for this task. Traditional drug-like scaffolds made of aromatic, flat, heterocyclic systems, are clearly not appropriate because with such systems, it is impossible to project the three residues in the same direction and in a tridimensional manner. An ingenious approach was proposed by Hamilton and coworkers, who designed and studied terphenyl or terpyridine scaffolds [9–13]. Related work, involving compounds where three suitably decorated aryl or heteroaryl groups are joined together by amidic bonds have also been reported [14]. On the other hand, ladder-like polycyclic ethers were developed by Hiram and co-workers to mimic the i , $i + 4$, and $i + 8$ positions of an α -helix [15].

L. Banfi (✉) · A. Basso · V. Cerulli · G. Guanti ·
P. Lecinska · R. Riva
Department of Chemistry and Industrial Chemistry, University
of Genova, via Dodecaneso 31, 16146 Genova, Italy
e-mail: banfi@chimica.unige.it

I. Monfardini
Department of Oncology, Biology and Genetics, University of Genova,
Largo Rosanna Benzi, 10, 16132 Genova, Italy

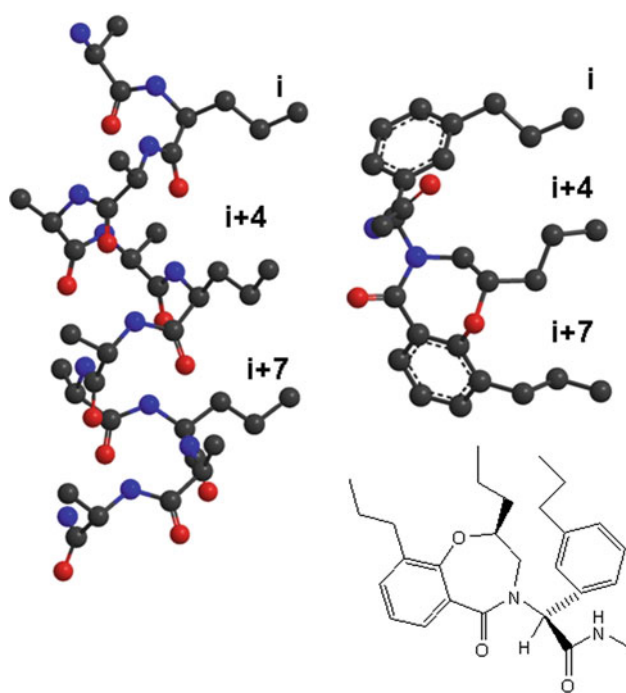


Fig. 1 Comparison of a model α -helix with a simplified model of the compounds prepared in the present work

Possible drawbacks of Hamilton's approach are the following: (a) the systems are not very rigid where only one out of several conformations is the active entity; (b) synthetic pathways are not convergent, and (c) exploration of several diverse substituents requires *de novo* syntheses.

An interesting alternative is to find a suitable, conformationally biased, non-planar heterocyclic system that serves as scaffold to hold the three needed residues [16–18]. For example, dihydrobenzodiazepines have been used towards that goal [19–21]. This approach is particularly attractive when the assembly of the heterocyclic system benefits from a convergent synthetic pathway. This would allow the introduction of the diversity inputs in just a few steps, and thus the easy generation of libraries of α -helix mimics [22,23].

Multicomponent reactions [24] are very well suited for diversity-oriented synthesis, being characterized by high atom- and step-economy and allowing the simultaneous introduction of several diversity inputs. Among them, a central role is played by those based on the peculiar reactivity of isocyanides (IMCRs). Their usefulness is demonstrated by their extensive use in the synthesis of libraries of potential drug candidates [25–29].

We have recently reported a series of very short entries into bicyclic non-planar heterocyclic systems by combining the Ugi reaction with a Mitsunobu or Mitsunobu-like intramolecular nucleophilic substitution [30–33]. In particular, dihydrobenzoxazepinones of general formula **1** ($R^1 = R^3 = H$, $R^2 = H$ or Me) may be accessed in only two synthetic steps

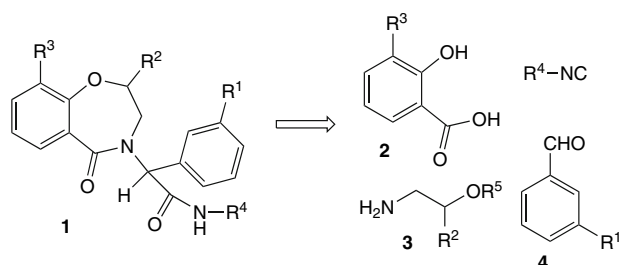
and in good overall yields starting from isocyanides, salicylic acid, benzaldehyde, and ethanolamine or 1-amino-2-propanol. A thorough conformational study, already discussed in a previous article [31], indicates that this system is conformationally biased, not only as far as it concerns the seven-membered ring, but also with regards to the aryl group and the secondary amide that lie outside the ring. The NH group and the ring carbonyl are involved in a γ -turn-like hydrogen bond, whereas the hydrogen atom evidenced in Fig. 1 is directed towards the ring carbonyl, for allylic strain reasons. All this evidence [31] makes these compounds promising as conformationally biased peptidomimetics.

The developed synthetic methodology allows the introduction of up to four diversity inputs in just two linear steps. Therefore, this scaffold seemed appropriate for the development of small molecule α -helix mimics.

We designed compounds of general formula **1**, where substituents R^1 , R^2 and R^3 should mimic the i , $i + 4$ and $i + 7$ residues. The fourth appendage (R^4) could alternatively afford an additional interaction point or be precious to modulate the solubility/pharmacokinetic profile. Figure 1 shows the predicted favoured conformation for a specific stereoisomer of a simplified model of **1**, with three propyl groups as the R^1 , R^2 , and R^3 substituents, and CH_3 as R^4 . This conformation was minimized with ChemBio3D Ultra v.11 [34], using the MOPAC Pro module,¹ taking also into account the previously reported NMR results on the simpler analog with $R^1 = R^2 = H$, $R^3 = CH_3$ and $R^4 = cyclohexyl$ [31]. As it can be observed, the R^1 , R^2 and R^3 groups are directed approximatively in the same direction. The distances between the first carbon of R^1 and R^2 and of R^1 and R^3 are in agreement with what was predicted, in an α -helix (Fig. 1), for the distances between the i and $i + 4$ residue and the i and $i + 7$ residues. R^2 and R^3 are somewhat closer in **1** than in the α -helix, but the length of the lipophilic side chains that we planned to introduce (see below) was expected to correct this small deviation from ideality.

As first target to test the validity of this approach, we chose Bcl-xL [35,36] because of our particular interest in finding ligands for this anti-apoptotic protein [8]. Bcl-xL is overexpressed in many types of cancer, where it leads to uncontrolled cell growth even in the presence of apoptotic signals.

¹ The MOPAC Pro plug-in of ChemBio3D Ultra 11., which implements semi-empirical methodologies for analysing molecular models, was used. PM3 was employed as potential energy function. It should be noted that the conformation indicated in Fig. 1 is purely indicative, since there are several other conformations very similar in local minima energy, due to the flexibility of the three propyl groups. Apart from that, evidence gained during previously reported studies [31] has shown that the structure is fairly rigid. The only freely rotatable bond should be the one that connects the aryl group containing R^1 group. Two minima with a very similar energy should be present. Obviously, only one of these two minima (the one shown in Fig. 1) would project the R^1 group in the correct direction.



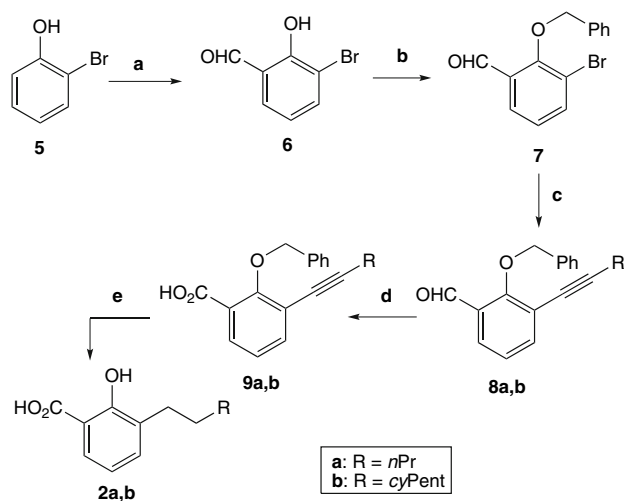
Scheme 1

Previous studies on the natural Bcl-xL ligand, that is Bak-peptide, have shown that Bak's BH3 domain is responsible for binding [37]. Moreover, it is clear that four hydrophobic residues of the BH3 domain (Val (*i*), Leu (*i* + 4), Ile (*i* + 7), Ile (*i* + 11)) along one edge of the helix are involved in binding. In addition, an Asp (*i* + 9) residue forms an ion pair with a lysine residue of Bcl-xL. In this first study, we chose to focus only on the hydrophobic interactions, putting three alkyl or aralkyl groups at the *i*, *i* + 4 and *i* + 7 (or *i* + 11, *i* + 7, *i* + 4) positions, because a carboxylate group could lead to unspecific binding. The inclusion of an aspartic mimetic was planned to be done later to increase potency. At this starting point, our goal was to detect even weak binding interactions to have an experimental rationale to be used for further refinements. For this reason, and since the four lipophilic residues in Bak could also be mimicked by our three lipophilic residues in more than a possible mode, we chose also to test all the possible stereoisomers of our designed α -helix mimetics. We worked on racemic mixtures, and tested (separately) both possible diastereoisomers.

For the synthesis of a small collection of compounds **1**, the development of efficient methodologies for the preparation of the three commercially unavailable substrates, **2**, **3** and **4** was needed. We needed robust, general pathways that could be used without problems for library extension, and that could allow the introduction of diverse alkyl or aralkyl chains without changing the reaction conditions.

3-Alkyl salicylic acids have been previously prepared from phenol by a sequence of Friedel-Crafts acylation, Wolff-Kishner reduction and Kolbe carboxylation [38]. This pathway was not ideal for our purposes because of several drawbacks: (a) the lack of regioselectivity in the Friedel-Crafts acylation; (b) the lack of convergency, since the alkyl substituent is introduced in the very first step; (c) the need for high pressure in the Kolbe carboxylation step; and (d) the use of toxic hydrazine in Wolff-Kishner reaction. Moreover, the yields of this sequence were not reported, and the harsh conditions were not very promising.

Thus, we decided to set up a different pathway based on the Sonogashira coupling of an alkyne with a 3-bromosalicylic acid derivative (Scheme 2). However, the preparation of 3-bromosalicylic acid from 2-bromophenol requires again a Kolbe carboxylation. Also, since a free carboxylic acid could



Scheme 2 Synthesis of 3-substituted salicylic acids a: (HCHO)_n, MgCl₂, CH₃CN, Et₃N, 67%. b: Cs₂CO₃, BnBr, DMF, MW (110°C, 30 min), 99%. c: 1-pentyne or *cyclo*-pentylacetylene, Et₃N, CuI, Pd(PPh₃)₂Cl₂, PPh₃, DMF, MW, 100°C, 2 h, 83% (8a), 79% (8b). d: NaClO₂, DME, CH₃CN, *t*BuOH, 2-butene, NaH₂PO₄, 100% (9a), 98% (9b). e: H₂, Pd-C, EtOH, 85% (2a), 82% (2b)

be troublesome in the Sonogashira step, we decided to use a formyl group as a synthetic equivalent since it is easy to incorporate and oxidize.

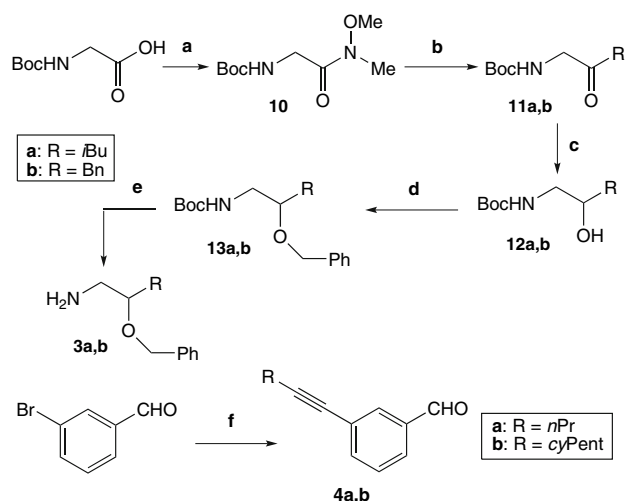
Among the various methods described in the literature for the regioselective *ortho*-formylation of phenols [39,40], we chose the one reported by Skattebøl [41], which had been already employed for the synthesis of aldehyde **6** (the yield was not reported) [42]. In our hands, after optimization, we reached an acceptable 67% yield.

Since the Sonogashira reaction did not work on the free phenol **6** [43], it was protected as the benzyl ether. This benzylation turned out to be less trivial than expected, because of the hindrance around the phenolic group. When we used K₂CO₃ and BnBr in refluxing DMF, the reaction was slow and the yield was only moderate, in line with what reported on the related iodide [44]. After several attempts, we found out that the use of a less coordinating cation (cesium) and MW heating afforded product almost quantitatively, provided that efficient magnetic stirring was used.²

The following Sonogashira coupling to give **8a** turned out to be the most challenging step: it was difficult to drive the reaction to completion, and it led, as side product, to substantial quantities of 2-pent-1-ynylbenzofuran-7-carbaldehyde [43], derived from debenzilation processes.

After a thorough investigation, we found out that best results were achieved with Pd(PPh₃)₂Cl₂ in DMF, with Et₃N as the base, using MW irradiation. The correct combination of solvent and base were particularly crucial. Under these

² Since the system was heterogeneous, insufficient stirring provoked overheating of the solid CsCO₃, leading to brownishing and to diminished yields.

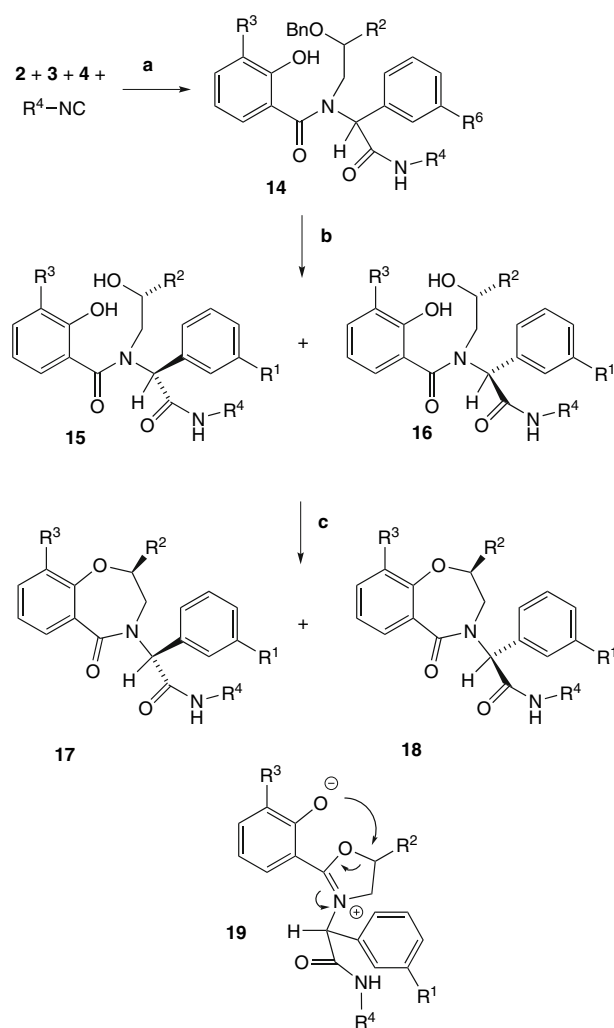


Scheme 3 Synthesis of protected aminoalcohols **3** a: carbonyl diimidazole, THF, Et₃N, MeONHMe·HCl, r.t., 19 h, 93%. b: RMgCl, THF, r.t., 4.5 h. c: NaBH₄, MeOH, 0°C, 99% (**12a**, from **10**), 83% (**12b**, from **10**). d: BnBr, NaH, *n*Bu₄NI, THF, 0°C → r.t., 85% (**13a**), 94% (**13b**). e: 1) 1.1 M HCl in dioxane-H₂O, MW, 80°C, 10 min; 2) basic extraction. f: R-C≡CH, piperidine, CuI, Pd(PPh₃)₂Cl₂, CH₃CN, MW, 90°C, 89% (**4a**), 92% (**4b**)

conditions, after 2 h at 100°C, the reaction was complete and the crude products were clean, although 5.5% of benzofuran was still present.

The synthesis was continued up to **9a** by oxidation. With the use of Jones reagent, the maximum yield was 69%, and the reaction was poorly reproducible. However, excellent results were achieved with NaClO₂ [45]. Moreover, this method involves an easier work-up and avoids the use of Cr(VI). Finally, **9a** was converted into **2a** by hydrogenation. The overall yield from known **6** to **2a** (determined after crystallization) was an excellent 70%. Following the same protocol, we also prepared compound **2b** in 63% overall yield this time bearing a branched alkyl chain.

Alcohols **3** (R⁵ = H) could be obtained in just two steps from aldehydes through hydrocyanation followed by LiAlH₄ reduction. However, in preliminary studies, we found that the use of unprotected aminoalcohols afforded unsatisfactory yields of the Ugi step. Moreover, purification of **3** (R⁵ = H) after LiAlH₄ reaction was troublesome. Therefore, we decided to set up a general synthesis of protected aminoalcohol **3** (R⁵ = benzyl) instead (Scheme 3). This synthesis is also more suitable to be modified for the obtainment of enantiomerically pure products (i.e. through enzymatic resolution of **12** or asymmetric reduction of **11**). The synthesis starts from known Weinreb hydroxamate **10** [46], which was prepared in high yields using carbonyl diimidazole as the coupling agent. In the next C–C forming step, it was necessary to use at least two equivalents of the Grignard reagent because of fast deprotonation of the urethane [46–48]. The reaction turned out to be quite clean in both cases tested (*isobutyl* and *benzyl* Grignards), and the crude ketones **11** were directly reduced



Scheme 4 Synthesis of dihydrobenzoxazepanones **17** and **18** a: MeOH, r.t. b: H₂, Pd-C, 96% EtOH, 0–414 KPa. c: PPh₃, DEAD, 0°C → rt

to their respective alcohols with NaBH₄. Finally, benzylation under a standard conditions [49] afforded **13a,b** in excellent overall yields (84–78%). The free amines **3a,b** were rapidly prepared just before their use, by Boc deprotection. For this step, we preferred a MW procedure that uses HCl instead of the typical CF₃CO₂H mediated urethane cleavage because the resulting amine was cleaner.

Finally, aldehydes **4a,b** were prepared in one step via Sonogashira couplings of alkynes with 3-bromobenzaldehydes. In this case, the reaction worked fine also under standard conditions (piperidine, CH₃CN). The alkynyl group was not hydrogenated since it was expected to be reduced after the Ugi step during the necessary benzyl hydrogenolysis.

Having in hand these three pairs of starting materials, we performed the combinatorial dihydrobenzodiazepinone synthesis of all possible combinations (8 diastereoisomeric pairs = 16 compounds), using *n*-butyl isocyanide (or *n*-pentyl isocyanide in one instance) as the isonitrile component. In addition to these 16 adducts, as control we also prepared two

Table 1 Synthesis of dihydrobenzoxazepinones 17 and 18

Final comp.	R ¹	R ²	R ³	R ⁴	Yield Ugi	Yield Hydrogenolysis		Yield Mitsunobu	
						15	16	17	18
17a–18a	H	<i>i</i> Bu	H	<i>n</i> Bu	80%	90%		49% ^a	
								(d.r.: 58:42)	
17b–18b	<i>n</i> Pent	<i>i</i> Bu	H	<i>n</i> Bu	90%	82%		50% ^a	
								(d.r.: 55:45)	
17c–18c	<i>n</i> Pent	<i>i</i> Bu	<i>n</i> Pent	<i>n</i> Bu	81%	99%		55% ^a	
								(d.r.: 45:55)	
17d–18d	<i>n</i> Pent	<i>i</i> Bu	<i>cy</i> Pent(CH ₂) ₂	<i>n</i> Bu	75%	93%		29% ^a	
								(d.r.: 59:41)	
17e–18e	<i>cy</i> Pent(CH ₂) ₂	<i>i</i> Bu	<i>n</i> Pent	<i>n</i> Bu	70%	94%		24% ^a	
								(d.r.: 42:58)	
17f–18f	<i>cy</i> Pent(CH ₂) ₂	<i>i</i> Bu	<i>cy</i> Pent(CH ₂) ₂	<i>n</i> Bu	85%	36%	39%	33% ^b	46% ^b
17g–18g	<i>n</i> Pent	Bn	<i>n</i> Pent	<i>n</i> Bu	92%	43%	48%	35% ^b	48% ^b
17h–18h	<i>n</i> Pent	Bn	<i>cy</i> Pent(CH ₂) ₂	<i>n</i> Pent	91%	41%	46%	31% ^b	51% ^b
17i–18i	<i>cy</i> Pent(CH ₂) ₂	Bn	<i>n</i> Pent	<i>n</i> Bu	91%	41%	43%	39% ^b	50% ^b
17j–18j	<i>cy</i> Pent(CH ₂) ₂	Bn	<i>cy</i> Pent(CH ₂) ₂	<i>n</i> Bu	90%	33%	38%	24% ^b	42% ^b
17k–18k	<i>n</i> Pent	<i>i</i> Bu	<i>n</i> Pent	<i>t</i> Bu	69%	42%	50%	30% ^b	33% ^b

^a Sum of the isolated yield of the two diastereoisomers, which were separated in all cases^b The reaction was carried out separately on 15 and 16

truncated diastereoisomeric pairs (**17–18a,b**) as well as a pair derived from *t*-butyl isocyanide (**17–18k**) (Scheme 4 and Table 1). The first ones were intended to serve as ‘blanks’, since they lack one or two of the needed appendages, whereas the latter was prepared to detect any influence of the nature of the isocyanide derived substituent.

The synthesis of truncated compounds **17a–18a** and **17b–18b** took place uneventfully in good yields in three steps: (a) Ugi reaction in MeOH at r.t.; (b) hydrogenolysis of the benzyl group; and (c) Mitsunobu cyclization.

It should be noted that in both cases the Ugi reaction gave much better yields than when the corresponding unprotected aminoalcohol was employed [31], confirming the usefulness of the benzyl protection. Hydrogenolysis took place at standard pressure without problems (in the case of **15b–16b**, triple bond saturation occurred faster than benzyl deprotection) and finally, Mitsunobu cyclization under the usual conditions (0°C, 45 min) gave the dihydrobenzoxazepinones in moderate yields. Both possible diastereoisomers have been obtained (they are easily separated by chromatography) due to poor selectivity of the Ugi step.

When we passed to the fully diversified compounds, some important differences were observed. Firstly, hydrogenolysis became much more difficult, and higher pressures and long reaction times were needed in most cases. Secondly, contrary to what happened for **15–16a,b**, the two diastereoisomers were in all cases chromatographically well separated already at this level. However, separation at the level of

17–18 was somewhat less pronounced. For this reason, and also for an easier purification from side-products arising during the Mitsunobu step and eluting close to the products, in some instances, we preferred to separate the two diastereoisomers **15–16** after hydrogenation and to carry out the Mitsunobu reactions separately.

The last difference from the synthesis of truncated analogues was observed during the Mitsunobu cyclization. A careful optimization was carried out on compounds **15–16c**. When typical reaction times (45–60 min at 0°C), substantial amounts of starting diols **15c–16c** were recovered after chromatography, despite of the fact that TLC indicated the reactions were complete. This strange and unpredicted fact made the purification of the final products also troublesome, since, contrary to what happened for the truncated analogues **a** and **b**, the diols **15c–16c** eluted quite close to dihydrobenzoxazepinones **17c–18c**.³ TLC indicated that after 30 min at 0°C there was complete disappearance of starting **15c–16c**, and the formation of **17c–18c** as well as of two more polar spots. However, after quenching with H₂O and stirring for several hours, the substrates **15c–16c** appeared again at the apparent expense of the polar spots. The yield was poor (25–29%).

³ Interestingly, while in all the cases **16c–k** eluted faster than **15c–k**, the order was inverted after cyclization, with **17c–k** eluting faster than **18c–k**. As a consequence, **15** and **17** were separated well enough, but **16** and **18** were rather close. Diastereoisomers with relative configuration **17** were faster eluting than **18** also for truncated compounds, **a** and **b**, as well as for the simpler, previously reported, adducts (ref. 31).

On the other hand, an improved yield and nearly no presence of starting materials, even after quenching and prolonged stirring in the presence of water, could be achieved by letting the reaction proceed for a long time (24 h) at r.t. The yield was improved up to 55%. A possible explanation of this behaviour is that **15c–16c** react fast giving not only the expected **17c–18c**, but also the polar adducts **19c** (diastereoisomeric mixture). Compounds **19c** are then converted, albeit very slowly, into **17c–18c** by prolonged stirring under anhydrous conditions, whereas they are hydrolysed slowly, upon quenching or by interaction with silica, to give back **15c–16c**. A possible chemical formula for compounds **19** is depicted in Scheme 4: they could be zwitterionic isoxazolinium ions formed by cyclization of the alcohol onto the carbonyl of the tertiary amide.

From a practical point of view, long reaction times solved, at least in part, the problem affording nearly complete conversion of **15c–16c** or of **19** into the desired products **17c–18c**, thus avoiding the troublesome separation of these products from the starting material. The yields were in any case lower than those obtained for the truncated analogues, indicating that substitution at position 3 of the salicylic acid has a negative effect, probably for steric reasons. Although the overall yields ranged from 55% (**17c–18c**) to 24% (**17e–18e**), the procedure was in general satisfactory based on both the very short sequence (only 3 steps) and the high yields of the Ugi and hydrogenation steps. Table 1 reports the results.

The relative configuration of the prepared compounds was determined by NMR [31] and chromatographic [50] analogies with the previously characterized simpler analogues (see Table 2). All these data are in perfect accord with the expected preferred conformation [31]. Thus, this indicates that the addition of the substituents does not have a significant conformational impact in these systems.

Having in hand a collection of 22 different compounds, we examined them for their potential binding to Bcl-xL. The binding affinity of these molecules for Bcl-xL was assessed by a fluorescence polarization assay using a fluorescein-labelled 16-mer peptide, corresponding to the BH3 domain of Bak. Displacement of this probe through competitive binding of our compounds with Bcl-xL would lead to a decrease in fluorescence anisotropy which in turn can be related to the known affinity of the 16-mer BH3/Bcl-xL complex. In our tests we always used a 2×10^{-7} M concentration of Bcl-xL and a 2×10^{-8} M concentration of BH3 peptide. We first analysed our compounds at a 2×10^{-6} M concentration. Unfortunately, no significant reduction of fluorescence anisotropy was observed. This result was not completely unexpected since we have anticipated that the lack of the Asp mimetic would have a negative impact on potency, compared to other ligands previously tested. Moreover, the competitive ligand has a strong affinity with Bcl-xL ($K_D = 120$ nM) [35].

Thus, we tried the assays at a higher concentration of **17** and **18** (2×10^{-5} M). However, test compounds tend to precipitate resulting in unreliable fluorescence anisotropy data.

In conclusion, we have demonstrated the feasibility of a short and convergent combinatorial synthesis of benzoxazinones of general formula **1** endowed with four diversity points, also thanks to the development of general and efficient processes for the preparation of 3-substituted salicylic acids **2**, and protected aminoalcohols **3** as inputs. However, preliminary biochemical binding assays have shown no significant activity of the members of this small library towards anti-apoptotic protein Bcl-xL.

Experimental

All reactions in dry solvents were performed under a nitrogen atmosphere (or argon atmosphere when specified). In the case of extractions, the aqueous phase was always re-extracted twice with the indicated organic solvent.

^1H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively. CDCl_3 was used as solvent and TMS as internal standard. Chemical shifts are reported in ppm (δ scale), coupling constants are reported in Hertz. Signal assignments were made with the aid of gCOSY and gHSQC experiments. In AB system, proton A is upfield.

GC-MS was carried out using an HP-1 column (11.85 m long, 0.2 mm wide), electron impact at 70 eV, and a mass temperature of about 170°C. Only $m/z > 33$ were detected. All the analyses were performed (unless otherwise stated) with a constant He flow of 0.9 ml/min with initial temp. of 100°C, init. time 2 min, rate 20°C/min, final temp. 280°C, inj. temp. 250°C, det. temp. 280°C.

HR-MS spectra were recorded on a MicroMass Autospec with electronic impact (EI). TLC analyses were carried out on silica gel plates and developed in this way: (A) U.V. (254 nm) (B) by dipping into a solution of 900 mg of ninhydrin in 300 mL of *n*-butanol and 9 mL of acetic acid and warming. (C) by dipping into a solution of $(\text{NH}_4)_4\text{MoO}_4 \cdot 4\text{H}_2\text{O}$ (21 g) and $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$ (1 g) in H_2SO_4 (31 mL) and H_2O (469 mL) and warming. R_f were measured after an elution of 7–9 cm. Column chromatography was done with the 'flash' methodology using 220–400 mesh silica.

Microwave irradiated reactions (MW) reactions were carried out in a CEM Discover apparatus using the normal mode (not the PowerMAX™ mode!) and air cooling. Unless otherwise stated, the reactions were carried out in closed vessel with an initial power of 50 W.

Hydrogenation was carried out in a Parr apparatus.

3-Bromo-2-hydroxybenzaldehyde **6**

A solution of 2-bromophenol **5** (caution: it has a very persistent smell!!) (8.00 g, 46.24 mmol) in dry acetonitrile (150 mL) was treated in this order with MgCl_2 (6.60 g, 69.36 mmol) (exothermic reaction), paraformaldehyde (8.33 g, 277.4 mmol), and Et_3N (16.1 mL, 115.6 mmol). The mixture was heated to reflux for 6 h. Then, the suspension was poured into an Erlenmeyer flask containing 100 mL of water, 12 mL of 37% HCl, and 50 g of ice. The aqueous phase was saturated with NaCl and extracted with Et_2O . The organic layers were dried (Na_2SO_4), filtered (washing also with some AcOEt) and evaporated to dryness to give an oil. The residue was chromatographed through a silica gel (160 g) column, packed with PE/acetone 90:10 and eluted with PE/acetone 90:10 + 0.5% 96% EtOH to PE/acetone 85:15 + 0.75% 96% EtOH to give pure **6** as a yellowish solid (6.122 g, 67%). Crystallization ($\text{Et}_2\text{O}/\text{ETP}$) afforded an analytical pure sample. M. p. 54.4–54.8 °C (lit.: 54–55 °C) [50]. R_f 0.49 (PE/acetone 80:20, dev. A, C) (R_f of 2-bromophenol: 0.41). ^1H NMR: δ 11.62 [1 H, s, OH]; 9.87 [1 H, s, CH=O]; 7.79 [1 H, dd, H -6, J 1.6, 7.9]; 7.56 [1 H, dd, H -4, J 1.5, 7.8]; 6.96 [1 H, t, H -5, J 7.8]. ^{13}C NMR: δ 196.0 [C=O]; 158.1 [C–O], 140.0, 132.9, 120.8 [CH]; 121.3, 111.2 [quat.]. GC–MS: R_t 3.68; m/z : 202 ($M^+ + 2$, 100.0); 201 (80.1); 200 (99.7); 199 (73.2); 184 (10.8); 182 (10.7); 156 (8.7); 154 (9.0); 145 (15.2); 143 (15.5); 119 (5.8); 92 (30.7); 91 (6.5); 87 (6.4); 86 (6.7); 75 (14.7); 74 (6.1); 53 (12.9); 50 (6.4); 46 (6.5); 39 (22.5); 38 (14.0); 37 (9.0).

3-Bromo-2-benzyloxybenzaldehyde **7**

A solution of bromophenol **6** (1.9027 g, 9.465 mmol) in dry DMF (12 mL) in a round-bottomed flask was treated with Cs_2CO_3 (3.70 g, 11.4 mmol) and benzyl bromide (1.35 mL, 11.4 mmol). The solution was heated in open vessel with MW for 30 min at 110 °C under strong magnetic stirring. The suspension was poured into 50 mL of 5% (NH_4) H_2PO_4 + 1 mL of 36% HCl. Extraction with Et_2O , followed by washing with 0.5 M NaOH and with saturated aqueous NH_4Cl , drying (Na_2SO_4) and evaporation, gave a crude oil, that was chromatographed through silica gel (84 g) with PE/ Et_2O 90:10 to 82:18. Pure **7** (low melting solid) was obtained (2.736 g, 99%). R_f 0.52 (PE/ Et_2O 80:20, dev. A), 0.54 (PE/AcOEt 85:15). ^1H NMR: δ 10.12 [1 H, s, CH=O]; 7.86 [1 H, dd, H -6, J 1.6, 7.6]; 7.78 [1 H, dd, H -4, J 1.6, 7.9]; 7.47–7.36 [5 H, m, benzyl aromatics]; 7.16 [1 H, t, H -5, J 7.8]; 5.14 [2 H, s, CH_2Ph]. ^{13}C NMR: δ 189.0 [C=O]; 158.4 [C-2], 139.5 [C-4]; 135.2 [quat. of benzyl]; 131.6 [C-1]; 129.0, 128.9 (x2), 128.8 (x2) [CH of benzyl]; 127.6 [C-6]; 125.9 [C-5]; 118.5 [C-3]; 77.8 [CH_2Ph]. GC–MS: R_t 7.78; m/z : 292 (0.6); 290 (0.6) (M^+); 263 (3.2); 261 (3.3); 201 (3.1); 199 (3.3); 91 (100.0); 65 (12.2); 63 (5.8); 39 (3.7). I.r.: ν_{max} 3021,

3007, 2873, 1682, 1586, 1436, 1368, 1118, 1069, 961 cm^{-1} . Anal. Calcd for $\text{C}_{14}\text{H}_{11}\text{BrO}_2$: C, 57.76; H, 3.81. Found: C, 57.8; H, 3.85.

2-(Benzyloxy)-3-(pent-1-ynyl)benzaldehyde **8a**

Compound **7** (790.4 mg, 2.71 mmol) was weighed in a 6-mL MW tube, dissolved in dry DMF (3 mL) and treated with CuI (26 mg, 136 μmol), PPh_3 (35.6 mg, 136 μmol), and $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (95.3 mg, 136 μmol). The suspension was flushed with Ar for 5 min, put under an Ar atmosphere, and introduced via syringe Et_3N (1.13 mL, 8.13 mmol) and finally 1-pentyne (401 μL , 4.065 mmol). After about 1 min, the system became a clear solution, but soon afterwards, it turned again into a pink suspension. After 5–10 min at r.t, it was treated at 100 °C for 1.5 h in a MW apparatus (closed vessel). After cooling, the brownish-red suspension was diluted with Et_2O (15 mL) and AcOEt (15 mL) and filtered through a celite cake, washing with Et_2O and AcOEt. The solution was washed with a mixture of 20 mL of saturated NH_4Cl and 20 mL of H_2O . The aqueous phase was re-extracted twice with Et_2O . The united organic phases were dried (Na_2SO_4), evaporated to dryness, and chromatographed through 58 g of silica with PE/ Et_2O 93:7 to 90:10 to give nearly pure **8a** as a yellow oil (661 mg). GC–MS showed a purity of 95%, the main side-product (3.5%) being 2-pent-1-ynylbenzofuran-7-carbaldehyde. Taking into account the purity, the yield was 83%.

R_f 0.60 (PE/AcOEt 85:15, dev. A). ^1H NMR: δ 10.19 [1 H, s, CH=O]; 7.72 [1 H, dd, H -6, J 1.6, 7.6]; 7.67 [1 H, dd, H -4, J 2.0, 7.6]; 7.44–7.32 [5 H, m, benzyl aromatics]; 7.14 [1 H, t, H -5, J 8.1]; 5.32 [2 H, s, CH_2Ph]; 2.45 [2 H, t, $\text{H}_3\text{CCH}_2\text{CH}_2$, J 7.0]; 1.65 [2 H, sextuplet, H_3CCH_2 , J 7.3]; 1.05 [3 H, t, CH_3 , J 7.4]. ^{13}C NMR: δ 189.7 [C=O]; 162.4 [C-2]; 139.9 [C-4]; 136.0 [quat. of benzyl]; 129.7 [C-1]; 128.60 (x2), 128.58, 128.5 (x2) [CH benzyl]; 127.2 [C-6], 124.0 [C-5]; 119.2 [C-3]; 97.1 [C \equiv CAr]; 76.9 [CH_2Ph]; 76.0 [C \equiv CAr]; 22.0 [$\text{H}_3\text{C}-\text{CH}_2$]; 21.7 [$\text{H}_3\text{C}-\text{CH}_2-\text{CH}_2$]; 13.6 [CH_3]. GC–MS: R_t 8.98; m/z : 278 (M^+ , 2.1%); 249 (13.2); 236 (4.4); 235 (3.7); 221 (4.5); 187 (2.1); 158 (2.4); 130 (2.3); 115 (4.2); 91 (100.0); 65 (14.3); 39 (4.5). I.r.: ν_{max} 3013, 2960, 2930, 2866, 2228, 1682, 1575, 1465, 1455, 1436, 1367, 1195, 1073, 969 cm^{-1} . Anal. Calcd for $\text{C}_{19}\text{H}_{18}\text{O}_2$: C, 81.99; H, 6.52. Found: C, 81.6; H, 6.6.

2-(Benzyloxy)-3-(cyclopentylethynyl)benzaldehyde **8b**

It was prepared in 79% yield from cyclopentylacetylene using the same procedure employed for **8a**. This time the chromatographed product was >98% pure as analysed by GC–MS.

R_f 0.32 (PE/ Et_2O 9:1, dev. A, C). ^1H NMR: δ 10.19 [1 H, s, CH=O]; 7.71 [1H, dd, H -6, J 1.8, 7.5]; 7.65 [1 H,

dd, *H*-4, *J* 1.8, 7.5]; 7.46–7.33 [5 H, m, benzyl *CH*]; 7.13 [1 H, dt, *H*-5, *J*_t 7.8, *J*_d 0.9]; 5.32 [2 H, s, *CH*₂Ph]; 2.89 [1 H, quint., *CH* cyclopentyl, *J* 6.9]; 2.10–1.94 [2 H, m]; 1.84–1.56 [6 H, m]. ¹³C NMR: δ 189.8 [*CH* = O]; 162.3 [*C*-2]; 139.8 [*C*-4]; 136.1 [quat. of benzyl]; 129.7 [*C*-1]; 128.61 (x2), 128.56, 128.45 (x2) [*CH* of benzyl]; 127.2 [*C*-6]; 123.9 [*C*-5]; 119.2 [*C*-3]; 101.3, 75.4 [*C* ≡ *C*]; 76.7 [*CH*₂Ph]; 33.7, 25.1 [cyclopentyl *CH*₂]; 31.0 [*CH*]. GC–MS: *R*_f 10.06; *m/z* 304 (*M*⁺, 1.0%); 275 (3.0); 262 (1.4); 236 (4.4); 235 (4.2); 213 (1.9); 171 (1.9); 128 (2.0); 115 (2.6); 91 (100.0); 89 (2.2); 65 (7.4); 63 (1.9). I.r.: *ν*_{max} 3030, 2940, 2850, 1660, 1610, 1430, 1155, 905 cm⁻¹. Anal. Calcd for C₂₁H₂₀O₂: 82.86; H, 6.62. Found: C, 82.7; H, 6.7.

2-(Benzyloxy)-3-(pent-1-ynyl)benzoic acid **9a**

Aldehyde **8a** (1.154 g, 4.146 mmol, 95% pure) was dissolved in CH₃CN (16 mL), and treated with *t*-BuOH (16 mL), dimethoxyethane (4 mL), 2-methyl-2-butene (4 mL) and NaH₂PO₄ monohydrate (2.86 g, 20.73 mmol). The resulting suspension was cooled to 0°C and treated with a solution of NaClO₂ (1.125 g, 12.44 mmol) in water (7 mL). After 15 min, the cooling bath was removed, and the biphasic system was vigorously stirred for further 45 min at r.t. Finally, it was poured into 60 mL of saturated aqueous NaCl and extracted three times with PE/AcOEt 1:1 (120 mL + 50 mL + 50 mL). The organic phases were dried (Na₂SO₄), evaporated to dryness and immediately chromatographed through 45 g of silica with PE/AcOEt 70:3 + 1% AcOH to give pure **9a** as a yellow oil of 95% purity (GC–MS) (1.212 g, 100%) (the main side-product (3.5%) was 2-pent-1-ynylbenzofuran-7-carboxylic acid). *R*_f 0.57 (PE/AcOEt 60:40 + 2% AcOH, dev. A). ¹H NMR: δ 11.0 [1 H, very broad s, *OH*]; 8.05 [1 H, dd, *H*-6, *J* 1.6, 8.0]; 7.66 [1 H, dd, *H*-4, *J* 1.6, 7.7]; 7.51–7.38 [5 H, m, benzyl aromatics]; 7.21 [1 H, t, *H*-5, *J* 8.1]; 5.41 [2 H, s, *CH*₂Ph]; 2.47 [2 H, t, H₃CCH₂CH₂, *J* 6.9]; 1.66 [2 H, sextuplet, H₃CCH₂, *J* 7.3]; 1.05 [3 H, t, *CH*₃, *J* 7.5]. ¹³C NMR: δ 165.5 [*C*=O]; 158.2 [*C*-2]; 139.2 [*C*-4]; 134.7 [quat. of benzyl]; 132.2 [*C*-6]; 129.2, 128.9 (x2), 128.8 (x2) [*CH* benzyl]; 124.8 [*C*-5]; 122.7 [*C*-1]; 118.7 [*C*-3]; 97.8 [*C* ≡ *C*Ar]; 77.3 [*CH*₂Ph]; 75.7 [*C* ≡ *C*Ar]; 22.0 [H₃C-CH₂]; 21.7 [H₃C-CH₂ - CH₂]; 13.7 [*CH*₃]. GC–MS: *R*_f 9.81; *m/z*: 294 (*M*⁺, 10.7%); 251 (1.5); 248 (3.1); 247 (5.5); 158 (1.6); 115 (1.6); 91 (100.0); 77 (1.8); 65 (6.0); 63 (1.7); 51 (1.6); 39 (1.6). I.r.: *ν*_{max} 3212, 3003, 2958, 2930, 2871, 2226, 1741, 1578, 1455, 1430, 1378, 1230, 1197, 1130, 1076, 943, 911 cm⁻¹. Anal. Calcd for C₁₉H₁₈O₃: 77.53; H, 6.16. Found: C, 77.3; H, 6.1.

2-(Benzyloxy)-3-(cyclopentylethynyl)benzoic acid **9b**

It was prepared as a pale pink, low melting, solid in 98% yield from **8b** using the same procedure employed for **9a**.

*R*_f 0.57 (PE/AcOEt 7:3 + 2% AcOH, dev. A, C). ¹H NMR (*OH* is very broad and, therefore, non-visible): δ 8.04 [1 H, dd, *H*-6, *J* 1.9, 8.0]; 7.64 [1 H, dd, *H*-4, *J* 1.8, 7.5]; 7.51–7.46 [2 H, m]; 7.44–7.3.7 [3 H, m]; 7.20 [1 H, t, *H*-5, *J* 7.8]; 5.42 [2 H, s, *CH*₂Ph]; 2.90 [1 H, quint., cyclopentyl *CH*, *J* 7.2]; 2.10–1.94 [2 H, m]; 1.86–1.58 [6 H, m]. ¹³C NMR: δ 166.3 [*C* = O]; 158.2 [*C*-2]; 139.0 [*C*-4]; 135.0 [benzyl quat.]; 131.9 [*C*-6]; 129.0, 128.7 (x4) [benzyl *CH*]; 124.5 [*C*-5]; 122.9 [*C*-3]; 119.0 [*C*-1]; 101.8, 75.1 [*C* ≡ *C*]; 76.9 [*CH*₂Ph]; 33.6, 25.0 [cyclopentyl *CH*₂]; 30.9 [cyclopentyl *CH*]. I.r.: *ν*_{max} 3212, 3000, 2957, 2869, 2223, 1738, 1578, 1376, 1186, 1028, 922 cm⁻¹. Anal. Calcd for C₂₁H₂₀O₃: 78.73; H, 6.29. Found: C, 78.6; H, 6.35.

2-(Hydroxy)-3-(pent-1-yl)benzoic acid **2a**

A solution of **9a** (mg 958, 3.25 mmol, 95% purity) in 96% EtOH (20 mL) was hydrogenated over 10% Pd-C (100 mg) at r.t. under the slight overpressure generated by an inflated balloon. After 5 h, the reaction was complete. After filtration of the catalyst and evaporation to dryness, the residue (665 mg) was purified by trituration (CH₂Cl₂/*n*-pentane) to give pure **2a** as a white solid (545 mg, 85% taking into account the purity of the starting material). *M.p.*: 104.2–104.7°C. *R*_f 0.52 (PE / AcOEt 60:40 + 2% AcOH, dev.: A). ¹H NMR: δ 12.0 [1 H, very broad s, carboxylic *OH*]; 10.65 [1 H, s, phenolic *OH*]; 7.79 [1 H, dd, *H*-6, *J* 1.6, 8.0]; 7.38 [1 H, dd, *H*-4, *J* 1.5, 7.5]; 6.86 [1 H, t, *H*-5, *J* 7.6]; 2.66 [2 H, t, ArCH₂, *J* 7.8]; 1.62 (mc) [2 H, m, *CH*₂]; 1.44–1.28 [4 H, m, *CH*₂]; 0.90 [3 H, t, *CH*₃, *J* 6.8]. ¹³C NMR: δ 175.1 [*C*=O]; 160.5 [*C*-2]; 137.0 [*C*-4]; 131.5 [*C*-3]; 128.4 [*C*-6]; 118.9 [*C*-5]; 110.6 [*C*-1]; 31.7, 29.6, 29.1, 22.6 [*CH*₂]; 14.1 [*CH*₃]. GC–MS: *R*_f 6.46; *m/z*: 208 (*M*⁺, 37.2); 190 (18.2); 175 (5.7); 173 (9.3); 162 (41.1); 161 (11.0); 151 (8.0); 148 (9.2); 147 (28.7); 134 (82.7); 133 (100.0); 119 (8.6); 106 (27.0); 105 (20.3); 91 (8.0); 77 (26.4); 65 (7.1); 51 (13.0); 41 (7.3); 39 (7.4). I.r.: *ν*_{max} 3080, 3016, 2926, 2856, 1655, 1612, 1444, 1291, 1191, 1130 cm⁻¹. Anal. Calcd for C₁₂H₁₆O₃: 69.21; H, 7.74. Found: C, 69.3; H, 7.8.

2-(Hydroxy)-3-(cyclopentylethyl)benzoic acid **2b**

It was prepared in 82% yield from **9b** using the same procedure employed for **2a**.

Mp: 139.4–139.9°C. *R*_f 0.56 (PE/AcOEt 1:1 + 2% AcOH, dev. A, C). ¹H NMR (carboxylic *OH* is very broad and, therefore, non-visible): δ 10.63 [1 H, s, phenolic *OH*]; 7.77 [1 H, dd, *H*-6, *J* 1.8, 8.1]; 7.38 [1 H, dd, *H*-4, *J* 0.6, 7.5]; 6.84 [1 H, t, *H*-5, *J* 7.6]; 2.68 [2 H, t, ArCH₂, *J* 7.9]; 1.90–1.70 [3 H, m]; 1.70–1.44 [6 H, m]; 1.25–1.07 [2 H, m]. ¹³C NMR: δ 175.4 [*C*=O]; 160.4 [*C*-2]; 136.9 [*C*-4]; 131.7 [*C*-3]; 128.4 [*C*-6]; 119.0 [*C*-5]; 110.7 [*C*-1]; 39.9 [*CH*]; 36.0 [*CH*₂CH₂Ar];

32.7, 25.3 [cyclopentyl CH_2]; 28.9 [ArCH_2]. Anal. Calcd for $\text{C}_{14}\text{H}_{18}\text{O}_3$: C, 71.77; H, 7.74; Found: C, 71.8; H, 7.7.

N-Methoxy- *N*-methyl-2-[(*tert*-Butoxycarbonyl)amino]acetamide **10**

A solution of *N*-(Boc)glycine (6.726 g, 38.39 mmol) in dry THF (50 mL) was cooled to 0°C and treated, in six portions, with 1,1'-carbonyldiimidazole (7.47 g, 46.1 mmol). After stirring for 15 min at 0°C and 60 min at r.t., *N*, *O*-dimethylhydroxylamine hydrochloride (4.497 g, 46.1 mmol) was added, followed by Et_3N (6.96 mL, 49.91 mmol). The suspension was stirred for 19 h at r.t. Then, it was filtered and the filtrate evaporated to dryness. It was taken up in AcOEt, and washed with a mixture of 5% aqueous $(\text{NH}_4)_2\text{HPO}_4$ (30 mL) + saturated aq. NaCl (30 mL) + 37% HCl (7 mL). The resulting pH of the aqueous layer was 5. The organic layer was washed with saturated aqueous NaHCO_3 , dried (Na_2SO_4) and evaporated to dryness to give a solid (8.50 g). Trituration ($\text{Et}_2\text{O}/\text{PE}$) afforded a white solid (7.794 g, 93%). M.p.: 99.2 – 100.1°C . Lit.: 99 – 100°C [46] or 100 – 101°C [51]. R_f 0.33 (PE/AcOEt 1:1, dev.: B).

tert-Butyl 2-hydroxy-4-methylpentylcarbamate **12a**

A solution of hydroxamate **2** (2.014 g, 9.23 mmol) in dry THF (15 mL) was cooled to -15°C (compound **10** tends to precipitate) and treated, during 4 min, with a 2 M solution of *iso*-butyl magnesium chloride in THF (13.8 mL, 27.7 mmol). After stirring for 10 min at -15°C , 15 min at 0°C , and finally 4.5 h at r.t., the mixture was poured into saturated aqueous NH_4Cl (50 mL) and treated with 1 M aqueous HCl (30 mL). Extraction with Et_2O , drying (Na_2SO_4) and evaporation to dryness afforded crude ketone **11a** as an oil (1.975 g). R_f 0.63 (PE/AcOEt 2:1, dev.: B). It was taken up in dry MeOH (25 mL), cooled to 0°C and treated with NaBH_4 (688 mg, 18.2 mmol). After stirring for 75 min at 0°C , the mixture was poured into a mixture of 5% aqueous $(\text{NH}_4)_2\text{HPO}_4$ (50 mL) + 37% HCl (1.5 mL). The system was saturated with NaCl and extracted with Et_2O . The organic layers were washed with saturated aqueous NaCl, dried (Na_2SO_4) and evaporated to dryness. The crude product (containing also some droplets of water) was directly chromatographed through a silica gel (42 g) column, packed with PE/AcOEt 70:30 and eluted with PE/AcOEt 70:30 to 65:35 + 1% of 96% EtOH to give pure **12a** as a white, low melting, solid (1.983 g, 99% from **10**).

R_f 0.42 (PE/AcOEt 2:1, dev.: B). ^1H NMR: δ 4.98 [1 H, broad s, *NH*]; 3.83–3.71 [1 H, m (mc: 3.77), *CHOH*]; 3.29 [1 H, ddd, *CHHN*, *J* 2.7, 6.6, 14.1]; 2.98 [1 H, ddd, *CHHN*, *J* 5.4, 7.5, 14.1]; 2.18 [1 H, broad s, *OH*]; 1.85–1.68 [1 H, m (mc: 1.78), *CH(CH}_3)_2*]; 1.45 [9 H, s, $(\text{CH}_3)_3\text{CH}$]; 1.39 [1 H, ddd, *CHH-iPr*, *J* 5.7, 8.7, 13.5]; 1.21 [1 H, ddd, *CHH-iPr*, *J* 4.5, 8.4, 13.5]; 0.94 & 0.91 [6 H, 2 d, $(\text{CH}_3)_2\text{CH}$,

J 6.5]. ^{13}C NMR: δ 156.8 [*C* = *O*]; 79.6 [$\text{C}(\text{CH}_3)_3$]; 69.7 [*CHOH*]; 47.1 [CH_2N]; 43.8 [CH_2 – *iPr*]; 28.4 [$\text{C}(\text{CH}_3)_3$]; 24.4 [$\text{CH}(\text{CH}_3)_2$], 23.3, 22.1 [$(\text{CH}_3)_2\text{CH}$]. GC–MS: R_t 5.16; *m/z*: 161 (M^+ – 56, 6.2); 144 (6.4); 131 (7.4); 104 (10.0); 83 (6.6); 75 (99.2); 74 (9.9); 69 (14.4); 57 (100.0); 56 (8.5); 55 (8.1); 45 (6.7); 43 (20.7); 41 (36.3); 39 (8.9). I.r.: ν_{max} 3453, 2953, 2865, 1701, 1496, 1367, 1158, 1106, 781 cm^{-1} . Anal. Calcd for $\text{C}_{11}\text{H}_{23}\text{NO}_3$: C, 60.80; H, 10.67; N, 6.45. Found: C, 61.0; H, 10.5; N, 6.3.

tert-Butyl 2-hydroxy-3-phenylpropylcarbamate **12b**

It was prepared as an oil in 83% overall yield from **10** and benzyl magnesium chloride following the same procedure used for **12a**.

R_f 0.61 (PE/AcOEt 1:1, dev. A, B). ^1H NMR: δ 7.39–7.18 [5 H, m]; 4.96 [1 H, broad s, *NH*]; 4.00–3.86 [1 H, *CHOH*]; 3.37 [1 H, ddd, *CHHNH*, *J* 2.7, 6.6, 14.1]; 3.06 [1 H, ddd, *CHHNH*, *J* 5.1, 7.2, 13.8]; 2.80, 2.72 [2 H, AB part of an ABX syst., CH_2Ph , J_{AB} 14.0 J_{AX} 8.0, J_{BX} 4.9]; 2.51 [1 H, broad s, *OH*]; 1.45 [9 H, s, $(\text{CH}_3)_3\text{CH}$]. ^{13}C NMR: δ 156.8 [*C*=*O*]; 137.7 [quat.]; 129.3 (x2), 128.7 (x2), 126.6 [benzyl *CH*]; 79.7 [$\text{C}(\text{CH}_3)_3$]; 72.4 [*CHOH*]; 45.9 [NHCH_2]; 41.3 [CH_2Ph]; 28.4 [$(\text{CH}_3)_3\text{CH}$]. GC–MS: R_t 10.3 (the analysis was performed initial temp. of 70°C , init. time 2 min, rate $15^\circ\text{C}/\text{min}$, inj. temp. 250°C); *m/z*: 195 (M^+ – 56, 0.2%); 177 (M^+ – 74, 13.3); 160 (12.8); 121 (11.6); 116 (28.8); 104 (80.7); 103 (10.9); 92 (20.4); 91 (36.6); 76 (17.0); 75 (43.1); 74 (8.6); 65 (7.9); 60 (23.7); 59 (17.4); 57 (100.0); 41 (20.8); 39 (6.6). I.r.: ν_{max} 3452, 2970, 2930, 1698, 1493, 1367, 1188, 1083 cm^{-1} . Anal. Calcd for $\text{C}_{14}\text{H}_{21}\text{NO}_3$: C, 66.91; H, 8.42; N, 5.57. Found: C, 66.9; H, 8.4; N, 5.5.

tert-Butyl 2-benzyloxy-4-methylpentylcarbamate **13a**

A solution of alcohol **12a** (1.655 g, 7.62 mmol) in dry THF (20 mL) was cooled to 0°C and treated with 60% NaH in mineral oil (320 mg, 8.00 mmol). After 5 min, benzyl bromide (0.99 mL, 8.38 mmol) was added, followed by tetra-*n*-butylammonium iodide (282 mg, 0.762 mmol). After stirring at 0°C for 50 min and at r.t. for 3.5 h, the suspension was poured into saturated aqueous NH_4Cl and extracted with Et_2O . After drying (Na_2SO_4), the organic layer was evaporated and immediately chromatographed through a silica gel (50 g) column, packed with PE/AcOEt 90:10 and eluted with PE/AcOEt 90:10 to 87:13 to give pure **13a** as a colourless oil (1.997 g, 85%). R_f 0.50 (PE/AcOEt 7:1, dev. A (weak) or B). ^1H NMR: δ 7.34 [5 H, s, aromatics]; 4.80 [1 H, broad s, *NH*]; 4.54 [2 H, s, CH_2Ph]; 3.61–3.43 [1 H, *CHOBN*]; 3.39 [1 H, dt, *CHHNH*, J_d 14.1, J_t 4.8]; 3.12 [1 H, dt, *CHHNH*, J_d 14.1, J_t 5.7]; 1.75 [1 H, heptuplet, $\text{CH}(\text{CH}_3)_2$, *J* 6.6]; 1.52 [1 H, dt, *CHH-iPr*, J_d 7.0, J_t 14.1]; 1.25 [1 H, ddd, *CHH-iPr*, *J* 14.1, 7.5, 5.9]; 0.91 [3 H, d, CH_3 , *J* 6.6]; 0.88 [3 H, d,

CH_3 , J 6.6]. ^{13}C NMR: δ 156.1 [C=O]; 138.4 [quat.]; 128.4 (x2), 127.8 (x2), 127.7 [aromatic CH]; 79.2 [C(CH₃)₃]; 76.3 [CH₂Ph]; 71.2 [CHOBn]; 43.3 [CH₂NH]; 41.3 [CH₂—*i*Pr]; 28.4 [C(CH₃)₃]; 24.5 [CH(CH₃)₂]; 23.0, 22.7 [(CH₃)₂CH]. GC–MS: R_t 7.71; m/z : 251 (M^+ —56, 0.9%); 250 (M^+ —57, 0.8); 234 (0.6); 206 (1.7); 177 (2.4); 145 (3.9); 144 (2.3); 104 (3.8); 100 (2.8); 91 (100.0); 85 (4.0); 65 (5.9); 59 (4.9); 57 (57.9); 41 (9.1); 39 (3.0). I.r.: ν_{max} 3447, 3039, 2953, 2865, 1704, 1492, 1367, 1158, 1090 cm^{-1} . Anal. Calcd for C₁₈H₂₉NO₃: C, 70.32; H, 9.51; N, 4.56. Found: C, 70.15; H, 9.6; N, 4.5.

tert-Butyl 2-benzyloxy-3-phenylpropylcarbamate **13b**

It was prepared as a yellow oil in 94% yield from **12b** following the same procedure used for **13a**.

R_f 0.48 (PE/AcOEt 8:2, dev. A, B). ^1H NMR: δ 7.36–7.17 [10 H, m]; 4.82 [1 H, broad t, NH]; 4.48, 4.44 [2 H, AB syst., OCH₂Ph]; 3.78–3.62 [1 H, m, CHOBn]; 3.36 [1 H, ddd, CHHNH, J 3.9, 5.7, 13.8]; 3.12 [1 H, dt, CHHNH, J_t 6.0, J_d 14.1]; 2.89, 2.79 [2 H, AB part of an ABX syst., CH₂Ph, J_{AB} 13.8, J_{AX} 6.0, J_{BX} 6.6]; 1.43 [9 H, s, (CH₃)₃C]. ^{13}C NMR: 156.0 [C = O]; 138.1, 138.0 [quat.]; 129.5 (x2), 128.4 (x4), 127.8 (x2), 127.7, 126.4 [aromatic CH]; 79.24 [C(CH₃)₃]; 79.15 [OCH₂Ph]; 71.8 [CHOBn]; 43.2 [CH₂N]; 38.7 [CH₂Ph]; 28.4 [(CH₃)₃C]. GC–MS: R_t 13.40 (the analysis was performed at initial temp. of 70°C, init. time 2 min, rate 15°C/min, inj. temp. 250°C); m/z : 284 (M^+ —57, 0.5%); 240 (M^+ —101, 0.8); 194 (10.3); 177 (5.8); 118 (4.0); 104 (2.6); 91 (100.0); 65 (6.2); 57 (38.4); 41 (5.6). I.r.: ν_{max} 3452, 2969, 1703, 1599, 1491, 1391, 1367, 1192, 1084, 919 cm^{-1} . Anal. Calcd for C₂₁H₂₇NO₃: C, 73.87; H, 7.97; N, 4.10. Found: C, 73.6; H, 8.0; N, 4.05.

3-(Pent-1-ynyl)benzaldehyde **4a**

A solution of 3-bromobenzaldehyde (1.50 mL, 2.381 g, 12.87 mmol) in dry acetonitrile (5 mL) in a pear-shaped flask was treated with CuI (125 mg, 0.656 mmol) and Pd(PPh₃)₂Cl₂ (451 mg). The resulting suspension was put under argon with a series of vacuum/argon cycles. Then, piperidine (3.81 mL, 38.6 mmol) was added (exothermic reaction). The suspension became suddenly a brown-red solution. Finally, 1-pentyne (1.775 mL, 18.00 mmol) was added. The solution became suddenly yellow, but then in a few minutes, it became darker. It was rapidly transferred and evenly divided, via syringe, into two 6-mL MW tubes. They were capped and separately treated at 90°C for 30 min in the MW apparatus. At the end, the contents of the two tubes were united and treated with saturated aqueous NH₄Cl (50 mL), 1 M HCl (35 mL) and Et₂O (35 mL). The mixture was carefully acidified with 37% HCl to pH 6. This could be easily done since, at pH >7, the aqueous phase was blue, whereas at pH <5, it was yellow. The phases were

separated and the dark organic phases were washed with a mixture of saturated NaCl (40 mL) and 5% (NH₄)₂HPO₄ (30 mL). Drying over Na₂SO₄, evaporation, and chromatography through a silica gel (70 g) column, packed with PE/Et₂O 97:3 and eluted with PE/Et₂O 97:3 to 93:7 afforded pure aldehyde **4a** as a yellow liquid (1.970 g, 89%). R_f 0.38 (PE/Et₂O 95:5, dev. A) (R_f of *m*-bromobenzaldehyde: 0.32). ^1H NMR: δ 9.98 [1 H, s, CH=O]; 7.89 [1 H, t, *H*-2, J 1.5]; 7.78 [1 H dt, *H*-6, J_d 7.5, J_t 1.5]; 7.63 [1 H, dt, *H*-4, J_d 7.8, J_t 1.5]; 7.45 [1 H, t, *H*-5, J 7.6]; 2.41 [2 H, t, C≡C—CH₂, J 6.9]; 1.64 [2 H, sextuplet, CH₂CH₂CH₃, J 7.2]; 1.06 [3 H, t, CH₃, J 7.5]. ^{13}C NMR: 191.8 [CH=O], 137.2, 133.0, 128.9, 128.2 [aromatic CH], 136.3, 125.3 [aromatic quat.]; 92.2, 79.4 [C≡C]; 22.1, 21.4 [CH₂]; 13.6 [CH₃]. GC–MS: R_t 5.34 min; m/z : 172 (M^+ , 69.9); 157 (3.9); 153 (4.9); 143 (100.0); 142 (8.1); 141 (7.6); 129 (60.4); 128 (63.1); 127 (16.3); 115 (41.2); 114 (18.1); 113 (10.2); 101 (6.0); 89 (15.7); 88 (9.8); 87 (7.4); 77 (8.8); 75 (10.4); 74 (7.8); 65 (10.8); 63 (23.4); 62 (9.1); 51 (11.5); 39 (12.8). I.r.: ν_{max} 3030, 2955, 2928, 2868, 2830, 2726, 2228, 1694, 1596, 1574, 1422, 1379, 1328, 1274, 1190, 1156, 1103, 895 cm^{-1} . Anal. Calcd for C₁₂H₁₂O: C, 83.69; H, 7.02. Found: C, 82.9; H, 6.85.

3-(Cyclopentylethynyl)benzaldehyde **4b**

It was prepared as a yellow liquid in 92% yield from cyclopentylacetylene following the same procedure employed for **4a**.

R_f 0.53 (PE/Et₂O 9:1, dev. A). ^1H NMR: δ 9.97 [1 H, s, CH=O]; 7.88 [1 H, t, *H*-2, J 1.4]; 7.76 [1 H, dt, *H*-6, J_d 7.8, J_t 1.4]; 7.62 [1 H, dt, *H*-4, J_d 7.8, J_t 1.5]; 7.44 [1 H, t, *H*-5, J 7.7]; 2.84 [1 H, quint., CH, J 7.5]; 2.07–1.94 [2 H, m]; 1.85–1.56 [6 H, m]. ^{13}C NMR: δ 191.7 [C=O]; 137.0, 132.8, 128.8, 128.0 [aromatic CH]; 136.2, 125.3 [quat.]; 96.4, 78.7 [C≡C]; 33.7, 25.0 [CH₂]; 30.6 [CH]. Anal. Calcd for C₁₄H₁₄O: C, 84.81; H, 7.12. Found: C, 84.4; H, 7.0.

(*R**,*S**) and (*R**,*R**) 3,4-Dihydro-4-(1-((butylamino) carbonyl)-1-phenylmethyl)-2-isobutylbenzo[*f*][1,4]oxazepin-5(2H)-ones, **17a** and **18a**

A solution of Boc derivative **13a** (409.8 mg, 1.333 mmol) was dissolved in 1,4-dioxane (3 mL) and treated with 37% aqueous HCl (300 μL). The solution was heated at 80°C in a microwave apparatus (open vessel) for 10 min. Then, it was poured into saturated aqueous NaCl (30 mL) + 1 M NaOH (8 mL). The mixture was extracted with Et₂O, washed with saturated aqueous NaCl and evaporated to dryness at the rotatory evaporator, avoiding to strip the solvent too much. The residue was taken up in dry MeOH (2.5 mL), and treated with benzaldehyde (118 μL , 1.17 mmol) and with freshly activate powdered 3 Å mol. sieves (227 mg). After stirring for 30 min, salicylic acid (153 mg, 1.11 mmol) was added, followed by

n-butyl isocyanide (139 μ L, 1.33 mmol). The suspension was stirred for 25 h at r.t. Then, it was diluted with CH_2Cl_2 , filtered and evaporated to dryness. Chromatography on silica gel (40 g) with PE/acetone ratio from 80:20 to 72:38 afforded the inseparable diastereoisomeric mixture of Ugi adducts **14a** as a white foam (457 mg). This foam was taken up in 96% EtOH (10 mL), treated with 10% Pd-C (93 mg) and hydrogenated at room temperature, under the slight overpressure given by an inflated balloon, for 24 h. After filtration and evaporation, the residue was taken up twice with CH_2Cl_2 / benzene and evaporated again. Finally, the residue was taken up in dry CH_2Cl_2 (10 mL), cooled to 0°C and treated with PPh_3 (304 mg, 1.16 mmol) and DEAD (183 μ L, 1.16 mmol). After 45 min, the reaction was quenched with H_2O (150 μ L), and stirred for 20 min at r.t. Benzene (10 mL) was added, and the mixture evaporated to dryness. Chromatography through silica gel (40 g) (PE/acetone ratio from 8:2 to 76:24) afforded pure **17a** (108.7 mg) and **18a** (89.0 mg) as oils. Overall yield from salicylic acid: 44%.

17a: R_f 0.60 (PE/AcOEt 60:40, dev.: A, C). ^1H NMR: δ 7.80 [1 H, dd, *H*-7, *J* 1.8, 7.8]; 7.42 [1 H, ddd, *H*-9, *J* 1.5, 7.2, 7.8]; 7.38–7.31 [5 H, m, phenyl CH]; 7.16 [1 H, dt, *H*-8, *J*_d 1.2, *J*_t 7.5]; 6.96 [1 H, dd, *H*-10, *J* 1.2, 8.2]; 6.57 [1 H, broad t, NH]; 6.52 [1 H, s, CH-Ph]; 4.74 [1 H, tt, *H*-2, *J* 3.9, 9.3]; 3.49 [1 H, dd, *H*-3, *J* 3.4, 15.6]; 3.32 [2 H, q, CH_2NH , *J* 6.6]; 3.06 [1 H, dd, *H*-3, *J* 9.3, 15.6]; 1.91–1.77 [1 H, m, $\text{CH}(\text{CH}_3)_2$]; 1.56–1.25 [5 H, m]; 0.99–0.91 [1 H, m, $\text{CHH-}i\text{Pr}$]; 0.93 & 0.92 [2 x 3 H, 2 d, $(\text{CH}_3)_2\text{CH}$, *J* 6.9 and 6.3]; 0.90 [3 H, t, CH_3CH_2 , *J* 7.2]. ^{13}C NMR: δ 169.9, 169.1 [C=O]; 153.0 [C-11]; 135.6, 127.6 [quat. aromatics]; 132.8 [C-9]; 130.9 [C-7]; 128.9 (x2), 128.7 (x2), 128.3 [phenyl CH]; 123.5 [C-8]; 122.8 [C-9]; 82.4 [C-2]; 60.6 [CHPh]; 48.3 [C-3]; 40.7 [$\text{CH}_2 - i\text{Pr}$]; 39.5 [CH_2N]; 31.4 [$\text{CH}_2\text{CH}_2\text{N}$]; 24.5 [$\text{CH}(\text{CH}_3)_2$]; 23.2, 22.0 [$(\text{CH}_3)_2\text{CH}$]; 20.0 [CH_2CH_3]; 13.7 [CH_2CH_3]. HRMS: m/z 408.2422 (EI) (M^+ . $\text{C}_{25}\text{H}_{32}\text{N}_2\text{O}_3$ requires 408.2413).

18a: R_f 0.40 (PE/AcOEt 60:40, dev.: A, C). ^1H NMR: δ 7.77 [1 H, dd, *H*-7, *J* 1.7, 7.6]; 7.57–7.50 [2 H, m, phenyl CH]; 7.46–7.35 [4 H, m, phenyl CH and *H*-9]; 7.17 [1 H, dt, *H*-8, *J*_d 1.2, *J*_t 7.6]; 6.88 [1 H, dd, *H*-10, *J* 1.2, 7.9]; 6.40 [1 H, s, CH-Ph]; 6.05 [1 H, broad t, NH]; 3.62–3.50 [2 H, m, *H*-2 and 1 *H*-3]; 3.45–3.19 [3 H, m, 1 *H*-3 and CH_2NH]; 1.61–1.44 [3 H, m, $\text{CH}(\text{CH}_3)_2 + \text{CH}_2\text{CH}_2\text{N}$]; 1.40–1.25 [3 H, m, $\text{CHH-}i\text{Pr} + \text{CH}_2\text{CH}_3$]; 0.91 [3 H, t, CH_3CH_2 , *J* 6.9]; 0.92–0.77 [1 H, m, $\text{CHH-}i\text{Pr}$]; 0.66 & 0.65 [2 x 3 H, 2 d, $(\text{CH}_3)_2\text{CH}$, *J* 6.9]. ^{13}C NMR: δ 169.6, 169.4 [C=O]; 152.2 [C-11]; 135.2, 128.4 [quat. aromatics]; 132.8 [C-9]; 131.0 [C-7]; 129.5 (x2), 129.0 (x2), 128.8 [phenyl CH]; 123.9 [C-8]; 123.2 [C-9]; 83.0 [C-2]; 60.2 [CHPh]; 48.0 [C-3]; 40.4 [$\text{CH}_2 - i\text{Pr}$]; 39.4 [CH_2N]; 31.4 [$\text{CH}_2\text{CH}_2\text{N}$]; 24.3 [$\text{CH}(\text{CH}_3)_2$]; 22.5, 22.1 [$(\text{CH}_3)_2\text{CH}$]; 20.0 [CH_2CH_3]; 13.7 [CH_2CH_3].

HRMS: m/z 408.2431 (EI) (M^+ . $\text{C}_{25}\text{H}_{32}\text{N}_2\text{O}_3$ requires 408.2413).

(R^*,S^*) and (R^*,R^*) 3,4-Dihydro-4-(1-((butylamino) carbonyl)-1-[[3-(pent-1-yl)phenyl]methyl]-2-isobutylbenzo[*f*][1,4]oxazepin-5(2H)-ones **17b** and **18b**

They were prepared starting from **13a–4a**, salicylic acid and *n*-butyl isocyanide following the same procedure used for **17a–18a**. The overall yield from salicylic acid was 53%. Diast. ratio **17b**:**18b**: 58:42.

17b: R_f 0.49 (PE/AcOEt 75:25, dev.: A, C). ^1H NMR: δ 7.82 [1 H, dd, *H*-7, *J* 1.7, 7.7]; 7.41 [1 H, dt, *H*-9, *J*_d 1.7, *J*_t 7.7]; 7.27 [1 H, t, *H*-5', *J* 8.1]; 7.22–7.11 [4 H, m, *H*-8, *H*-2', *H*-4', *H*-6']; 6.96 [1 H, dd, *H*-10, *J* 1.2, 8.1]; 6.46 [1 H, s, CH-Ar]; 6.33 [1 H, broad t, NH, *J* 5.4]; 4.74 [1 H, tt, *H*-2, *J* 3.9, 9.0]; 3.46 [1 H, dd, *H*-3, *J* 3.4, 15.5]; 3.40–3.25 [2 H, m, CH_2NH]; 3.05 [1 H, dd, *H*-3, *J* 9.0, 15.5]; 2.58 [2 H, t, ArCH_2 , *J* 7.6]; 1.90–1.75 [1 H, m, $\text{CH}(\text{CH}_3)_2$]; 1.64–1.24 [11 H, m]; 0.97–0.84 [13 H, m, $\text{CHH-}i\text{Pr}$, $(\text{CH}_3)_2\text{CH}$, CH_3CH_2]. ^{13}C NMR: δ 169.8, 169.3 [C=O]; 153.1 [C-11]; 143.8, 135.3, 127.6 [quat. aromatics]; 132.8 [C-9]; 130.9 [C-7]; 128.9, 128.8, 128.5, 126.2 [CH of pentylphenyl]; 123.5 [C-8]; 122.7 [C-10]; 82.4 [C-2]; 60.8 [CHAr]; 48.2 [C-3]; 40.7 [$\text{CH}_2 - i\text{Pr}$]; 39.4 [CH_2N]; 35.9 [CH_2Ar]; 31.5, 31.4, 31.2 [CH_2]; 24.5 [$\text{CH}(\text{CH}_3)_2$]; 23.2, 22.0 [$(\text{CH}_3)_2\text{CH}$]; 22.5, 20.1 [CH_2CH_3]; 14.0, 13.7 [CH_2CH_3]. HRMS: m/z 478.3202 (EI) (M^+ . $\text{C}_{30}\text{H}_{42}\text{N}_2\text{O}_3$ requires 478.3195).

18b: R_f 0.27 (PE/AcOEt 75:25, dev.: A, C). ^1H NMR: δ 7.77 [1 H, dd, *H*-7, *J* 1.8, 7.8]; 7.39 [1 H, dt, *H*-9, *J*_d 1.8, *J*_t 7.8]; 7.34–7.30 [3 H, m]; 7.23–7.18 [1 H, m]; 7.16 [1 H, dt, *H*-8, *J*_d 1.2, *J*_t 7.5]; 6.88 [1 H, dd, *H*-10, *J* 1.2, 8.2]; 6.38 [1 H, s, CH-Ar]; 6.02 [1 H, broad t, NH, *J* 5.5]; 3.62–3.50 [2 H, m, *H*-2 and 1 *H*-3]; 3.40 [1 H, dq, CHHNH , *J*_d 13.8, *J*_q 6.7]; 3.32–3.18 [2 H, m, *H*-3 and CHHNH]; 2.61 [2 H, t, CH_2Ar , *J* 7.8]; 1.64–1.45 [5 H, m, $\text{CH}(\text{CH}_3)_2 + 4 \text{H of } \text{CH}_2$]; 1.40–1.24 [7 H, m]; 0.91 [3 H, t, CH_3CH_2 , *J* 7.4]; 0.89 [3 H, t, CH_3CH_2 , *J* 7.4]; 0.91–0.76 [1 H, m, $\text{CHH-}i\text{Pr}$]; 0.66 & 0.65 [2 x 3 H, 2 d, $(\text{CH}_3)_2\text{CH}$, *J* 6.3 and 6.6]. ^{13}C NMR: δ 169.6, 169.5 [C=O]; 152.3 [C-11]; 144.0, 135.0, 128.4 [quat. aromatics]; 132.7 [C-9]; 131.0 [C-7]; 129.5, 128.9 (x2), 126.8 [CH of pentylphenyl]; 123.8 [C-8]; 123.2 [C-9]; 83.0 [C-2]; 60.3 [CHAr]; 48.0 [C-3]; 40.5 [$\text{CH}_2 - i\text{Pr}$]; 39.4 [CH_2N]; 35.9 [CH_2Ar]; 31.52, 31.46, 31.41 [CH_2]; 24.3 [$\text{CH}(\text{CH}_3)_2$]; 22.46, 22.10 [$(\text{CH}_3)_2\text{CH}$]; 22.52, 20.0 [CH_2CH_3]; 14.0, 13.7 [CH_2CH_3]. HRMS: m/z 478.3190 (EI) (M^+ . $\text{C}_{30}\text{H}_{42}\text{N}_2\text{O}_3$ requires 478.3195).

General procedure for the synthesis of compounds 17–18c-k

A solution of Boc derivative **13a** or **13b** (1.25 mmol) was dissolved in 1,4-dioxane (3 mL) and treated with 37% aqueous HCl (300 μ L). The solution was heated at 80°C in a microwave apparatus (open vessel) for 10 min. Then, it was poured into saturated aqueous NaCl (30 mL) + 1 M NaOH (8

mL). The mixture was extracted twice with Et₂O and twice with AcOEt, washed with saturated aqueous NaCl, and evaporated to dryness at the rotatory evaporator, avoiding to strip the solvent too much. The residue was treated with a solution of aldehyde **4a** or **4b** (1.20 mmol) in dry MeOH (2.5 mL) and with freshly activated powdered 3 Å mol. sieves (200 mg). After stirring for 10 min, carboxylic acid **2a** or **2b** (1.00 mmol) was added, followed by *n*-butyl or *n*-pentyl or *t*-butyl isocyanide (1.20 mmol). The suspension was stirred for 24 h at r.t. Then, it was diluted with CH₂Cl₂, filtered and evaporated to dryness. Chromatography on 50 g of silica gel using PE/acetone (typically from 95:5 to 85:15) afforded the corresponding Ugi adducts **14** as inseparable diastereoisomeric mixture (yields are reported in Table 1). These Ugi products were not characterized by NMR. Actually, owing to restricted rotation around the tertiary amides, and around the bonds connecting the aryl groups, NMR gave broad peaks at r.t., and nearly complete coalescence could be reached only at 125°C. Even at this temperature, the spectra were rather complex. Thus, we preferred to perform a full characterization only at the level of final products **17** and **18**, where these problems are no longer present.

Then, the Ugi products **14** were taken up in 96% EtOH (15 mL), treated with 10% Pd-C (100 mg) and hydrogenated at room temperature at an overpressure of 0–414 KPa. **14c**: 0 KPa, 24 h. **14d**: 0 KPa, 24 h. **14e**: 0 KPa, 218 h. **14f**: 0 KPa, 66 h. **14g**: 240 KPa, 18 h. **14h**: 414 KPa, 233 h. **14i**: 414 KPa, 233 h. **14j**: 414 KPa, 256 h. **14k**: 0 KPa, 24 h.

After filtration and evaporation, the residue was taken up twice with CH₂Cl₂ / benzene and evaporated again to give crude **15–16**. In the case of **15c–e–16c–e**, this crude product was used as such for the next Mitsunobu reaction, whereas in the other cases, the two diastereoisomers were separated by chromatography (from PE/AcOEt 80:20 + 0.25% EtOH to PE/AcOEt 80:20 + 0.25% EtOH). For **15c–e–16c–e**, the yields reported in Table 1 refer to the crude product; in the other cases, to the chromatographed products. Compounds **16** had in all cases higher *R_f* compared with **15** with PE/AcOEt 80:20 or 70:30. For example, **16d** and **15d** had, respectively, *R_f* 0.54 and 0.36 with PE/AcOEt 70:30.

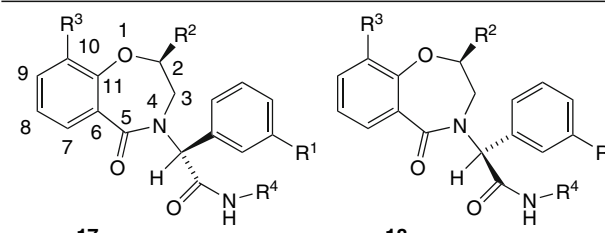
Phenolic alcohols **15** or **16** (0.5 mmol) were taken up in dry CH₂Cl₂ (10 mL), cooled to 0°C and treated with PPh₃ (0.75 mmol) and DEAD (0.75 mmol). After 1 h, the cooling bath was removed and the orange solution stirred at r.t. for 24 h. Then the reaction was quenched with H₂O (150 µL), and stirred for 2 h at r.t. Benzene (10 mL) was added, and the mixture evaporated to dryness. Chromatography through silica gel (PE/AcOEt) afforded pure **17** and **18** as oils. Compounds **17** had in all the cases higher *R_f* compared with **18**. The yields are reported in Table 1.

17c: *R_f* 0.62 (PE/AcOEt 70:30, dev.: A, C). ¹H NMR: δ 7.62 [1 H, dd, *H*-7, *J* 2.1, 7.8]; 7.32–7.11 [4 H, m, aromatics]; 7.05 [1 H, t, *H*-8, *J* 7.5]; 6.42 [1 H, s, *CH*–

Ar]; 6.26 [1 H, broad t, *NH*, *J* 5.7]; 4.51 [1 H, ddt, *H*-2, *J_t* 4.2, *J_d* 6.0, 10.2]; 3.55 [1 H, dd, *H*-3, *J* 3.8, 15.5]; 3.40–3.25 [2 H, m, *CH*₂NH]; 3.20 [1 H, dd, *H*-3, *J* 6.1, 15.5]; 2.70–2.47 [4 H, m, ArCH₂]; 1.75–1.65 [1 H, m, *CH*(CH₃)₂]; 1.64–1.40 [6 H, m]; 1.40–1.20 [11 H, m]; 0.93–0.82 [15 H, m, (CH₃)₂CH, CH₃CH₂]; 0.74–0.64 [1 H, m, *CHH*_{*i*}Pr]. ¹³C NMR: δ 170.5, 169.5 [C=O]; 151.7 [C-11]; 143.8, 135.5, 135.3, 127.8 [quat. aromatics]; 133.3 [C-9]; 129.4, 128.8, 128.7, 128.6, 126.8 [CH of pentylphenyl and C-7]; 123.0 [C-8]; 82.2 [C-2]; 61.0 [CHAr]; 47.7 [C-3]; 41.6 [CH₂ – *i*Pr]; 39.4 [CH₂N]; 35.9, 30.4 [CH₂Ar]; 31.8, 31.5, 31.4, 31.2, 30.2 [CH₂]; 24.3 [CH(CH₃)₂]; 23.1, 21.8 [(CH₃)₂CH]; 22.53, 22.51, 20.0 [CH₂]; 14.0 (x2), 13.7 [CH₂CH₃]. HRMS: *m/z* 548.3979 (EI) (M⁺, 10.6%. C₃₅H₅₂N₂O₃ requires 548.3978). Base peak = 448.3246 (M⁺–100) (C₃₀H₄₂NO₂ requires 448.3210).

18c: *R_f* 0.47 (PE/AcOEt 70:30, dev.: A, C). ¹H NMR: δ 7.59 [1 H, dd, *H*-7, *J* 1.9, 7.7]; 7.36–7.27 [4 H, m]; 7.23–7.16 [1 H, m]; 7.06 [1 H, t, *H*-8, *J* 7.6]; 6.39 [1 H, s, *CH*–Ar]; 6.03 [1 H, broad t, *NH*, *J* 5.4]; 3.70–3.58 [1 H, m, *H*-2]; 3.56 [1 H, dd, *H*-3, *J* 3.6, 15.9]; 3.46–3.18 [3 H, m, *H*-3 and CH₂NH]; 2.72–2.51 [3 H, m, CH₂*n*Bu]; 2.41 [1 H, ddd, *CHH* – *n*Bu, *J* 6.0, 9.6, 13.6]; 1.66–1.40 [7 H, m]; 1.40–1.22 [11 H, m]; 0.96–0.82 [10 H, m, CH₃CH₂ + *CHH*–*i*Pr]; 0.63 [3 H, d, CH₃CHCH₃, *J* 6.6]; 0.59 [3 H, d, CH₃CHCH₃, *J* 6.6]. ¹³C NMR: δ 169.9, 169.6 [C=O]; 150.4 [C-11]; 144.0, 135.9, 134.9, 128.2 [quat. aromatics]; 133.1 [C-9]; 129.6, 128.9 (x2), 126.9 [CH of pentylphenyl]; 128.8 [C-7]; 123.1 [C-8]; 83.6 [C-2]; 60.3 [CHAr]; 47.7 [C-3]; 41.7 [CH₂ – *i*Pr]; 39.3 [CH₂N]; 35.9 and 30.2 [CH₂Ar]; 31.8, 31.51, 31.45, 31.36, 30.0, 22.5 (x2), 20.0 [CH₂]; 24.4 [CH(CH₃)₂]; 22.3, 22.2 [(CH₃)₂CH]; 14.0 (x2), 13.7 [CH₂CH₃]. HRMS: *m/z* 548.3983 (EI) (M⁺, 9.8%. C₃₅H₅₂N₂O₃ requires 548.3978). Base peak = 448.3238 (M⁺–100) (C₃₀H₄₂NO₂ requires 448.3210).

17d: *R_f* 0.67 (PE/AcOEt 70:30, dev. A, C). ¹H NMR: δ 7.61 [1 H, dd, *H*-7, *J* 1.6, 7.9]; 7.32–7.12 [5 H, m, aromatics]; 7.04 [1 H, t, *H*-8, *J* 7.5]; 6.42 [1 H, s, *CH*–Ar]; 6.33 [1 H, broad t, *NH*, *J* 5.7]; 4.52 [1 H, ddt, *H*-2, *J_t* 4.2, *J_d* 6.2, 10.5]; 3.56 [1 H, dd, *H*-3, *J* 3.8, 15.5]; 3.40–3.15 [2 H, m, CH₂NH]; 3.20 [1 H, dd, *H*-3, *J* 6.3, 15.6]; 2.75–2.48 [4 H, m, ArCH₂]; 1.85–1.67 [4 H, m]; 1.66–1.41 [10 H, m]; 1.40–1.22 [7 H, m]; 1.20–1.02 [2 H, m]; 0.92–0.80 [12 H, m, CH₃]; 0.79–0.65 [1 H, m]. ¹³C NMR: δ 170.5, 169.5 [C = O]; 151.6 [C-11]; 143.7, 135.7, 135.4, 127.8 [quat. aromatics]; 133.2 [C-9]; 129.4, 128.8, 128.7, 128.6, 126.7 [CH of pentylphenyl and C-7]; 123.0 [C-8]; 82.2 [C-2]; 61.0 [CHAr]; 47.7 [C-3]; 41.7 [CH₂ – *i*Pr]; 40.0 [cyclopentyl CH]; 39.4 [CH₂N]; 35.9, 29.6 [CH₂Ar]; 37.0, 32.6 (x2), 31.5, 31.4, 31.2, 25.2 (x2), 22.5, 20.0 [other CH₂]; 24.3 [CH(CH₃)₂]; 23.1, 21.9 [(CH₃)₂CH]; 14.0, 13.7 [CH₂CH₃]. HRMS: *m/z* 574.4140 (EI) (M⁺, 5.4%. C₃₇H₅₄N₂O₃ requires 574.4134). Base peak = 474.3487 (M⁺–100) (C₃₂H₄₄NO₂ requires 474.3367).

Table 2 Selected ^1H NMR chemical shifts of dihydrobenzoxazepinones **17** and **18**


Compound	<i>H</i> -2	$\text{CH}_2\text{CH}(\text{CH}_3)_2$	$\text{CH}_2\text{CH}(\text{CH}_3)_2$	$\text{CH}_2\text{CH}(\text{CH}_3)_2$
17a	4.74	1.50, ^a 0.98 ^a	1.84	0.93, 0.92
18a	3.58	1.32, ^a 0.82 ^a	1.53	0.66, 0.65
17b	4.74	1.42, ^a 0.90 ^a	1.83	0.90 ^a
18b	3.58	1.32, ^a 0.82 ^a	1.55	0.66, 0.65
17c	4.51	1.32, ^a 0.68 ^a	1.62 ^a	0.90 ^a
18c	3.63	1.33, ^a 0.85 ^a	1.46	0.63, 0.59
17d	4.52	1.32, ^a 0.68 ^a	1.73 ^a	0.82, 0.85 ^a
18d	3.65	1.35, ^a 0.84 ^a	1.48 ^a	0.63, 0.59
17e	4.51	1.32, ^a 0.69 ^a	1.73 ^a	0.82, 0.85 ^a
18e	3.64	1.35, ^a 0.88 ^a	1.50	0.63, 0.60
17f	4.52	1.32, ^a 0.70	1.75 ^a	0.85, 0.83
18f	3.67	1.36, ^a 0.85 ^a	1.47 ^a	0.63, 0.59
17k	4.47	1.32, ^a 0.70	1.75 ^a	0.85 ^a
18k	3.61	1.32, ^a 0.83 ^a	1.48 ^a	0.62, 0.59

Compound	<i>H</i> -2	CH_2Ph	<i>H</i> ortho of benzyl
17g	4.68	2.62, 2.22 ^a	7.12 ^{a,b} or 7.24 ^{a,b}
18g	3.91	2.94, 2.38 ^a	6.78
17h	4.72	2.67, 2.23	7.12 ^{a,b} or 7.22 ^{a,b}
18h	3.92	2.96, 2.38 ^a	6.78
17i	4.68	2.61, 2.20	7.12 ^{a,b} or 7.21 ^{a,b}
18i	3.91	2.93, 2.39	6.78
17j	4.71	2.66, 2.21	7.11 ^{a,b} or 7.22 ^{a,b}
18j	3.92	2.95, 2.40	6.77

^a Values determined by gHSQC or gCOSY^b In this case we were not able to assign the signals to the *ortho* or *meta* benzyl protons

18d: R_f 0.44 (PE/AcOEt 70:30, dev. A, C). ^1H NMR: δ 7.54 [1 H, dd, *H*-7, *J* 1.8, 7.8]; 7.36–7.30 [4 H, m]; 7.27 [1 H, dd, *H*-9, *J* 1.6, 7.7]; 7.03 [1 H, t, *H*-8, *J* 7.6]; 6.43 [1 H, s, *CH*-Ar]; 6.27 [1 H, broad dd, *NH*]; 3.70–3.54 [2 H, m, *H*-2, *H*-3]; 3.46–3.17 [3 H, m, *H*-3 and CH_2NH]; 2.68 [1 H, ddd, *CHH*Ar, *J* 5.7, 10.2, 13.5]; 2.61 [2 H, t, other *ArCH*₂, *J* 7.8]; 2.43 [1 H, ddd, *CHH*Ar, *J* 6.3, 10.5, 13.8]; 1.85–1.67 [4 H, m]; 1.66–1.42 [10 H, m]; 1.40–1.23 [7 H, m]; 1.17–1.02 [2 H, m]; 0.94–0.80 [1 H, m]; 0.89 [3 H, t, CH_3CH_2 , *J* 6.5]; 0.88 [3 H, t, CH_3CH_2 , *J* 7.4]; 0.63 [3 H, d, CH_3CHCH_3 , *J* 6.9]; 0.59 [3 H, d, CH_3CHCH_3 , *J* 6.3]. ^{13}C NMR: δ 169.9, 169.6 [*C*=O]; 150.4 [*C*-11]; 143.9, 136.0, 135.1, 128.2 [quat. aromatics]; 133.1 [*C*-9]; 129.6, 128.82, 128.78, 128.75, 126.9 [*CH* of pentylphenyl and *C*-

7]; 123.1 [*C*-8]; 83.6 [*C*-2]; 60.3 [*CH*Ar]; 47.7 [*C*-3]; 41.8 [CH_2 - *i*Pr]; 40.06 [cyclopentyl *CH*]; 39.3 [CH_2N]; 35.9 and 29.4 [CH_2Ar]; 36.8, 32.6, 32.5, 31.5, 31.4, 31.3, 25.2 (x2), 22.5, 20.0 [other CH_2]; 24.4 [$\text{CH}(\text{CH}_3)_2$]; 22.3, 22.2 [$(\text{CH}_3)_2\text{CH}$]; 14.0, 13.7 [CH_2CH_3]. HRMS: m/z 574.4145 (EI) (M^+ , 5.2%. $\text{C}_{37}\text{H}_{54}\text{N}_2\text{O}_3$ requires 574.4134). Base peak = 474.3443 (M^+ —100) ($\text{C}_{32}\text{H}_{44}\text{NO}_2$ requires 474.3367).

17e: R_f 0.56 (PE/AcOEt 80:20, dev. A, C). ^1H NMR: δ 7.63 [1 H, dd, *H*-7, *J* 1.9, 7.6]; 7.32–7.12 [5 H, m, aromatics]; 7.05 [1 H, t, *H*-8, *J* 7.8]; 6.40 [1 H, s, *CH*-Ar]; 6.22 [1 H, broad dd, *NH*]; 4.51 [1 H, ddt, *H*-2, *J*_f 3.9, *J*_d 6.0, 9.9]; 3.54 [1 H, dd, *H*-3, *J* 3.8, 15.8]; 3.40–3.20 [2 H, m, CH_2NH]; 3.20 [1 H, dd, *H*-3, *J* 6.3, 15.6]; 2.70–2.46 [4 H, m, *ArCH*₂]; 1.86–1.68 [4 H, m]; 1.68–1.42 [10 H, m];

1.42–1.20 [7 H, m]; 1.20–1.00 [2 H, m]; 0.96–0.79 [12 H, m, CH₃]; 0.76–0.62 [1 H, m]. ¹³C NMR: δ 170.5, 169.5 [C=O]; 151.7 [C-11]; 143.9, 135.5, 135.4, 127.8 [quat. aromatics]; 133.2 [C-9]; 129.4, 128.8 (x2), 128.6, 126.7 [C-7 and other CH]; 123.0 [C-8]; 82.2 [C-2]; 61.1 [CHAr]; 47.7 [C-3]; 41.7 [CH₂ - *i*Pr]; 39.7 [cyclopentyl CH]; 39.4 [CH₂N]; 35.1, 30.4 [CH₂Ar]; 38.1, 32.6 (x2), 31.8, 31.5, 30.2, 25.2 (x2), 22.5, 20.0 [other CH₂]; 24.3 [CH(CH₃)₂]; 23.1, 21.8 [(CH₃)₂CH]; 14.0, 13.7 [CH₂CH₃].

HRMS: *m/z* 574.4140 (EI) (M⁺, 5.8%. C₃₇H₅₄N₂O₃ requires 574.4134). Base peak = 474.3462 (M⁺—100)(C₃₂H₄₄NO₂ requires 474.3367).

18e: R_f 0.34 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.56 [1 H, dd, *H*-7, J 1.8, 7.8]; 7.36–7.30 [3 H, m]; 7.27 [1 H, dd, *H*-9, J 1.6, 7.3]; 7.23–7.15 [1 H, m]; 7.04 [1 H, t, *H*-8, J 7.8]; 6.42 [1 H, s, *CH*-Ar]; 6.19 [1 H, t, *NH*, J 5.5]; 3.70–3.54 [2 H, m, *H*-2, *H*-3]; 3.46–3.17 [3 H, m, *H*-3 and CH₂NH]; 2.75–2.62 [3 H, m, *CHH*Ar and other CH₂Ar]; 2.41 [1 H, ddd, *CHH*Ar, J 6.3, 9.6, 13.5]; 1.86–1.70 [4 H, m]; 1.70–1.41 [10 H, m]; 1.40–1.20 [7 H, m]; 1.20–1.02 [2 H, m]; 0.94–0.80 [1 H, m]; 0.89 [3 H, t, CH₃CH₂, J 6.9]; 0.87 [3 H, t, CH₃CH₂, J 7.2]; 0.63 [3 H, d, CH₃CHCH₃, J 6.3]; 0.60 [3 H, d, CH₃CHCH₃, J 6.6]. ¹³C NMR: δ 169.9, 169.6 [C = O]; 150.5 [C-11]; 144.0, 135.9, 135.0, 128.1 [quat. aromatics]; 133.1 [C-9]; 129.5, 128.85, 128.79 (x2), 126.9 [C-7 and other CH]; 123.0 [C-8]; 83.6 [C-2]; 60.3 [CHAr]; 47.7 [C-3]; 41.7 [CH₂ - *i*Pr]; 39.7 [cyclopentyl CH]; 39.3 [CH₂N]; 35.0 and 30.2 [CH₂Ar]; 38.2, 32.6 (x2), 31.8, 31.4, 30.0, 25.2 (x2), 22.5, 20.0 [other CH₂]; 24.4 [CH(CH₃)₂]; 22.3, 22.2 [(CH₃)₂CH]; 14.0, 13.7 [CH₂CH₃]. HRMS: *m/z* 574.4146 (EI) (M⁺, 5.4%. C₃₇H₅₄N₂O₃ requires 574.4134). Base peak = 474.3477 (M⁺—100)(C₃₂H₄₄NO₂ requires 474.3367).

18f: R_f 0.39 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.61 [1 H, dd, *H*-7, J 2.0, 7.7]; 7.32–7.12 [5 H, m, aromatics]; 7.05 [1 H, t, *H*-8, J 7.8]; 6.42 [1 H, s, *CH*-Ar]; 6.33 [1 H, broad t, *NH*, J 5.4]; 4.52 [1 H, ddt, *H*-2, J_t 4.2, J_d 6.2, 10.5]; 3.55 [1 H, dd, *H*-3, J 3.9, 15.6]; 3.40–3.22 [2 H, m, CH₂NH]; 3.20 [1 H, dd, *H*-3, J 6.2, 15.8]; 2.75–2.48 [4 H, m, ArCH₂]; 1.86–1.68 [6 H, m]; 1.68–1.41 [14 H, m]; 1.40–1.21 [4 H, m]; 1.20–1.02 [4 H, m]; 0.89 [3 H, t, CH₃CH₂, J 7.2]; 0.85 [3 H, d, CH₃CHCH₃, J 6.6]; 0.83 [3 H, d, CH₃CHCH₃, J 6.6]; 0.70 [1 H, ddd, *CHH*-*i*Pr, J 4.2, 8.7, 13.8]. ¹³C NMR: δ 170.5, 169.5 [C=O]; 151.6 [C-11]; 143.9, 135.7, 135.4, 127.8 [quat. aromatics]; 133.2 [C-9]; 129.4, 128.8, 128.7, 128.5, 126.7 [C-7 and other CH]; 123.0 [C-8]; 82.2 [C-2]; 61.0 [CHAr]; 47.7 [C-3]; 41.7 [CH₂ - *i*Pr]; 40.0, 39.7 [cyclopentyl CH]; 39.4 [CH₂N]; 35.1, 29.6 [CH₂Ar]; 38.1, 37.0, 32.6 (x4), 31.4, 25.2 (x4), 20.0 [other CH₂]; 24.3 [CH(CH₃)₂]; 23.1, 21.9 [(CH₃)₂CH]; 13.7 [CH₂CH₃]. HRMS: *m/z* 600.4286 (EI) (M⁺, 7.7%. C₃₉H₅₆N₂O₃ requires 600.4291). Base peak = 500.3531 (M⁺—100)(C₃₄H₄₆NO₂ requires 500.3523).

18f: R_f 0.25 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.53 [1 H, dd, *H*-7, J 1.6, 7.6]; 7.36–7.30 [3 H, m]; 7.27 [1

H, dd, *H*-9, J 2.1, 7.5]; 7.22–7.10 [1 H, m]; 7.03 [1 H, t, *H*-8, J 7.6]; 6.45 [1 H, s, *CH*-Ar]; 6.36 [1 H, broad dd, *NH*]; 3.70–3.54 [2 H, m, *H*-2, *H*-3]; 3.46–3.17 [3 H, m, *H*-3 and CH₂NH]; 2.68 [1 H, ddd, *CHH*Ar, J 5.7, 9.9, 13.5]; 2.62 [2 H, t, other ArCH₂, J 7.8]; 2.43 [1 H, ddd, *CHH*Ar, J 6.0, 10.2, 13.5]; 1.86–1.68 [6 H, m]; 1.68–1.41 [14 H, m]; 1.41–1.22 [4 H, m]; 1.22–1.00 [4 H, m]; 0.94–0.80 [1 H, m]; 0.88 [3 H, t, CH₃CH₂, J 7.2]; 0.63 [3 H, d, CH₃CHCH₃, J 6.6]; 0.59 [3 H, d, CH₃CHCH₃, J 6.6]. ¹³C NMR: δ 169.9, 169.6 [C = O]; 150.4 [C-11]; 144.0, 136.0, 135.1, 128.2 [quat. aromatics]; 133.0 [C-9]; 129.5, 128.8, 128.7 (x2), 126.8 [C-7 and other CH]; 123.0 [C-8]; 83.7 [C-2]; 60.2 [CHAr]; 47.7 [C-3]; 41.7 [CH₂ - *i*Pr]; 40.1, 39.6 [cyclopentyl CH]; 39.3 [CH₂N]; 35.0 and 29.4 [CH₂Ar]; 38.2, 36.8, 32.6 (x4), 31.4, 25.2 (x4), 20.0 [other CH₂]; 24.4 [CH(CH₃)₂]; 22.3, 22.2 [(CH₃)₂CH]; 13.7 [CH₂CH₃]. HRMS: *m/z* 600.4279 (EI) (M⁺, 8.1%. C₃₉H₅₆N₂O₃ requires 600.4291). Base peak = 500.3534 (M⁺—100)(C₃₄H₄₆NO₂ requires 500.3523).

17g: R_f 0.35 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.60 [1 H, dd, *H*-7, J 1.8, 7.8]; 7.30–7.08 [10 H, m, aromatics]; 7.04 [1 H, t, *H*-8, J 7.8]; 6.41 [1 H, s, *CH*-Ar]; 6.27 [1 H, t, *NH*, J 5.8]; 4.68 [1 H, dq, *H*-2, J_d 9.2, J_q 4.6]; 3.59 [1 H, dd, *H*-3, J 3.9, 15.9]; 3.42–3.18 [3 H, m, *H*-3 and NHCH₂]; 2.62 [1 H, dd, *CHH*Ph, J 9.3, 14.7]; 2.57 [2 H, t, CH₂Ar, J 7.6]; 2.46–2.17 [3 H, m, *CHH*Ph and CH₂Ar]; 1.62–1.51 [2 H, m]; 1.52–1.42 [2 H, m]; 1.38–1.24 [8 H, m]; 1.23–1.12 [2 H, m]; 1.12–1.00 [2 H, m]; 0.89 [3 H, t, CH₃CH₂, J 7.2]; 0.86 [3 H, t, CH₃CH₂, J 6.9]; 0.82 [3 H, t, CH₃CH₂, J 7.2]. ¹³C NMR: δ 170.5, 169.6 [C = O]; 151.7 [C-11]; 143.9, 137.8, 135.6, 135.3, 127.6 [quat. aromatics]; 133.3 [C-9]; 129.7, 128.9 (x2), 128.82, 128.76, 128.73, 128.2 (x2), 126.9, 126.3 [C-7 and other CH]; 123.1 [C-8]; 84.2 [C-2]; 61.4 [CHAr]; 47.4 [C-3]; 39.4 [CH₂NH]; 39.1 [CH₂Ph]; 35.9, 30.2 [CH₂Ar]; 31.6, 31.5, 31.4, 31.2, 30.2 [CH₂]; 22.49, 22.47, 20.0 [CH₂]; 14.0 (x2), 13.7 [CH₂CH₃]. HRMS: *m/z* 582.3839 (EI) (M⁺, 4.6%. C₃₈H₅₀N₂O₃ requires 582.3821). Base peak = 482.3071 (M⁺—100)(C₃₃H₄₀NO₂ requires 482.3054).

18g: R_f 0.24 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.56 [1 H, dd, *H*-7, J 2.0, 7.6]; 7.29 [1 H, dd, *H*-9, J 1.7, 7.4]; 7.20–7.08 [7 H, m, aromatics]; 7.06 [1 H, t, *H*-8, J 7.6]; 6.78 (mc) [2 H, m, *H* ortho of benzyl group]; 6.34 [1 H, s, *CH*-Ar]; 6.16 [1 H, t, *NH*, J 5.7]; 3.91 (mc) [1 H, m, *H*-2]; 3.48 [1 H, dd, *H*-3, J 3.9, 15.9]; 3.47–3.26 [2 H, m, *H*-3 and *CHH*NH]; 3.26–3.11 [1 H, m, *CHH*NH]; 2.94 [1 H, dd, *CHH*Ph, J 6.2, 14.0]; 2.66 [1 H, ddd, *CHH*Ar, J 6.0, 9.3, 13.2]; 2.60–2.46 [2 H, m, CH₂Ar]; 2.46–2.30 [2 H, m, *CHH*Ph and *CHH*Ar]; 1.62–1.36 [4 H, m]; 1.36–1.15 [12 H, m]; 0.89 [3 H, t, CH₃CH₂, J 6.9]; 0.85 [3 H, t, CH₃CH₂, J 6.9]; 0.84 [3 H, t, CH₃CH₂, J 6.9]. ¹³C NMR: δ 169.9, 169.5 [C = O]; 150.2 [C-11]; 143.8, 136.3, 135.9, 134.6, 128.2 [quat. aromatics]; 133.2 [C-9]; 129.6, 128.8 (x2), 128.6, 128.5 (x4), 126.7, 126.4 [C-7 and other CH];

123.3 [C-8]; 85.5 [C-2]; 60.6 [CHAr]; 47.0 [C-3]; 39.4 [CH₂Ph]; 39.3 [CH₂NH]; 35.8, 30.0 [CH₂Ar]; 31.7, 31.5, 31.4, 31.2, 30.1 [CH₂]; 22.51, 22.48, 20.0 [CH₂]; 14.0 (x2), 13.6 [CH₂CH₃]. HRMS: m/z 582.3844 (EI) (M^+ , 4.0%. C₃₈H₅₀N₂O₃ requires 582.3821). Base peak = 482.3067 (M^+ —100)(C₃₃H₄₀NO₂ requires 482.3054).

17h: R_f 0.36 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.60 [1 H, dd, *H*-7, *J* 1.8, 7.8]; 7.30–7.08 [10 H, m, aromatics]; 7.03 [1 H, t, *H*-8, *J* 7.8]; 6.41 [1 H, s, *CH*-Ar]; 6.30 [1 H, broad t, *NH*]; 4.72 [1 H, dq, *H*-2, *J_d* 9.0, *J_q* 5.1]; 3.58 [1 H, dd, *H*-3, *J* 3.9, 15.6]; 3.40–3.18 [3 H, m, *H*-3 and *NHCH*₂]; 2.67 [1 H, dd, *CHHPh*, *J* 8.7, 14.1]; 2.56 [2 H, t, *CH*₂Ar, *J* 7.6]; 2.51–2.28 [2 H, m, *CH*₂Ar]; 2.23 [1 H, dd, *CHHPh*, *J* 5.0, 14.2]; 1.70–1.40 [11 H, m]; 1.40–1.20 [10 H, m]; 1.02–0.88 [2 H, m]; 0.86 [6 H, t, *CH*₃CH₂, *J* 6.8]. ¹³C NMR: δ 170.5, 169.6 [C=O]; 151.6 [C-11]; 143.8, 137.7, 135.8, 135.3, 127.7 [quat. aromatics]; 133.2 [C-9]; 129.7, 129.0 (x2), 128.8, 128.73, 128.67, 128.2 (x2), 126.8, 126.3 [C-7 and other CH]; 123.1 [C-8]; 84.2 [C-2]; 61.4 [CHAr]; 47.4 [C-3]; 39.8 [cyclopentyl CH]; 39.7 [CH₂NH]; 39.1 [CH₂Ph]; 35.9, 29.4 [CH₂Ar]; 36.9, 32.5, 32.4, 31.5, 31.2, 29.1, 29.0, 25.2 (x2), 22.5, 22.3 [CH₂]; 14.0, 13.9 [CH₂CH₃]. HRMS: m/z 622.4151 (EI) (M^+ , 3.7%. C₄₁H₅₄N₂O₃ requires 622.4134). Base peak = 508.3280 (M^+ —114)(C₃₅H₄₂NO₂ requires 508.3210).

18h: R_f 0.28 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.60 [1 H, dd, *H*-7, *J* 1.8, 7.8]; 7.31 [1 H, dd, *H*-9, *J* 1.8, 7.8]; 7.20–7.08 [7 H, m, aromatics]; 7.09 [1 H, t, *H*-8, *J* 7.2]; 6.78 (mc) [2 H, m, *H* ortho of benzyl group]; 6.29 [1 H, s, *CH*-Ar]; 5.88 [1 H, broad s, *NH*]; 3.92 (mc) [1 H, m, *H*-2]; 3.50–3.13 [4 H, m, *H*-3 and *CH*₂NH]; 2.96 [1 H, dd, *CHHPh*, *J* 6.0, 13.8]; 2.69 [1 H, ddd, *CHHAr*, *J* 5.4, 10.2, 13.5]; 2.62–2.34 [4 H, m, *CH*₂Ar, *CHHAr* and *CHHPh*]; 1.78–1.40 [11 H, m]; 1.40–1.17 [10 H, m]; 1.12–0.95 [2 H, m]; 0.89 [3 H, t, *CH*₃CH₂, *J* 6.9]; 0.85 [3 H, t, *CH*₃CH₂, *J* 6.9]. ¹³C NMR: δ 169.8, 169.5 [C=O]; 150.2 [C-11]; 143.7, 136.3, 135.9, 134.7, 128.2 [quat. aromatics]; 133.0 [C-9]; 129.5, 128.71, 128.69, 128.5, 128.4 (x4), 126.6, 126.3 [C-7 and other CH]; 123.3 [C-8]; 85.5 [C-2]; 60.4 [CHAr]; 46.9 [C-3]; 39.9 [cyclopentyl CH]; 39.5 [CH₂NH]; 39.4 [CH₂Ph]; 35.8, 29.3 [CH₂Ar]; 36.7, 32.6, 32.5, 31.5, 31.2, 29.0, 28.9, 25.1 (x2), 22.5, 22.2 [CH₂]; 14.0, 13.9 [CH₂CH₃]. HRMS: m/z 622.4147 (EI) (M^+ , 3.7%. C₄₁H₅₄N₂O₃ requires 622.4134). Base peak = 508.3264 (M^+ —114)(C₃₅H₄₂NO₂ requires 508.3210).

17i: R_f 0.39 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.60 [1 H, dd, *H*-7, *J* 1.8, 7.8]; 7.30–7.08 [10 H, m, aromatics]; 7.03 [1 H, t, *H*-8, *J* 7.8]; 6.42 [1 H, s, *CH*-Ar]; 6.32 [1 H, broad t, *NH*]; 4.68 [1 H, dq, *H*-2, *J_d* 9.0, *J_q* 4.4]; 3.59 [1 H, dd, *H*-3, *J* 3.9, 15.3]; 3.42–3.19 [3 H, m, *H*-3 and *NHCH*₂]; 2.61 [1 H, dd, *CHHPh*, *J* 9.3, 14.1]; 2.58 [2 H, t, *CH*₂Ar, *J* 8.1]; 2.46–2.16 [3 H, m, *CH*₂Ar and *CHHPh*]; 1.82–1.67 [3 H, m]; 1.66–1.41 [8 H, m]; 1.37–

1.00 [10 H, m]; 0.88 [3 H, t, *CH*₃CH₂, *J* 7.5]; 0.82 [3 H, t, *CH*₃CH₂, *J* 7.5]. ¹³C NMR: δ 170.5, 169.6 [C=O]; 151.6 [C-11]; 144.0, 137.8, 135.6, 135.3, 127.6 [quat. aromatics]; 133.3 [C-9]; 129.6, 128.9 (x2), 128.8, 128.7 (x2), 128.2 (x2), 126.8, 126.2 [C-7 and other CH]; 123.1 [C-8]; 84.2 [C-2]; 61.4 [CHAr]; 47.4 [C-3]; 39.7 [cyclopentyl CH]; 39.4 [CH₂NH]; 39.1 [CH₂Ph]; 35.1, 30.3 [CH₂Ar]; 38.2, 32.6 (x2), 31.6, 31.4, 30.3, 25.2 (x2), 22.5, 20.0 [CH₂]; 14.0, 13.7 [CH₂CH₃]. HRMS: m/z 608.3989 (EI) (M^+ , 4.8%. C₄₀H₅₂N₂O₃ requires 608.3978). Base peak = 508.3250 (M^+ —100)(C₃₅H₄₂NO₂ requires 508.3210).

18i: R_f 0.28 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.53 [1 H, dd, *H*-7, *J* 1.8, 7.5]; 7.28 [1 H, dd, *H*-9, *J* 1.5, 7.5]; 7.22–7.08 [7 H, m, aromatics]; 7.05 [1 H, t, *H*-8, *J* 7.5]; 6.78 (mc) [2 H, m, *H* ortho of benzyl group]; 6.36 [1 H, s, *CH*-Ar]; 6.29 [1 H, t, *NH*, *J* 5.7]; 3.91 (mc) [1 H, m, *H*-2]; 3.49 [1 H, dd, *H*-3, *J* 3.6, 15.6]; 3.47–3.27 [2 H, m, *CHHNH* and *H*-3]; 3.24–3.08 [1 H, m, *CHHNH*]; 2.93 [1 H, dd, *CHHPh*, *J* 5.7, 13.8]; 2.73–2.47 [3 H, m, *CHHAr*]; 2.46–2.30 [1 H, m, *CHHAr*]; 2.39 [1 H, dd, *CHHPh*, *J* 9.0, 13.8]; 1.86–1.69 [3 H, m]; 1.69–1.10 [18 H, m]; 0.84 [6 H, t, *CH*₃CH₂, *J* 6.9]. ¹³C NMR: δ 169.8, 169.5 [C=O]; 150.2 [C-11]; 143.9, 136.3, 135.8, 134.6, 128.1 [quat. aromatics]; 133.1 [C-9]; 129.4, 128.8 (x2), 128.6, 128.4 (x4), 126.6, 126.4 [C-7 and other CH]; 123.3 [C-8]; 85.5 [C-2]; 60.5 [CHAr]; 47.0 [C-3]; 39.7 [cyclopentyl CH]; 39.3 [CH₂NH]; 39.2 [CH₂Ph]; 35.0, 30.0 [CH₂Ar]; 38.0, 32.6 (x2), 31.7, 31.4, 30.1, 25.2 (x2), 22.5, 20.0 [CH₂]; 14.0, 13.6 [CH₂CH₃]. HRMS: m/z 608.3976 (EI) (M^+ , 5.4%. C₄₀H₅₂N₂O₃ requires 608.3978). Base peak = 508.3257 (M^+ —100)(C₃₅H₄₂NO₂ requires 508.3210).

17j: R_f 0.45 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.60 [1 H, dd, *H*-7, *J* 1.8, 7.8]; 7.28–7.08 [10 H, m, aromatics]; 7.04 [1 H, t, *H*-8, *J* 7.8]; 6.40 [1 H, s, *CH*-Ar]; 6.26 [1 H, t, *NH*, *J* 5.4]; 4.71 [1 H, dq, *H*-2, *J_d* 9.3, *J_q* 5.1]; 3.57 [1 H, dd, *H*-3, *J* 3.9, 15.9]; 3.42–3.19 [3 H, m, *H*-3 and *NHCH*₂]; 2.66 [1 H, dd, *CHHPh*, *J* 8.7, 14.1]; 2.58 (mc) [2 H, m, *CH*₂Ar]; 2.50–2.25 [2 H, m, *CH*₂Ar]; 2.21 [1 H, dd, *CHHPh*, *J* 4.8, 14.1]; 1.82–1.68 [3 H, m]; 1.68–1.40 [15 H, m]; 1.40–1.20 [4 H, m]; 1.20–1.00 [2 H, m]; 1.00–0.82 [2 H, m]; 0.89 [3 H, t, *CH*₃CH₂, *J* 7.5]. ¹³C NMR: δ 170.5, 169.6 [C = O]; 151.6 [C-11]; 144.0, 137.7, 135.8, 135.3, 127.7 [quat. aromatics]; 133.2 [C-9]; 129.6, 129.0 (x2), 128.8, 128.7, 128.6, 128.2 (x2), 126.8, 126.3 [C-7 and other CH]; 123.1 [C-8]; 84.2 [C-2]; 61.4 [CHAr]; 47.3 [C-3]; 39.8, 39.7 [cyclopentyl CH]; 39.4 [CH₂NH]; 39.1 [CH₂Ph]; 35.1, 29.4 [CH₂Ar]; 38.2, 36.9, 32.6 (x2), 32.5, 32.4, 31.4, 25.2 (x4), 20.0 [CH₂]; 13.7 [CH₂CH₃]. HRMS: m/z 634.4106 (EI) (M^+ , 5.3%. C₄₂H₅₄N₂O₃ requires 634.4134). Base peak = 534.3353 (M^+ —100)(C₃₇H₄₄NO₂ requires 534.3372).

18j: R_f 0.32 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.55 [1 H, dd, *H*-7, *J* 1.8, 7.8]; 7.30 [1 H, dd, *H*-9, *J* 1.8, 7.5]; 7.22–7.08 [7 H, m, aromatics]; 7.06 [1 H, t, *H*-8, *J* 7.5]; 6.77 (mc) [2 H, m, *H* ortho of benzyl group]; 6.34 [1

H, s, *CH*-Ar]; 6.17 [1 H, t, *NH*, J 5.7]; 3.92 (mc) [1 H, m, *H*-2]; 3.46 [1 H, dd, *H*-3, J 3.9, 15.9]; 3.45–3.27 [2 H, m, *CHHNH* and *H*-3]; 3.24–3.10 [1 H, m, *CHHNH*]; 2.95 [1 H, dd, *CHHPh*, J 6.0, 13.8]; 2.69 [1 H, ddd, *CHHAr*, J 5.3, 10.5, 13.8]; 2.60–2.47 [2 H, m, *CHHAr*]; 2.46–2.32 [2 H, m, *CHHAr*, *CHHPh*]; 1.83–1.36 [22 H, m]; 1.33–1.19 [2 H, m]; 1.19–0.98 [2 H, m]; 0.85 [6 H, t, *CH*₃*CH*₂, J 6.9]. ¹³C NMR: δ 169.8, 169.5 [C=O]; 150.2 [C-11]; 143.8, 136.2, 135.9, 134.6, 128.2 [quat. aromatics]; 133.0 [C-9]; 129.5, 128.8, 128.7, 128.5, 128.45 (x2), 128.41 (x2), 126.6, 126.4 [C-7 and other *CH*]; 123.3 [C-8]; 85.5 [C-2]; 60.5 [CHAr]; 46.9 [C-3]; 39.9, 39.7 [cyclopentyl *CH*]; 39.4 [CH₂Ph]; 39.2 [CH₂NH]; 35.0, 29.4 [CH₂Ar]; 38.0, 36.8, 32.6 (x3), 32.5, 31.4, 25.1 (x4), 20.0 [CH₂]; 13.6 [CH₂CH₃]. HRMS: *m/z* 634.4113 (EI) (*M*⁺, 5.5%. C₄₂H₅₄N₂O₃ requires 634.4134). Base peak = 534.3349 (*M*⁺—100) (C₃₇H₄₄NO₂ requires 534.3372).

17k: *R*_f 0.37 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.65 [1 H, dd, *H*-7, J 1.8, 7.8]; 7.32–7.11 [4 H, m, aromatics]; 7.06 [1 H, t, *H*-8, J 7.8]; 6.28 [1 H, s, *CH*-Ar]; 5.91 [1 H, s, *NH*]; 4.47 (mc) [1 H, m, *H*-2]; 3.55 [1 H, dd, *H*-3, J 3.3, 15.3]; 3.20 [1 H, dd, *H*-3, J 6.3, 15.3]; 2.70–2.47 [4 H, m, ArCH₂]; 1.80–1.47 [5 H, m]; 1.40–1.23 [9 H, m]; 1.38 [9 H, s, (CH₃)₃C]; 0.93–0.80 [15 H, m, (CH₃)₂CH, CH₃CH₂]; 0.76–0.64 [1 H, m, *CHHPr*]. ¹³C NMR: δ 170.5, 168.8 [C = O]; 151.7 [C-11]; 143.7, 135.6, 135.5, 127.7 [quat. aromatics]; 133.2 [C-9]; 129.4, 128.80, 128.75, 128.5, 126.7 [C-7 and other *CH*]; 122.9 [C-8]; 82.2 [C-2]; 61.3 [CHAr]; 51.7 [C(CH₃)₃]; 47.7 [C-3]; 41.7 [CH₂ — *iPr*]; 35.9, 30.4 [CH₂Ar]; 31.8, 31.5, 31.2, 30.2 [CH₂]; 28.6 [C(CH₃)₃]; 24.3 [CH(CH₃)₂]; 23.1, 21.8 [(CH₃)₂CH]; 22.54, 22.51 [CH₂]; 14.0 (x2) [CH₂CH₃].

HRMS: *m/z* 548.3966 (EI) (*M*⁺, 3.1%. C₃₅H₅₂N₂O₃ requires 548.3978). Base peak = 449.3378 (*M*⁺—99) (C₃₀H₄₃NO₂ requires 449.3294).

18k: *R*_f 0.27 (PE/AcOEt 80:20, dev. A, C). ¹H NMR: δ 7.61 [1 H, dd, *H*-7, J 1.6, 7.6]; 7.35–7.30 [3 H, m]; 7.27 [1 H, dd, *H*-9, J 2.0, 7.4]; 7.23–7.16 [1 H, m]; 7.05 [1 H, t, *H*-8, J 7.5]; 6.31 [1 H, s, *CH*-Ar]; 5.72 [1 H, s, *NH*]; 3.65–3.50 [1 H, m, *H*-2, *H*-3]; 3.32 [1 H, dd, *H*-3, J 11.0, 16.4]; 2.66 [1 H, ddd, *CHHAr*, J 5.7, 9.3, 13.5]; 2.61 [2 H, t, CH₂Ar, J 7.6]; 2.40 [1 H, ddd, *CHHAr*, J 6.0, 9.3, 13.8]; 1.66–1.40 [5 H, m]; 1.40–1.20 [9 H, m]; 1.38 [9 H, s, C(CH₃)₃]; 0.96–0.82 [1 H, m, *CHH-iPr*]; 0.89 [3 H, t, CH₃CH₂, J 6.9]; 0.86 [3 H, t, CH₃CH₂, J 6.9]; 0.62 [3 H, d, CH₃CHCH₃, J 6.3]; 0.59 [3 H, d, CH₃CHCH₃, J 6.6].

¹³C NMR: δ 169.8, 169.1 [C=O]; 150.5 [C-11]; 144.0, 135.8, 135.4, 128.2 [quat. aromatics]; 133.1 [C-9]; 129.6, 128.9 (x 2), 128.8, 126.9 [C-7 and other *CH*]; 123.0 [C-8]; 83.5 [C-2]; 60.5 [CHAr]; 51.8 [C(CH₃)₃]; 47.7 [C-3]; 41.7 [CH₂-*iPr*]; 35.9 and 30.2 [CH₂Ar]; 31.8, 31.5, 31.3, 30.0, 22.5 (x2) [CH₂]; 28.7 [C(CH₃)₃]; 24.4 [CH(CH₃)₂]; 22.25, 22.21 [(CH₃)₂CH]; 14.03, 13.98 [CH₂CH₃].

HRMS: *m/z* 548.3963 (EI) (*M*⁺, 2.8%. C₃₅H₅₂N₂O₃ requires 548.3978). Base peak = 449.3381 (*M*⁺—99) (C₃₀H₄₃NO₂ requires 449.3294).

Competitive assays

Recombinant Bcl-XL (ΔTM): protein expression and purification

A DNA fragment corresponding to the coding sequence for human Bcl-XL (Accession No. Z23115.1), with the C-terminus truncated, was amplified by PCR using the following oligonucleotides:

forward 5'-ACATGCATGCTTCAGAGCAACCGGGA-GC-3' (SphI site)

reverse 5'-GAAGATCTGCGGTTGAAGCGTTCCTG-3' (BglII site).

The second codon of Bcl-XL was altered to create a SphI site. The amplified product and the His6 vector pQE-70 were digested with SphI and BglII, followed by ligation and transformation into JM109 strain *E. coli* in Luria–Bertani medium, supplemented with ampicillin (100 μg/mL) and chloramphenicol (25 μg/mL). Colonies were screened by restriction enzyme digestion of plasmid DNA. The correctness of the DNA sequence was confirmed through sequencing analysis. The PCR product was inserted in the PCRII vector (TA cloning system; Invitrogen, San Diego, CA) and then cloned in the bacterial expression vector pET-14b(+)-His-TAG (Novagen; Madison, WI), between the Nde I and BamH I sites. Vectors were sequenced by an ALF DNA sequencer (LKB-Pharmacia Biotech) using both vectors and internal primers. *E. coli* BL21 (pLys) were transformed with a purified plasmid and recombinant protein expression was induced for up to 15 h by adding 100 mM IPTG to exponentially growing bacteria at room temperature. Bacteria were collected by centrifugation and re-suspended in 5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl (pH=7.9); samples were sonicated on ice. Following centrifugation at 14,000g for 15 min, bacterial lysates were then applied to a charged and equilibrated Chelating Sepharose (Pharmacia Biotech) chromatography column.

The column was subsequently washed with 10 volumes of 5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl (pH=7.9) (binding buffer), and 10 volumes of 60 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl (pH=7.9) (washing buffer). The bound protein was eluted with 10 volumes of 0.5 M imidazole, 0.25 M NaCl, 10 mM Tris-HCl (pH=7.9) (elution buffer). Fractions were collected and purity of recombinant His6-Bcl-XL (ΔTM) protein preparation was determined by SDS-polyacrylamide gel electrophoresis. The eluted recombinant protein (26,000 Mr) was found predominantly monomeric by centrifugal filtration on a 30,000 NMWL Millipore filter unit and capable of immunoreaction with Bcl-XL antibodies.

BakF-BH3 Fluo

As binding partner, we used the 5-carboxyfluorescein-labelled 16-mer peptide Bak-BH3 D84A (sequence 72 GQVGRQLAIIGDAINR 87 derived from the Bak BH3 domain). It was prepared by conventional peptide synthesis.

Competition assays

For the Fluorescence Anisotropy assays, a Polarion Instrument (Tecan, Austria) was used, and the following parameters were set: excitation wavelength: 485 nm, emission wavelength: 535 nm, number of flash: 25, plate shaking time: 5 sec, resting time upon shaking: 5 sec, temperature: 30°C. Experiments were performed in 96-wells black non-binding plates (Corning, N.Y., USA) with a capacity of 200 µL per well. Anisotropy measurements were carried out at different incubation times (every 15 min for 2 h). A 1-mM mother solution of compounds **17–18** in DMSO was prepared and subsequently diluted to a 2×10^{-6} M working solution in the assay buffer (25 mM NaHCO₃, 120 mM KCl, 1 mM KH₂PO₄, 10 mM MgCl₂ and 20 mM Hepes, pH=7.4). Anisotropy values for the blank binding assay were registered for a 2×10^{-8} M concentration of Bak-BH3F and for a 2×10^{-7} concentration of Bcl-xL.

Competition assays were performed incubating the reference peptide Bak-BH3F ($C = 2 \times 10^{-8}$ M) with Bcl-xL ($C = 2 \times 10^{-7}$ M) and with compounds **17–18** (2×10^{-6} M). The measured values of anisotropy in the competition assay for all the compounds were too close to the blank values to be able to detect any weak binding with Bcl-xL.

Acknowledgements We thank Prof. Silvio Parodi, Department of Oncology, Biology and Genetics, University of Genova, for his invaluable suggestions, stemming from his great experience in the field of Bcl-2 family proteins and protein–protein interactions. Financial assistance by the University of Genova, the Ministry of University and Research (PRIN 2006 project ‘Diversity-oriented synthetic methodologies and strategies for the preparation of biologically active compounds’ and a Ph.D. scholarship to V.C. (‘Progetto Giovani’)), Fondazione CARIGE (for a Ph.D. scholarship to P.L.), Istituto Superiore di Oncologia (for a Ph.D. scholarship to I.M.); and Fondazione S. Paolo (for a contribution to the purchase of NMR and HPLC instruments) is gratefully acknowledged.

References

- Yin H, Hamilton AD (2005) Strategies for targeting protein–protein interactions with synthetic agents. *Angew Chem Int Ed Engl* 44: 4130–4163. doi:10.1002/anie.200461786
- Boger DL, Desharnais J, Capps K (2003) Solution-phase combinatorial libraries: modulating cellular signaling by targeting protein–protein or protein–DNA interactions. *Angew Chem Int Ed Engl* 42: 4138–4176. doi:10.1002/anie.200300574
- Pagliaro L, Felding J, Audouze K, Nielsen SJ, Terry RB, Krogh-Jensen C, Butcher S (2004) Emerging classes of protein–protein interaction inhibitors and new tools for their development. *Curr Opin Chem Biol* 8:442–449. doi:10.1016/j.cbpa.2004.06.006
- Fletcher S, Hamilton AD (2005) Protein surface recognition and proteomimetics: mimics of protein surface structure and function. *Curr Opin Chem Biol* 9:632–638. doi:10.1016/j.cbpa.2005.10.006
- Khorchid A, Beuparlant P (2004) Bcl-2-targeted cancer therapeutics. *Expert Opin Ther Patents* 14:805–818. doi:10.1517/13543776.14.6.805
- Shepherd NE, Hoang HN, Desai VS, Letouze E, Young PR, Fairlie DP (2006) Modular alpha-helical mimetics with antiviral activity against respiratory syncytial virus. *J Am Chem Soc* 128:13284–13289. doi:10.1021/ja064058a
- Sadowsky JD, Fairlie WD, Hadley EB, Lee HS, Umezawa N, Nikolovska-Coleska Z, Wang SM, Huang DCS, Tomita Y, Gellman SH (2007) (alpha/beta+alpha)-Peptide antagonists of BH3 Domain/Bcl-x(L) recognition: toward general strategies for foldamer-based inhibition of protein–protein interactions. *J Am Chem Soc* 129:139–154. doi:10.1021/ja0662523
- Ponassi R, Biasotti B, Tomati V, Bruno S, Poggi A, Malacarne D, Cimoli G, Salis A, Pozzi S, Miglino M et al (2008) A novel Bim-BH3-derived Bcl-X-L inhibitor biochemical characterization, in vitro, in vivo and ex-vivo anti-leukemic activity. *Cell Cycle* 7: 3211–3224
- Davis JM, Tsou LK, Hamilton AD (2007) Synthetic non-peptide mimetics of alpha-helices. *Chem Soc Rev* 36:326–334. doi:10.1039/b608043j
- Yin H, Lee GI, Sedey KA, Rodriguez JM, Wang HG, Sebt SM, Hamilton AD (2005) Terephthalamide derivatives as mimetics of helical peptides: disruption of the Bcl-x(L)/Bak interaction. *J Am Chem Soc* 127:5463–5468. doi:10.1021/ja0446404
- Yin H, Lee GI, Park HS, Payne GA, Rodriguez JM, Sebt SM, Hamilton AD (2005) Terphenyl-based helical mimetics that disrupt the p53/HDM2 interaction. *Angew Chem Int Ed Engl* 44:2704–2707. doi:10.1002/anie.200462316
- Davis JM, Truong A, Hamilton AD (2005) Synthesis of a 2,3′;6′, 3′′-terpyridine scaffold as an alpha-helix mimetic. *Org Lett* 7:5405–5408. doi:10.1021/ol0521228
- Yin H, Lee GI, Sedey KA, Kutzki O, Park HS, Omer BP, Ernst JT, Wang HG, Sebt SM, Hamilton AD (2005) Terphenyl-based bak BH3 alpha-helical proteomimetics as low-molecular-weight antagonists of Bcl-X-L. *J Am Chem Soc* 127:10191–10196. doi:10.1021/ja050122x
- Ahn JM, Han SY (2007) Facile synthesis of benzamides to mimic an alpha-helix. *Tetrahedron Lett* 48:3543–3547. doi:10.1016/j.tetlet.2007.03.108
- Oguri H, Tanabe S, Oomura A, Umetsu M, Hirama M (2006) Synthesis and evaluation of alpha-helix mimetics based on a trans-fused polycyclic ether: sequence-selective binding to aspartate pairs in alpha-helical peptides. *Tetrahedron Lett* 47:5801–5805. doi:10.1016/j.tetlet.2006.05.170
- Biros SM, Moisan L, Mann E, Carella A, Zhai D, Reed JC, Rebek J (2007) Heterocyclic alpha-helix mimetics for targeting protein–protein interactions. *Bioorg Med Chem Lett* 17:4641–4645. doi:10.1016/j.bmcl.2007.05.075
- Volonterio A, Moisan L, Rebek J (2007) Synthesis of pyridazine-based scaffolds as alpha-helix mimetics. *Org Lett* 9:3733–3736. doi:10.1021/ol701487g
- Prakesch M, Denisov AY, Naim M, Gehring K, Arya P (2008) The discovery of small molecule chemical probes of Bcl-X-L and Mcl-1. *Bioorg Med Chem* 16:7443–7449. doi:10.1016/j.bmc.2008.06.023
- Grasberger BL, Lu TB, Schubert C, Parks DJ, Carver TE, Koblish HK, Cummings MD, LaFrance LV, Milkiewicz KL, Calvo RR et al (2005) Discovery and cocrystal structure of benzodiazepinedione HDM2 antagonists that activate p53 in cells. *J Med Chem* 48:909–912. doi:10.1021/jm049137g

20. Cummings MD, Schubert C, Parks DJ, Calvo RR, LaFrance LV, Lattanze J, Milkiewicz KL, Lu TB (2006) Substituted 1,4-benzodiazepine-2,5-diones as alpha-helix mimetic antagonists of the HDM2-p53 protein-protein interaction. *Chem Biol Drug Design* 67:201–205. doi:[10.1111/j.1747-0285.2006.00365.x](https://doi.org/10.1111/j.1747-0285.2006.00365.x)
21. Raboisson P, Marugan JJ, Schubert C, Koblish HK, Lu TB, Zhao SY, Player MR, Maroney AC, Reed RL, Huebert ND et al (2005) Structure-based design, synthesis, and biological evaluation of novel 1,4-diazepines as HDM2 antagonists. *Bioorg Med Chem Lett* 15:1857–1861. doi:[10.1016/j.bmcl.2005.02.018](https://doi.org/10.1016/j.bmcl.2005.02.018)
22. Antuch W, Menon S, Chen QZ, Lu YC, Sakamuri S, Beck B, Schauer-Vukasinovic V, Agarwal S, Hess S, Domling A (2006) Design and modular parallel synthesis of a MCR derived alpha-helix mimetic protein-protein interaction inhibitor scaffold. *Bioorg Med Chem Lett* 16:1740–1743. doi:[10.1016/j.bmcl.2005.11.102](https://doi.org/10.1016/j.bmcl.2005.11.102)
23. Domling A, Antuch W, Beck B, Schauer-Vukasinovic V (2008) Isosteric exchange of the acylsulfonamide moiety in Abbott's Bcl-X-L protein interaction antagonist. *Bioorg Med Chem Lett* 18:4115–4117. doi:[10.1016/j.bmcl.2008.05.096](https://doi.org/10.1016/j.bmcl.2008.05.096)
24. Zhu J, Bienaymé H (2005) Multicomponent reactions. Wiley, Weinheim
25. Hulme C, Dietrich J (2009) Emerging molecular diversity from the intra-molecular Ugi reaction: iterative efficiency in medicinal chemistry. *Mol Divers* 13:195–207. doi:[10.1007/s11030-009-9111-6](https://doi.org/10.1007/s11030-009-9111-6)
26. Akritopoulou-Zanze I (2008) Isocyanide-based multicomponent reactions in drug discovery. *Curr Opin Chem Biol* 12:324–331. doi:[10.1016/j.cbpa.2008.02.004](https://doi.org/10.1016/j.cbpa.2008.02.004)
27. Domling A (2006) Recent developments in isocyanide based multicomponent reactions in applied chemistry. *Chem Rev* 106:17–89. doi:[10.1021/cr0505728](https://doi.org/10.1021/cr0505728)
28. Hulme C, Nixey T (2003) Rapid assembly of molecular diversity via exploitation of isocyanide-based multi-component reactions. *Curr Opin Drug Discov Develop* 6: 921–929
29. Zhu JP (2003) Recent developments in the isonitrile-based multicomponent synthesis of heterocycles. *Eur J Org Chem* 1133–1144. doi:[10.1002/ejoc.200390167](https://doi.org/10.1002/ejoc.200390167)
30. Banfi L, Basso A, Guanti G, Lecinska P, Riva R (2008) Multicomponent synthesis of benzoxazinones via tandem Ugi/Mitsunobu reactions: an unexpected cine-substitution. *Mol Divers* 12:187–190. doi:[10.1007/s11030-008-9087-7](https://doi.org/10.1007/s11030-008-9087-7)
31. Banfi L, Basso A, Guanti G, Lecinska P, Riva R, Rocca V (2007) Multicomponent synthesis of novel 2- and 3-substituted dihydrobenzo[1,4]oxazepinones and tetrahydrobenzo[1,4]diazepin-5-ones and their conformational analysis. *Heterocycles* 73: 699–728
32. Banfi L, Basso A, Guanti G, Kielland N, Repetto C, Riva R (2007) Ugi multicomponent reaction followed by an intramolecular nucleophilic substitution: convergent multicomponent synthesis of 1-sulfonyl 1,4-diazepan-5-ones and of their benzo-fused derivatives. *J Org Chem* 72:2151–2160. doi:[10.1021/jo062626z](https://doi.org/10.1021/jo062626z)
33. Banfi L, Basso A, Guanti G, Lecinska P, Riva R (2006) Multicomponent synthesis of dihydrobenzoxazepinones by coupling Ugi and Mitsunobu reactions. *Org Biomol Chem* 4:4236–4240. doi:[10.1039/b613056a](https://doi.org/10.1039/b613056a)
34. Developed and sold by CambridgeSoft, 100 CambridgePark Drive, Cambridge, MA 02140 USA. URL: <http://www.cambridgesoft.com/>
35. Kutzki O, Park HS, Ernst JT, Orner BP, Yin H, Hamilton AD (2002) Development of a potent Bcl-x(L) antagonist based on alpha-helix mimicry. *J Am Chem Soc* 124:11838–11839. doi:[10.1021/ja026861k](https://doi.org/10.1021/ja026861k)
36. Lugovskoy AA, Degterev AI, Fahmy AF, Zhou P, Gross JD, Yuan J, Wagner G (2002) A novel approach for characterizing protein ligand complexes: molecular basis for specificity of small-molecule Bcl-2 inhibitors. *J Am Chem Soc* 124:1234–1240. doi:[10.1021/ja011239y](https://doi.org/10.1021/ja011239y)
37. Feng W, Huang SY, Wu H, Zhang MJ (2007) Molecular basis of Bcl-xL's target recognition versatility revealed by the structure of Bcl-xL in complex with the BH3 domain of beclin-1. *J Mol Biol* 372:223–235. doi:[10.1016/j.jmb.2007.06.069](https://doi.org/10.1016/j.jmb.2007.06.069)
38. Buckley D, Thomas J (1971) Synthesis of alkylsalicylic acids as antimicrobial agents. *J Med Chem* 14:265. doi:[10.1021/jm00285a031](https://doi.org/10.1021/jm00285a031)
39. Trampusch KN, F. KH, Blau M (1983) Radioiodine-labeled N,N-dimethyl-N'-[2-hydroxy-3-alkyl-5-iodobenzyl]-1,3-propanediamines for brain perfusion imaging. *J Med Chem* 26:121–125. doi:[10.1021/jm00356a001](https://doi.org/10.1021/jm00356a001)
40. Gano KW, Myles DC (2000) Progress toward the synthesis of a biomimetic membrane. *Tetrahedron Lett* 41:4247–4250. doi:[10.1016/S0040-4039\(00\)00597-9](https://doi.org/10.1016/S0040-4039(00)00597-9)
41. Hofsløkken NU, Skattebøl L (1999) Convenient method for the ortho-formylation of phenols. *Acta Chem Scand* 53:258–262. doi:[10.3891/acta.chem.scand.53-0258](https://doi.org/10.3891/acta.chem.scand.53-0258)
42. Verner E, Katz BA, Spencer JR, Allen D, Hataye J, Hruzewicz W, Hui HC, Kolesnikov A, Li Y, Luong C et al (2001) Development of serine protease inhibitors displaying a multicentered short (<2.3 angstrom) hydrogen bond binding mode: inhibitors of urokinase-type plasminogen activator and factor Xa. *J Med Chem* 44:2753–2771. doi:[10.1021/jm0100638](https://doi.org/10.1021/jm0100638)
43. Kundu NG, Pal M, Mahanty JS, De M (1997) Palladium-catalysed heteroannulation with acetylenic compounds: synthesis of benzofurans. *J Chem Soc, Perkin Trans 1*:2815–2820. doi:[10.1039/a703305b](https://doi.org/10.1039/a703305b)
44. Feldman KS, Eastman KJ, Lessene G (2002) Diazonamide synthesis studies: use of Negishi coupling to fashion diazonamide-related biaryls with defined axial chirality. *Org Lett* 4:3525–3528. doi:[10.1021/ol026694t](https://doi.org/10.1021/ol026694t)
45. Lee YR, Wang X (2005) A short synthetic route to biologically active (±)-daurichromenic acid as highly potent anti-HIV agent. *Org Biomol Chem* 3:3955–3957. doi:[10.1039/b508577b](https://doi.org/10.1039/b508577b)
46. Incarvito C, Lam M, Rhatigan B, Rheingold AL, Qin CJ, Gavrilova AL, Bosnich B (2001) Bimetallic reactivity. Preparations, properties and structures of complexes formed by unsymmetrical binucleating ligands bearing 4- and 6-coordinate sites supported by alkoxide bridges. *J Chem Soc, Dalton Trans* 3478–3488. doi:[10.1039/b103164n](https://doi.org/10.1039/b103164n)
47. Buchanan JL, Vu CB, Merry TJ, Corpuz EG, Pradeepan SG, Mani UN, Yang M, Plake HR, Varkhedkar VM, Lynch BA et al (1999) Structure-activity relationships of a novel class of Src SH2 inhibitors. *Bioorg Med Chem Lett* 9:2359–2364. doi:[10.1016/S0960-894X\(99\)00389-3](https://doi.org/10.1016/S0960-894X(99)00389-3)
48. Liu J, Ikemoto N, Petrillo D, Armstrong JD (2002) Improved syntheses of alpha-BOC-aminoketones from alpha-BOC-amino-Weinreb amides using a pre-deprotonation protocol. *Tetrahedron Lett* 43:8223–8226. doi:[10.1016/S0040-4039\(02\)02031-2](https://doi.org/10.1016/S0040-4039(02)02031-2)
49. Bartoli G, Bosco M, Carlone A, Locatelli M, Melchiorre P, Sambri L (2004) Asymmetric catalytic synthesis of enantiopure N-protected 1,2-amino alcohols. *Org Lett* 6:3973–3975. doi:[10.1021/ol048322l](https://doi.org/10.1021/ol048322l)
50. Kauch M, Hoppe D (2006) Synthesis of halogenated phenols by directed ortho-lithiation and ipso-iododesilylation reactions of O-aryl N-isopropylcarbamates. *Synthesis* 1578–1589. doi:[10.1055/s-2006-926462](https://doi.org/10.1055/s-2006-926462)
51. Brown P, Davies DT, Ohanlon PJ, Wilson JM (1996) The chemistry of pseudomonic acid. 15. Synthesis and antibacterial activity of a series of 5-alkyl, 5-alkenyl, and 5-heterosubstituted oxazoles. *J Med Chem* 39:446–457. doi:[10.1021/jm9503862](https://doi.org/10.1021/jm9503862)