RSC Advances

PAPER

Cite this: RSC Adv., 2015, 5, 104187

Design, synthesis and conformational analyses of bifacial benzamide based foldamers[†]

Silvia Rodriguez-Marin,^{ab} Natasha S. Murphy,^{ab} Helena J. Shepherd^c and Andrew J. Wilson^{*ab}

The design, synthesis and conformational analyses of novel backbones represents a key focus of research that underpins efforts to exploit foldamers (i) in a biological setting *e.g.* as inhibitors of protein–protein interactions (PPIs) and (ii) for the purposes of constructing functional architectures that adopt defined tertiary and quaternary folds. The current manuscript addresses a need to develop aromatic oligoamide backbones that are regioisomeric in terms of backbone connectivity and/or functionalized on more than one face. We describe the design, synthesis and comparative conformational analyses of foldamers derived from 2-, 3- and 2,5-O-alkylated derivatives of *para*-aminobenzoic acid, and, derived from 2-,3- and 2,5-O-alkylated derivatives of 1,4-diaminobenzene/terephthalic acid monomers. Analysis of the accessible conformational space for these oligomers indicates that despite different connectivity they can adopt conformations that position side chains in a manner that mimic the *i*, *i* + 3, *i* + 4 of an α -helix.

Received 2nd October 2015 Accepted 20th November 2015

DOI: 10.1039/c5ra20451h

www.rsc.org/advances

Introduction

Protein-protein interactions (PPIs) are considered difficult targets for drug discovery due to their large and moderately convex surfaces, and the availability of fewer "obvious" welldefined binding sites when compared to classical enzymes and receptors.¹ Among the wide range of PPI recognition motifs, the α -helix is the most common secondary structure in nature and thus represents a good generic template for inhibitor design.^{2,3} The proteomimetic⁴ approach utilises suitably functionalized non-peptidic foldamers^{5,6} (oligomers that adopt well defined conformations) to topographically mimic the spatial orientation of the key recognition residues on the native a-helix surface (Fig. 1a). Most of these scaffolds mimic the i, i + 4 and i + 47 residues on a single face.^{4,7-12} In particular oligobenzamides have been described as effective proteomimetics by our group and others;9,13-17 they may be accessed through robust modular solution¹⁸ and solid phase syntheses,^{19,20} and, as is typical for aromatic oligoamide foldamers²¹⁻²⁴ adopt reasonably predictable conformations. Most published studies focus on the design of oligobenzamides mimicking the key residues located on one

face of the α -helix; however, there are also examples of these scaffolds mimicking more than one face.^{25–27} In the context of foldamer synthesis and structure, the construction of backbones functionalised with different side-chains on multiple faces of the scaffold represents an as yet unrealised approach to achieve control over secondary conformation and higher order tertiary/quaternary organisation. Similarly, there is an obvious need for PPI inhibiting helix mimetics that target more than one face of an interaction, such as the case of the estrogen receptor (ER), a ligand-activated transcription factor that plays a key role in the development of certain cancers.^{28,29} In response to binding with its natural ligand, ER undergoes a conformational change to promote recruitment of co-regulators, thereby up- or down-regulating the expression of specific genes.³⁰ The



Fig. 1 (a) Bifacial helix mimetics as inhibitors of PPIs (steroid ligand in orange, ER in purple, α -helix containing co-activator in red and key side chain residues represented as coloured circles). (b) Crystal structure of the ER α (in purple) bound to an LXXLL co-activator motif (in red) (PDB ID: 3ERD) (charge clamp shown in green).⁴⁰



View Article Online

View Journal | View Issue

[&]quot;School of Chemistry, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, UK. E-mail: J.Wilson@leeds.ac.uk

^bAstbury Centre for Structural Molecular Biology, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT, UK

^cSchool of Physical Sciences, University of Kent, Ingram Building, Canterbury, Kent, CT2 7NH, UK

[†] Electronic supplementary information (ESI) available: Synthetic procedures, characterisation, modelling and docking studies. CCDC 1426299. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c5ra20451h

nuclear receptor box is an alpha helical LXXLL motif (where L is leucine and X any amino acid), which acts as a recognition element between co-activators and their receptors (Fig. 1b).³¹ Direct inhibition of the receptor/co-activator protein-protein interaction,³²⁻³⁷ notably using helix mimetics^{35,36,38} is of potential therapeutic interest as an alternative to the use of competitive inhibitors for the ligand binding site.39 Herein, we introduce two bifacial proteomimetic scaffolds; bis-benzamide and N-(4-aminophenyl)terephthalamidic as novel foldamers designed as tools to (a) enhance our understanding of aromatic oligoamide foldamer conformation and (b) ligands that could mimic the key side chains at *i*, i + 3, i + 4 positions of α -helices that participate in PPIs mediated by such a side chain constellation. A comprehensive analysis of the different scaffolds reveals that different combinations of monomers lead to a plethora of side chain spatial relationships which effectively mimic the intended α -helix side-chains.

Results and discussion

A first generation of scaffold 1-4 (Fig. 2) was designed using the modular oligobenzamide synthetic methodology previously reported by our group (Schemes S2 and S3†).^{15,41,42} Combinations of 3-O-alkylated, 2-O-alkylated and 2,5-O-dialkylated monomers were used to obtain a regioisomeric set of compounds for conformational analyses. The regioisomer of compound 2 could not be obtained due to unsuccessful coupling between methyl 4-amino-2,5-diisobutoxybenzoate and 4-nitro-2-isobutoxybenzoic acid under multiple conditions. The tetrasubstituted

scaffold (4) was also synthesised to explore the role of a 4th side chain in helix mimicry.

A novel second generation scaffold was designed based on a N-(4-aminophenyl)terephthalamidic acid backbone, where the central amide bond is inverted in comparison with the bisbenzamide scaffold. The dimer is formed from a para-phenylenediamine monomer linked to a terephthalate monomer through an amide bond. The backbone can be functionalized at different positions using a variety of O-alkylated monomers. A convergent synthetic methodology was developed to provide the monomeric building blocks (Scheme 1). Noteworthy features of the monomer syntheses include: (i) the use of a Curtius rearrangement to convert monomer 6 to 7 rendering this synthetically efficient for both building blocks (ii) the use of a common starting material 8 to access monomers 9 and 10 for construction of different regioisomers (see below). Note also that no Nalkylation was observed on transformation of 8 to 9. For monosubstituted alkoxy derivatives of terephthalic acid, it was necessary to perform a sequence of protecting group manipulations.

To effect amide bond formation, the acyl chloride of the diacid monomer **6** was obtained using thionyl chloride before coupling to its amino-monomer partners **9** or **10** (Scheme 2). By using an excess of the diacid **6** it was possible to bias the product distribution towards the monoamide. The final products **14ba** and **14bb** were obtained by hydrogenation of the nitro group or hydrolysis of the Fmoc group respectively. Due to oxidation upon exposure to air, the diamine derivative of compound 7 was obtained through *in situ* Boc deprotection and direct reaction with



Fig. 2 Minimal benzamide foldamers comprising 3-*O*, 2-*O*, 2,5-*O* alkylated *p*-aminobenzoic acid monomers.



Scheme 1 Synthesis of monomers for N-(4-aminophenyl)terephthalamidic derived foldamers.

Paper



the acid chloride derivative of **12** or **13**; these were obtained by *in situ* activation using Ghosez's reagent. Again, the monoamide product was biased by using the starting diamine in excess. The final compounds **14aa** and **14ab** were obtained after appropriate deprotection sequences (Scheme 2) specifically acid mediated Boc deprotection and ethyl ester hydrolysis respectively. Despite numerous efforts we were unable to isolate a tetrasubstituted foldamer derived from **6** and **7**.

Previous studies on 2- and 3-O-alkylated trimers and model dimers on the oligobenzamide scaffold revealed intramolecular pseudo-six- or five-membered hydrogen bonding between the NH and adjacent O-alkyl group.15,43 This results in restricted rotation around one of the Ar-CO or Ar-NH bonds leaving the other free to rotate. The conformation of such scaffolds can be further restricted by introduction of a second alkoxy group leading to a "bifurcated" hydrogen bonding interaction, where the NH is located between two phenolic oxygens from adjacent monomers forming pseudo-six- and five-membered rings.42,44,45 In principle, the set of compounds discussed here can display a similar array of conformations, resulting in different projections of the alkoxy side chains and a distribution of 3D structures some of which effectively mimic an i, i + 3, i + 4 helical pharmacophore. Consequently, structural and conformational analyses were performed on each compound (summarised in Fig. 3a-d). Compounds 2 and 3 form pseudo-five- or sixmembered hydrogen-bonded rings whereas the ¹H-¹H 2D NOESY spectra for compound 1 were indicative of both pseudofive- and six-membered hydrogen-bonded rings being populated in solution.

An X-ray crystal structure of compound 1 reinforces this result with NH to O distances of 2.007 and 2.223 Å respectively for the S(6) and S(5) H-bonded rings (Fig. 3c). Intriguingly, similar NMR analyses for compound 4 were indicative only of bonding in pseudo-six-membered hydrogen solution. Compounds 14aa, 14bb form pseudo-five- or six-membered hydrogen-bonded rings as expected whereas 14ab (Fig. 3b) and 14ba showed evidence of only pseudo-six-membered intramolecular hydrogen bonded ring formation in solution. These results are supported by H/D exchange experiments performed on compounds 1, 2 and 14ba as models of the three types of intramolecular hydrogen bonding interaction (Fig. 3d, S1[†] and Table 1). The rate of exchange is entirely consistent with that which is observed for S(6) type hydrogen bonded rings for the regioisomeric oligomers derived from 2-O-alkoxy-4aminobenzoic acid and suggests the different electronic structure of the 2,5-dialkoxyterephthalamide monomer does not dramatically affect the strength of hydrogen-bonding.

Molecular modelling was also performed on all the compounds (Fig. 3a). The lowest energy conformations all adopt an extended structure, where the amide bond is *trans*. The low energy conformations for each compound are consistent with those that are accessible in solution phase according to the NOESY data. The nature of the structure permits the superimposition in both parallel and antiparallel N-to-C orientation



Fig. 3 (a) Preferred conformation and intramolecular hydrogen-bonding interactions of the compounds 1–3 and 14 supported by molecular modelling, 2D NMR studies and H/D exchange experiments. Distances and angles between side chains (green and black respectively), H-bonds (dashed red line) and free rotation axes (red arrow) are shown. (b) ${}^{1}H{}^{-1}H$ NOESY (10 mM, 500 MHz CDCl₃) of 14ba (c) X-ray structure of 1 (H-bonding distances (Å) are shown in red) (d) H/D exchange data for 1, 2 and 14ba.

Table 1 Kinetic constants and $t_{1/2}$ based on H/D exchange in 10% CD_3OD/CDCl_3

	$k_{\mathrm{H/D}} (\mathrm{min}^{-1})$	$t_{1/2}$ (min)	H bonding
1 2 14ba	$\begin{array}{c} 6.7857 \times 10^{-4} \pm 0.0000093 \\ 0.01485 \pm 0.00017 \\ 0.00305 \pm 0.00005 \end{array}$	$1021.5 \pm 14 \\ 46.7 \pm 0.5 \\ 228 \pm 3$	S(5)/S(6) S(5) S(6)

with respect to an α -helical peptide.¹⁹ Accordingly, both alignments were analysed using an ER α co-activator sequence. The match was assessed on the basis of the RMSD between α -carbons on the helix and oxygen atoms on the foldamer together with an evaluation on the quality of orientation with respect to the helical axis of the peptide (Table 2 and ESI† for details). For compounds **1–3**, **14aa** and **14bb**, in the poses presenting the best overlay, the three side chains overlap reasonably well with the leucine residues at positions *i*, *i* + 3 and *i* + 4 of the co-activator helix (Fig. 3a for **14aa** and Fig. S5–S11† for other dimers) and the distances between the oxygens of the dimers match the distance between the α -CH of those residues. Compounds **14ab** and **14ba**, matched less well in terms of alignment with the helical backbone.

Docking studies using the lowest energy conformation of each foldamer were also performed to ascertain the extent to which they might act as ERa/co-activator inhibitors (see ESI[†]). The results from the docking analyses reveal binding poses that display favourable interaction of the foldamers 1-3 and 14 with the coactivator binding groove. Electrostatic interactions are observed for both termini of the foldamer. However, in all cases only one of these involves the precise "charge clamp" residues from ER exploited by co-activator ligands. Shown in Fig. 4b is a good pose for 14aa; the three hydrophobic side chains of the foldamer occupy the hydrophobic space normally occupied by the coactivator peptide. The terminal carboxamide and aniline groups of the dimer are suitably positioned to form electrostatic interactions with glutamic acid (542) and glutamine 372 (rather than lysine 362) in the region of the "charge clamp". This behaviour is reproduced for the other compounds (e.g. Fig. S12[†] for 1).

To perform a preliminary assessment of the ability of these compounds to act as PPI inhibitors, we carried out fluorescence polarisation competition assays for three nuclear receptor/co-

	Alignment ^a	RMSD	
1	Antiparallel	1.322	
2	Parallel	1.625	
3	Antiparallel	2.084	
14aa	Antiparallel	1.038	
14ab	No good alignment	1.622	
14ba	No good alignment	1.027	
14bb	Parallel	2.046	

^{*a*} Where N and C termini of the benzamide and helix match, they are defined as being parallel and where they oppose, they are defined as being antiparallel.



Fig. 4 (a) Overlay of compound 14aa with a co-activator peptide. Coactivator residues are in dark colours and helix mimetic residues are in light colours (side and top views are given). (b) Potential binding mode of compound 14aa in the ER co-activator-binding groove with the native helix in transparent red.

activator interactions (ER α /SrcBox2, ER β /Src1B2 and RXR α /D22), however the compounds were not sufficiently potent to show a significant effect in these assays. Future studies will focus on the synthesis of libraries bearing different side-chains and terminal groups together with a broader array of biophysical and cellular assays to explain this observation and identify potent inhibitors.

Conclusions

In conclusion, we have described the design, synthesis and comparative structural/conformational analyses of two minimal bifacial foldamer scaffolds, using for first time a N-(4aminophenyl)terephthalamidic acid. The synthetic route to the N-(4-aminophenyl)terephthalamidic acid scaffold has been readily adapted to obtain a multitude of analogues displaying diverse side chain spacing. Furthermore, the ability of these scaffolds to mimic more than one face of an α-helix has been assessed using molecular modelling. These new foldamer backbones should be entirely compatible with the plethora of aromatic oligoamide backbones and could readily be utilised to construct well defined secondary and tertiary structures by exploiting side-chain/side-chain interactions as appropriate. In future, our own efforts will focus on exploiting these aromatic benzamide scaffolds as components of proteomimetic inhibitors of PPIs.

Acknowledgements

This work was supported by the European Research Council [ERC-StG-240324] and [ERC-PoC-632207]. We thank Prof Luc Brunsveld and Marcel Scheepstra (Technische Universiteit Eindhoven) for assistance in performing preliminary ER/co-activator assays. We also thank Dr Chris Pask (University of Leeds) for assistance with Crystallographic data deposition.

References

- 1 S. Surade and T. L. Blundell, Chem. Biol., 2012, 19, 42-50.
- 2 B. N. Bullock, A. L. Jochim and P. S. Arora, *J. Am. Chem. Soc.*, 2011, **133**, 14220–14223.
- 3 V. Azzarito, K. Long, N. S. Murphy and A. J. Wilson, *Nature Chem.*, 2013, 5, 161–173.
- 4 B. P. Orner, J. T. Ernst and A. D. Hamilton, *J. Am. Chem. Soc.*, 2001, **123**, 5382–5383.
- 5 S. H. Gellman, Acc. Chem. Res., 1998, 31, 173-180.
- 6 S. Fahs, Y. Patil-Sen and T. J. Snape, *ChemBioChem*, 2015, **16**, 1840–1853.
- 7 D. Xin, L. M. Perez, T. R. Ioerger and K. Burgess, *Angew. Chem. Int. Ed.*, 2014, **53**, 3594–3598.
- 8 G. Schäfer, J. Milić, A. Eldahshan, F. Götz, K. Zühlke, C. Schillinger, A. Kreuchwig, J. M. Elkins, K. R. Abdul Azeez, A. Oder, M. C. Moutty, N. Masada, M. Beerbaum, B. Schlegel, S. Niquet, P. Schmieder, G. Krause, J. P. von Kries, D. M. F. Cooper, S. Knapp, J. Rademann, W. Rosenthal and E. Klussmann, *Angew. Chem. Int. Ed.*, 2013, **52**, 12187–12191.
- 9 J. L. Yap, X. Cao, K. Vanommeslaeghe, K.-Y. Jung, C. Peddaboina, P. T. Wilder, A. Nan, A. D. MacKerell, W. R. Smythe and S. Fletcher, *Org. Biomol. Chem.*, 2012, 10, 2928–2933.
- 10 J.-M. Ahn and S.-Y. Han, *Tetrahedron Lett.*, 2007, **48**, 3543–3547.
- 11 W. E. Martucci, J. M. Rodriguez, M. A. Vargo, M. Marr, A. D. Hamilton and K. S. Anderson, *MedChemComm*, 2013, 4, 1247–1256.
- 12 P. Tošovská and P. S. Arora, Org. Lett., 2010, 12, 1588-1591.
- 13 F. Campbell, J. P. Plante, T. A. Edwards, S. L. Warriner and A. J. Wilson, *Org. Biomol. Chem.*, 2010, 8, 2344–2351.
- 14 J. P. Plante, T. Burnley, B. Malkova, M. E. Webb, S. L. Warriner, T. A. Edwards and A. J. Wilson, *Chem. Commun.*, 2009, 5091–5093.
- 15 V. Azzarito, P. Prabhakaran, A. I. Bartlett, N. S. Murphy, M. J. Hardie, C. A. Kilner, T. A. Edwards, S. L. Warriner and A. J. Wilson, *Org. Biomol. Chem.*, 2012, **10**, 6469–6472.
- 16 A. Barnard, K. Long, H. L. Martin, J. A. Miles, T. A. Edwards, D. C. Tomlinson, A. Macdonald and A. J. Wilson, *Angew. Chem. Int. Ed.*, 2015, 54, 2960–2965.
- 17 V. Azzarito, J. A. Miles, J. Fisher, T. A. Edwards, S. L. Warriner and A. J. Wilson, *Chem. Sci.*, 2015, **6**, 2434–2443.
- 18 G. M. Burslem and A. J. Wilson, Synlett, 2014, 25, 324-335.
- 19 N. S. Murphy, P. Prabhakaran, V. Azzarito, J. P. Plante, M. J. Hardie, C. A. Kilner, S. L. Warriner and A. J. Wilson, *Chem. Eur. J.*, 2013, **19**, 5546–5550.
- 20 K. Long, T. A. Edwards and A. J. Wilson, *Bioorg. Med. Chem.*, 2013, **21**, 4034–4040.

- 21 I. Huc, Eur. J. Org. Chem., 2004, 2004, 7.
- 22 A. Roy, P. Prabhakaran, P. K. Baruah and G. J. Sanjayan, *Chem. Commun.*, 2011, **47**, 11593–11611.
- 23 B. Gong, Chem. Eur. J., 2001, 7, 4336-4342.
- 24 D.-W. Zhang, X. Zhao, J.-L. Hou and Z.-T. Li, *Chem. Rev.*, 2012, **112**, 5271–5316.
- 25 S. Marimganti, M. N. Cheemala and J. M. Ahn, *Org. Lett.*, 2009, **11**, 4418–4421.
- 26 S. Thompson, R. Vallinayagam, M. J. Adler, R. T. W. Scott and A. D. Hamilton, *Tetrahedron*, 2012, **68**, 4501–4505.
- 27 I. C. Kim and A. D. Hamilton, Org. Lett., 2006, 8, 1751-1754.
- 28 E. P. Gelmann, J. Clin. Oncol., 2002, 20, 3001-3015.
- 29 M. H. Herynk and S. A. W. Fuqua, *Endocr. Rev.*, 2004, **25**, 869–898.
- 30 R. Evans, Science, 1988, 240, 889-895.
- 31 K. A. Green and J. S. Carroll, *Nat. Rev. Cancer*, 2007, 7, 713–722.
- 32 J. R. Gunther, T. W. Moore, M. L. Collins and J. A. Katzenellenbogen, *ACS Chem. Biol.*, 2008, **3**, 282–286.
- 33 A. A. Parent, J. R. Gunther and J. A. Katzenellenbogen, *J. Med. Chem.*, 2008, **51**, 6512–6530.
- 34 J. R. Gunther, A. A. Parent and J. A. Katzenellenbogen, *ACS Chem. Biol.*, 2009, 4, 435–440.
- 35 J. Becerril and A. D. Hamilton, *Angew. Chem. Int. Ed.*, 2007, **46**, 4471–4473.
- 36 M. Scheepstra, L. Nieto, A. K. H. Hirsch, S. Fuchs, S. Leysen, C. V. Lam, L. in het Panhuis, C. A. A. van Boeckel, H. Wienk, R. Boelens, C. Ottmann, L.-G. Milroy and L. Brunsveld, *Angew. Chem. Int. Ed.*, 2014, 53, 6443–6448.
- 37 P. Ravindranathan, T. K. Lee, L. Yang, M. M. Centenera, L. Butler, W. D. Tilley, J. T. Hsieh, J. M. Ahn and G. V. Raj, *Nat. Commun.*, 2013, 4, e1923.
- 38 A. B. Williams, P. T. Weiser, R. N. Hanson, J. R. Gunther and J. A. Katzenellenbogen, *Org. Lett.*, 2009, **11**, 5370–5373.
- 39 M.-E. Taplin, Nat Clin Prac Oncol, 2007, 4, 236-244.
- 40 A. K. Shiau, D. Barstad, P. M. Loria, L. Cheng, P. J. Kushner, D. A. Agard and G. L. Greene, *Cell*, 1998, **95**, 927–937.
- 41 J. Plante, F. Campbell, B. Malkova, C. Kilner, S. L. Warriner and A. J. Wilson, *Org. Biomol. Chem.*, 2008, **6**, 138–146.
- 42 P. Prabhakaran, A. Barnard, N. S. Murphy, C. A. Kilner, T. A. Edwards and A. J. Wilson, *Eur. J. Org. Chem.*, 2013, 3504–3512.
- 43 P. Prabhakaran, V. Azzarito, T. Jacobs, M. J. Hardie, C. A. Kilner, T. A. Edwards, S. L. Warriner and A. J. Wilson, *Tetrahedron*, 2012, **68**, 4485–4491.
- 44 K. Yamato, L. Yuan, W. Feng, A. J. Helsel, A. R. Sanford, J. Zhu, J. Deng, X. C. Zeng and B. Gong, *Org. Biomol. Chem.*, 2009, 7, 3643–3647.
- 45 J. Zhu, R. D. Parra, H. Zeng, E. Skrzypczak-Jankun, X. C. Zeng and B. Gong, *J. Am. Chem. Soc.*, 2000, **122**, 4219–4220.