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► **To cite this version:**

Olivier Roy, Geoffrey Dumonteil, Sophie Faure, Laurent Jouffret, Alexandre Kriznik, et al.. Homogeneous and Robust Polyproline Type I Helices from Peptoids with Nonaromatic  $\alpha$ -Chiral Side Chains. Journal of the American Chemical Society, 2017, 139 (38), pp.13533 - 13540. 10.1021/jacs.7b07475 . hal-01656039

**HAL Id: hal-01656039**

**<https://hal.science/hal-01656039>**

Submitted on 3 Feb 2022

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# Homogeneous and robust PolyProline type I helices from peptoids with non-aromatic $\alpha$ -chiral side chains

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**ABSTRACT:** Peptoids which are oligomers of *N*-substituted glycines represent a class of peptide mimics with great potential in areas ranging from medicinal chemistry to biomaterial science. Controlling the equilibria between the *cis* and *trans* conformations of their backbone amides is the major hurdle to overcome for the construction of discrete folded structures, particularly for the development of all-*cis* PolyProline-type I (PPI) helices, as tools for modulating biological functions. The prominent role of backbone to side chain electronic interactions ( $n \rightarrow \pi^*$ ) and side chains bulkiness in promoting *cis*-amides was essentially investigated with peptoid aromatic side chains among which the chiral 1-naphthylethyl (*Inpe*) group yielded the best results. We have explored for the first time the possibility to achieve similar performances with a sterically hindered  $\alpha$ -chiral aliphatic side chain. Herein, we report on the synthesis and detailed conformational analysis of a series of (*S*)-*N*-(1-*tert*-butylethyl)glycine (*Ns1tbe*) peptoid homooligomers. The X-ray crystal structure of an *Ns1tbe* pentamer revealed an all-*cis* PPI helix and the CD curves of the *Ns1tbe* oligomers also resemble those of PPI peptide helices. Interestingly, the CD data reported here are the first for any conformationally homogeneous helical peptoids containing only  $\alpha$ -chiral aliphatic side chains. Finally we also synthesized and analysed two mixed oligomers composed of *NtBu* and *Ns1tbe* monomers. Strikingly, the solid state structure of the mixed oligomer Ac-(*tBu*)<sub>2</sub>-(*s1tbe*)<sub>4</sub>-(*tBu*)<sub>2</sub>-COO*tBu*, the longest to be solved for any linear peptoid revealed a PPI helix of great regularity despite the presence of only 50% of chiral side chain in the sequence.

## 1. INTRODUCTION

Peptoids are artificial oligomers/polymers composed of *N*-Substituted Glycine (NSG) monomers that are becoming a valuable class of compounds in biosciences<sup>1</sup> as well as in important chemical areas including catalysis,<sup>2</sup> self-assembly<sup>3</sup> and metal binding.<sup>4</sup> Compared to natural peptides, the side chains are shifted from the C $\alpha$  carbons to the amide bond nitrogen atoms. This change has important implications on the conformational behaviour of this class of compounds whose main chain is more flexible than that of their parent peptides. This difference is essentially due to the presence of *N,N*-disubstituted amide bonds, often referred as tertiary amides, with no hydrogen bond donor atoms for establishing intramolecular H-bonding interactions, and also to the fact that in most

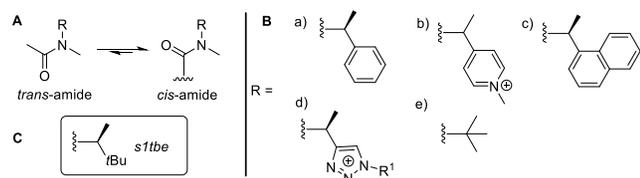
cases the *cis* and *trans* conformations of the amide bonds are very close in energy giving rise to *cis/trans* equilibria.<sup>5</sup> Despite their inherent flexibility, peptoids have been shown to adopt preferential folded conformations, including the PolyProline-type II (PPII)<sup>6</sup> and PolyProline-type I (PPI) helical structures.<sup>7</sup> The potential of foldamer helices from a drug discovery standpoint has been recognized but for some of them a limitation exists in term of diversity of accessible monomers.<sup>8</sup> In the case of peptoids a huge diversity of side chains can be incorporated but only few of them are capable of promoting a stable secondary structure. The canonical PPI helix with all-*cis* amide bonds was the first to be predicted by molecular mechanics calculations from prototypical oligopeptoids composed of chiral (*S*)-*N*-(1-phenylethyl)glycine residues (*spe*).<sup>7</sup> Soon after, the PPI-type conformation was con-

firmed by NMR of the (*Nspe*)<sub>5</sub> homopentamer, the all-*cis* structure being the major solution conformation among a mixture of *cis/trans* isomers.<sup>9</sup> In a recent past, intensive efforts have been made for a clear understanding of the local stereoelectronic factors that govern the *cis/trans* peptoid amides equilibria. This research led to the development of side chains capable of exercising a strong steric and/or electronic control over the amide geometry and consequently on the conformation in the vicinity of the studied amide. Thus, a number of side chains were examined with respect to their ability to promote the *cis* conformation. Those that yielded the best performances to date are presented in Figure 1B.<sup>10,11,12,13</sup> It must be pointed out here that most of them have an aromatic group in their structure which is a limitation in term of physico-chemical properties in a drug discovery context as for example the disruption of protein-protein interactions. In this work we have sought to identify a non-aromatic *cis*-peptoid amide inducer allowing the formation of discrete PPI helices of defined handedness. This challenge was never achieved from peptoid oligomers with  $\alpha$ -chiral aliphatic side chains. Thus our goal was also to describe more precisely the CD signature of non-aromatic PPI peptoid. In view of our understanding of the noncovalent interactions that govern local peptoid folding, the first objective was to identify a chiral aliphatic side chain bringing the maximum of steric hindrance. As a precursor of this ideal side chain, it was also important for this work to identify a commercially primary amine available in the *R* and *S* configurations. We report here the synthesis of a series of (*S*)-*N*-(1-*tert*-butylethyl)glycine (*Nsitbe*) homooligomers and two mixed oligomers composed of *Nsitbe* and *NtBu* monomers, and their detailed structural analysis by NMR spectroscopy, X-ray crystallography and CD analysis.

## 2. RESULTS AND DISCUSSIONS

### 2.1 Synthesis of peptoid oligomers

The commercially available (*2S*)-3,3-dimethylbutan-2-amine was used as the precursor of the *sitbe* side chain. Its enantiomeric *R* form is also commercially available. Peptoids are most often synthesized by the so-called solid-phase submonomer synthesis<sup>14</sup> but this method can result in low yields in case of very bulky amines like the 1-naphthylethyl amine or the *tert*-butyl amine for which



**Figure 1.** (A) *Cis-trans* peptoid amide equilibrium. (B) Chemical structure of side chains promoting the *cis* conformation: a) (*S*)-1-phenylethyl (*spe*); b) 4-methylpyridinium (*4mpy*);<sup>10</sup> c) (*S*)-1-naphthylethyl (*sinpe*);<sup>10,11</sup> d) with R<sup>1</sup> = Bn, 1-(benzyltriazolium)ethyl (*bte*<sup>+</sup>);<sup>12</sup> e) *tert*-butyl (*tBu*).<sup>13</sup>

all attempts of solid-phase synthesis yielded mixtures of truncated (*NtBu*) oligomers.<sup>15</sup> The solution-phase submonomer synthesis was therefore preferred for this study. In order to compare the efficacy of the new *sitbe* side chain to the past work with the *sinpe* side chain,<sup>11a</sup> which can be considered today as the reference for constructing robust peptoid helical structures, we chose to synthesize a series of acetamide-capped (*Nitbe*) homooligomers with *tBu* esters at their C-termini. This series comprises all the oligomers from the monomer till the hexamer (**1-6**), the octamer (**8**) and the nonamer (**9**) (Table 1). The synthesis started by substitution of the bromine atom of *tert*butyl bromoacetate by the *sitbe* amine, yielding a *Nitbe* monomer which was then elongated by the iterative submonomer method consisting in a two-step acylation-substitution cycle. Bromoacetic anhydride, freshly prepared from bromoacetic acid and DCC, was systematically used for the acylation steps and the substitutions were carried out with 3 equivalents of the *sitbe* amine.<sup>13</sup> After each iteration, with the exception of the heptamer stage, a portion of the formed amine was acetylated to yield peptoids **1-6**, **8** and **9**. The same procedure was applied for synthesizing the mixed oligomers **6'** and **8'** (Table 1) by using when required *tBuNH*<sub>2</sub> instead of the *sitbe* amine (see Scheme S1 for details). The *Nsch* hexamer **7** composed of (*S*)-*N*-(1-cyclohexylethyl)glycine units with an acetylated N-terminus and a *tert*-butyl ester at the C-terminus, was also synthesised as a key control to allow for spectroscopic comparison.<sup>16</sup>

**Table 1. Structures for peptoids 1-9, 6' and 8'**

peptoid	Monomer sequence
1	Ac- <i>sitbe</i> -COOtBu
2	Ac-( <i>sitbe</i> ) <sub>2</sub> -COOtBu
3	Ac-( <i>sitbe</i> ) <sub>3</sub> -COOtBu
4	Ac-( <i>sitbe</i> ) <sub>4</sub> -COOtBu
5	Ac-( <i>sitbe</i> ) <sub>5</sub> -COOtBu
6	Ac-( <i>sitbe</i> ) <sub>6</sub> -COOtBu
7	Ac-( <i>sch</i> ) <sub>6</sub> -COOtBu
6'	Ac- <i>tBu</i> ( <i>sitbe</i> ) <sub>4</sub> <i>tBu</i> -COOtBu
8	Ac-( <i>sitbe</i> ) <sub>8</sub> -COOtBu
8'	Ac-( <i>tBu</i> ) <sub>2</sub> ( <i>sitbe</i> ) <sub>4</sub> ( <i>tBu</i> ) <sub>2</sub> -COOtBu
9	Ac-( <i>sitbe</i> ) <sub>9</sub> -COOtBu

### 2.2. NMR analysis of peptoid oligomers

<sup>1</sup>H NMR of monomer **1** was recorded at a concentration of 10 mM in various solvents (CDCl<sub>3</sub>, CD<sub>3</sub>CN and CD<sub>3</sub>OD) for determining the *K*<sub>*cis/trans*</sub> ratios (Table 2). They were calculated by averaging the integration of three sets of related rotamer signals, including when possible the NC $\alpha$  methyne protons. The chemical shift of the methyne proton in monomer **1** is remarkably deshielded in the *cis* conformation relative to the *trans* (~ 1 ppm in CDCl<sub>3</sub>),

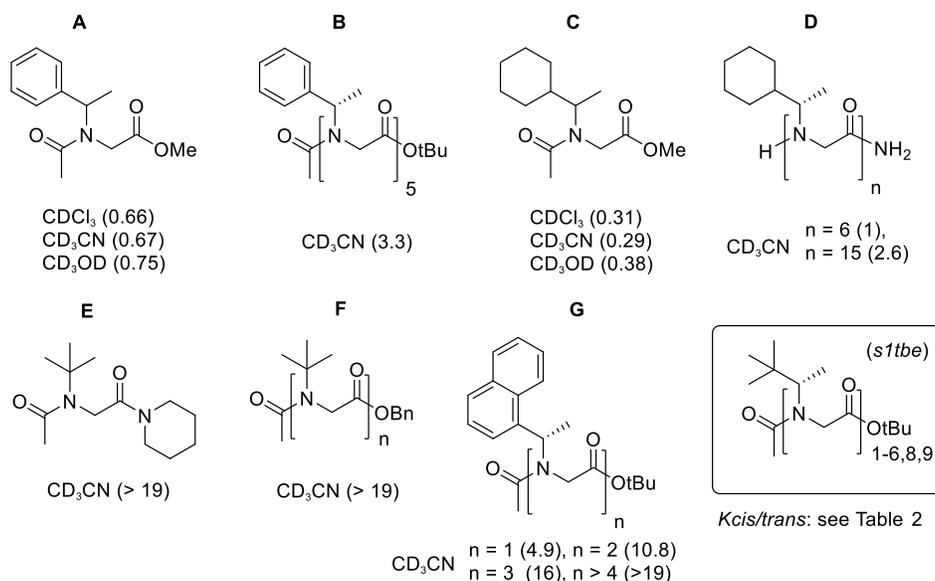
**Table 2. Overall  $K_{cis/trans}$  values as determined by  $^1\text{H}$  NMR integration and/or  $^1\text{H}$ -COSY in  $\text{CDCl}_3$ ,  $\text{CD}_3\text{CN}$  and  $\text{CD}_3\text{OD}$**

peptoid	$\text{CDCl}_3$	$\text{CD}_3\text{CN}$	$\text{CD}_3\text{OD}$
	$K_{cis/trans}$	$K_{cis/trans}$	$K_{cis/trans}$
1	0.64 <sup>a</sup>	0.67 <sup>a</sup>	0.77 <sup>a</sup>
2	0.98 <sup>a</sup>	1.60 <sup>a</sup>	1.68 <sup>a</sup>
3	1.95 <sup>a</sup>	2.61 <sup>a</sup>	3.00 <sup>a</sup>
4	>19 <sup>b</sup>	10.89 <sup>a</sup>	>19 <sup>b</sup>
5	18.92 <sup>a</sup>	7.96 <sup>a</sup>	>19 <sup>b</sup>
6	>19 <sup>a</sup>	10.39 <sup>a</sup>	>19 <sup>b</sup>
7	2.71 <sup>c</sup>	1.39 <sup>c</sup>	
6'	>19 <sup>b</sup>	>19 <sup>b</sup>	
8	>19 <sup>a</sup>	8.08 <sup>a</sup>	>19 <sup>b</sup>
8'	>19 <sup>b</sup>	>19 <sup>b</sup>	
9	>19 <sup>a</sup>	12.13 <sup>a</sup>	>19 <sup>b</sup>

<sup>a</sup>Determined by integration of 1D- $^1\text{H}$  NMR spectrum. <sup>b</sup>Determined by integration of 2D- $^1\text{H}$ -COSY spectrum. <sup>c</sup>Determined by integration of 2D-HSQC spectrum.

in accordance with previously  $^1\text{H}$  NMR reported data on aliphatic and aromatic side chains.<sup>10,11a,12,17</sup> The methyne protons resonances are thus a good way for discriminating the *cis* and *trans* rotamers of the *Nsitbe* family. Monomer **1** which exhibits  $K_{cis/trans}$  ranging between 0.64 and 0.77 displays only slight sensitivity with solvent polarity, in line with previous observations of ester-capped monomer models, relative to related amide-capped systems.<sup>10</sup> These values are similar to those observed for the *pe* side chain in the methyl ester model **A** (Figure 2) for which an  $n \rightarrow \pi^*_{Ar}$  interaction stabilizing the *cis* rotamer has been suggested.<sup>10</sup> Interestingly, the  $K_{cis/trans}$  for compound **1** are 2-fold higher than those for the model **C** containing the

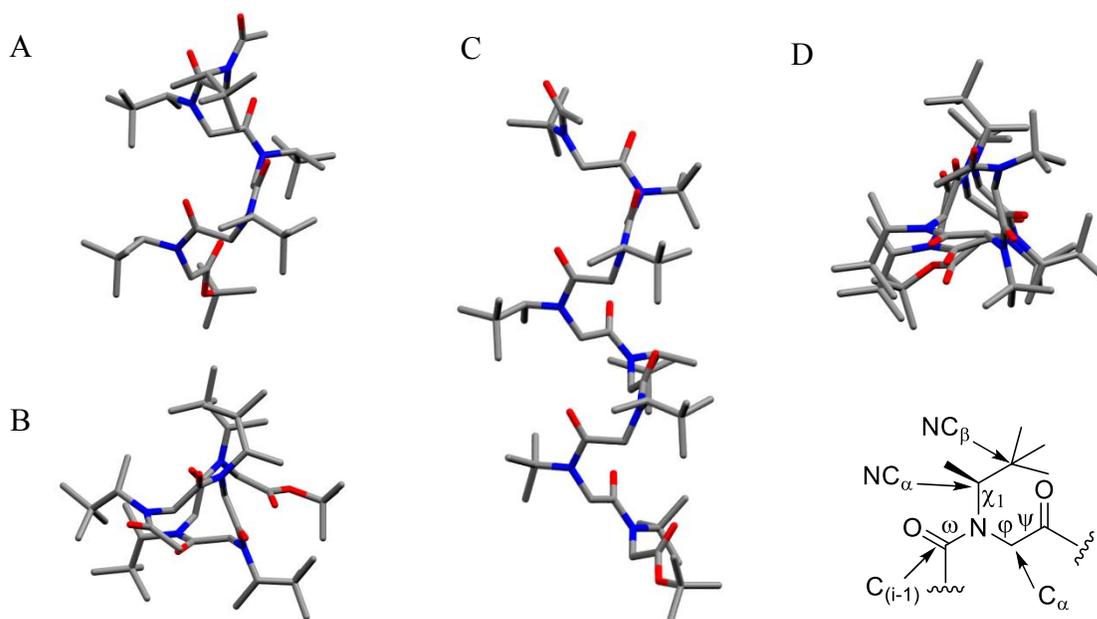
aliphatic cyclohexyl (*ch*) side chain, which served as a baseline indicator of chiral aliphatic side chain.<sup>16</sup> The bulky *sitbe* side chain in monomer **1**, however, cannot compete with the *tBu* side chain which suppressed completely the *trans* rotamers, based on steric hindrance ( $K_{cis/trans} \geq 19$  for **E** and **F**) and with the *sinpe* in the monomer compound **G** ( $n=1$ ) for which the high  $K_{cis/trans}$  value results from complementary steric and  $n \rightarrow \pi^*_{Ar}$  effects. A greater sensitivity to solvent polarity was observed from the dimer stage. Notably, the *Nsitbe* dimer exhibits a larger overall  $K_{cis/trans}$  value in acetonitrile ( $^{CD_3CN}K_{cis/trans} = 1.60$ ) relative to the value observed in chloroform ( $^{CDCl_3}K_{cis/trans} = 0.98$ ). One possible explanation is that acetonitrile, by minimizing inter amide  $n \rightarrow \pi^*_{C=O}$  interactions, would destabilize *trans*-amide rotamers. What is striking and deserves to be mentioned on how the overall backbone  $K_{cis/trans}$  evolve with the oligomer length, is the great leap between the trimer and tetramer stage, particularly in chloroform and methanol. Taking the example of chloroform, the  $K_{cis/trans}$  increased from 1.95 to  $\geq 19$ , which mean that overall, the population of *trans* rotamers decreased from 66% to < 4%. The same tendency, through less marked was observed in  $\text{CD}_3\text{CN}$  between trimer **3** and tetramer **4**. Extension of the trimer by one residue thus induced a dramatic increase of conformational ordering. This phenomenon is much more pronounced with the *Nsitbe* system as compared with the *Nsinpe* for which progression of the  $K_{cis/trans}$  is more regular going from the monomer to the tetramer (**G**, Figure 2). A direct comparison of these two systems is particularly relevant since they possess identical *N*- and *C*-capping groups and have been studied both in acetonitrile. A  $K_{cis/trans}$  of 3.3 for  $\text{Ac}-(\text{Nspe})_5\text{-CO}_2t\text{Bu}$  in  $\text{CD}_3\text{CN}$  (**B**, Figure 2), has also been described as part of the study of the *Nisinpe* system.<sup>11a</sup>



**Figure 2.**  $K_{cis/trans}$  values (the number in brackets) of various model peptoids and oligomers as determined by NMR measurements in different solvents. In the lower right corner, the structure of the homo-oligomers synthesized in this work.

This value is far lower than that of pentamer **5** ( $^{CD_3CN}K_{cis/trans} = 7.96$ ), and it should also be noted that switching from acetonitrile to chloroform or methanol increased  $K_{cis/trans}$  by 2.4-fold. As already mentioned the cyclohexylethyl side chain represents a baseline to assess the effectiveness of aliphatic side chains. A modest  $^{CD_3CN}K_{cis/trans}$  value of 1.0 was described for hexamer H-(Nsch)<sub>6</sub>-CONH<sub>2</sub> (**D**, Figure 2).<sup>16</sup> A very close value was measured in acetonitrile for the synthesized peptoid **7** ( $^{CD_3CN}K_{cis/trans} = 1.39$ ) and increased significantly in chloroform ( $^{CDCl_3}K_{cis/trans} = 2.71$ ) but still far behind the value measured for hexamer **6** ( $^{CDCl_3}K_{cis/trans} = >19$ ). The effec-

tiveness of the *sitbe* is definitely out of proportion with the *sch* and reached unprecedented levels for an aliphatic side chain. Remarkably, the heterooligomers Ac-(*tBu*)(*sitbe*)<sub>4</sub>(*tBu*)-CO<sub>2</sub>*tBu* (**6'**) and Ac-(*tBu*)<sub>2</sub>(*sitbe*)<sub>4</sub>(*tBu*)<sub>2</sub>-CO<sub>2</sub>*tBu* (**8'**) exhibit overall amide  $K_{cis/trans}$  higher than 19 in CDCl<sub>3</sub> and CD<sub>3</sub>CN which means in other words that the *trans*-amide conformation was undetectable. The overall  $K_{cis/trans}$  measured for **6'** and **8'** correspond in fact to those of the tetrameric central chiral segment of the oligomers since they were determined from the NMR



**Figure 3.** Solid-state structures of compounds **5** and **8'** determined by X-ray crystallography (A-D) and dihedral angles definition (lower right corner). Views perpendicular to the helical axis (A: compound **5**, C: compound **8'**). Views parallel to the helical axis from the N-terminus (B: compound **5**, D: compound **8'**). Atom colours: grey, carbon; red, oxygen; blue, nitrogen. Hydrogen atoms are omitted for clarity. Dihedral angles definition (upper left corner):  $\omega$  [ $C_{\alpha}(i-1)$ ;  $C(i-1)$ ;  $N$ ;  $C_{\alpha}$ ],  $\phi$  [ $C(i-1)$ ;  $N$ ;  $C_{\alpha}$ ;  $C$ ],  $\psi$  [ $N$ ;  $C_{\alpha}$ ;  $C$ ;  $N(i+1)$ ],  $\chi_1$  [ $C(i-1)$ ;  $N$ ;  $NC_{\alpha}$ ;  $NC_{\beta}$ ]

integration of the methyne protons of the *sitbe* side chains, but past studies from our group have shown that peptoid amides bearing the *tBu* side chain only populate the *cis* conformation.<sup>13</sup> The tetrameric central chiral segment in **6'** and **8'** is thus conformationally homogeneous in solution with all peptoid amides in the *cis* conformation in CDCl<sub>3</sub> and CD<sub>3</sub>CN. This is not the case of tetramer **4** which exhibits lower overall  $K_{cis/trans}$  in CD<sub>3</sub>CN (10.89). The *NtBu* residues at the extremities of **6'** and **8'** thus help promote a higher conformational homogeneity of the inner chiral segment of these oligomers relative to tetramer **4**.

### 2.3. X-ray studies for peptoids **5** and **8'**

Crystals of peptoid **5** were grown via slow evaporation from isopropanol solution and solved in the  $P2_12_1$  space group. As expected, compound **5** adopts a right-handed helix with  $\sim 3$  residues per turn and a pitch of  $\sim 6$  Å, simi-

lar to a PPI helix (Figure 3 A,B) and in accordance with the X-ray crystal structure of tetramer Ac-(*isnpe*)<sub>4</sub>-CO<sub>2</sub>*tBu*<sup>13a</sup> but slightly lower than that determined from the X-ray structure of the aliphatic *Nrch* pentamer ( $\sim 6.7$  Å).<sup>16</sup> The pitch of compound **5** in the solid was accurately measured between the methylene carbon atoms of residues 1 and 4 (6.1 Å) on the one hand and residues 2 and 5 (5.9 Å) on the other (Figure S3). Similarity of the values is a first indication of conformational regularity of the helical structure. This is reflected by the regularity of the dihedral angles and particularly those of the internal residues 2, 3 and 4 that do not differ by more than  $1.3^\circ$  for  $\omega$  and  $\phi$ ,  $4.2^\circ$  for  $\chi_1$ , and  $3.4^\circ$  for  $\psi$  (Table 3). In contrast with the reference pentamer H-(*rch*)<sub>5</sub>-CONH<sub>2</sub>,<sup>16</sup> the  $\omega$  amide dihedral angles of **5** significantly deviate from planarity, with values ranging from  $-15.8$  to  $-9.7$ . The most important deviations of  $\omega$  peptoid amides have been previously observed for constrained cyclic peptoids<sup>18</sup> and also

in the X-ray crystallography of *N*isnpe peptoids,<sup>11a</sup> commensurate with the bulkiness of the side chain. The  $\chi_1 \approx -103^\circ$  ( $C_{(i-1)}$ ; N; N-C $\alpha$ ;N-C $\beta$  dihedral angle as depicted in Figure 3) results in a conformation in which the amide carbonyl and the N-C $\alpha$ -H bonds are roughly eclipsed. Stabilization of this conformation is likely to be attributed in part to C-H $\cdots$ O=C interactions involving the methyne protons of *sitbe* side chains and the carbonyl peptoid amides as discussed in recent studies.<sup>19,11b</sup>

**Table 3.** Observed dihedral angles for Ac-(*sitbe*)<sub>5</sub>-COOtBu **5** as determined by X-ray crystallography

residue	$\omega$	$\phi$	$\psi$	$\chi_1$
1	-15.8(2)	-73.4(2)	-164.6(2)	-103.2(1)
2	-14.4(2)	-67.3(2)	-176.8(2)	-100.2(2)
3	-14.4(2)	-68.3(2)	-178.8(1)	-104.4(2)
4	-15.7(2)	-67.0(2)	175.4(2)	-103.2(2)
5	-9.7(2)	-81.9(2)	-	-103.9(2)

**Table 4.** Observed dihedral angles for Ac-(*tBu*)<sub>2</sub>(*sitbe*)<sub>4</sub>(*tBu*)<sub>2</sub>-COOtBu **8'** as determined by X-ray crystallography

monomer	$\omega$	$\phi$	$\psi$	$\chi_1$
<b>1</b> ( <i>tBu</i> )	-1.1(5)	-89.2(4)	-175.3(3)	
<b>2</b> ( <i>tBu</i> )	-14.0(4)	-76.6(3)	-170.0(3)	
<b>3</b> ( <i>sitbe</i> )	-4.7(4)	-71.7(3)	-176.8(3)	-99.9(3)
<b>4</b> ( <i>sitbe</i> )	-6.3(4)	-69.4(3)	177.4(3)	-98.4(3)
<b>5</b> ( <i>sitbe</i> )	-6.9(4)	-66.5(4)	171.0(3)	-100.9(3)
<b>6</b> ( <i>sitbe</i> )	-0.9(4)	-68.4(4)	169.2(3)	-105.2(3)
<b>7</b> ( <i>tBu</i> )	7.1(4)	-84.6(3)	-178.9(3)	
<b>8</b> ( <i>tBu</i> )	-2.0(4)	-85.4(3)	-172.4(4)	

However the  $\chi_1$  dihedral angles of **5** are about  $20^\circ$  smaller than the predicted values in gas phase for *N*sppe oligomers.<sup>7</sup> They are also significantly smaller ( $\sim 10$ - $25^\circ$ ) than those measured in the crystals of the *N*rch and *N*isnpe systems. Determination of the interactions responsible for this discrepancy is not obvious. This might be a consequence of attractive dispersion interactions between hydrocarbon moieties,<sup>20</sup> notably short intra-residue H $\cdots$ H contacts between the backbone CH<sub>2</sub> and the NC $\alpha$  methyl hydrocarbons of *sitbe* side chains which are in the range 2.1-2.3 Å (see Supporting Information, S33).

The conformation of the mixed octamer **8'** was also investigated by X-ray diffraction. Single crystals, suitable for X-ray crystallography were grown via slow evaporation from isopropanol solution of **8'**. They were solved in the *P*<sub>2</sub>,<sub>2</sub> space group. The X-ray structure of **8'** revealed the typical PPI-type helical conformation of peptoids with *cis*-amide bonds and approximately three residues per turn, but what is absolutely remarkable, is that this structure is the

longest to be solved for any linear peptoid to date. While the previous solved tetramer and pentamers revealed essentially one helical turn showing a nascent helix, the structure of **8'** is characterized by at least two well-formed turns. The screw-sense of the helical structure is logically right-handed as imposed by the *S* configuration of the *sitbe* side chains, resulting in negative  $\phi$  dihedral angles. Another noteworthy feature is that the achiral *NtBu* glycine residues at the extremities of **8'** (positions 1, 2, 7 and 8 of the sequence) adopt the same rotational state as the chiral *Nsitbe* residues 3-6. In other words, the crystal structure of **8'** doesn't show any screw sense inversion, the negative value of  $\phi$  imposed by the *sitbe* side chains (residues 3-6) is transmitted towards the achiral *NtBu* monomers. The structure of **8'** exhibits a remarkable conformational regularity without important jump of dihedral angles when moving along the sequence, even considering the residues at the extremities. The  $\chi_1$  values are again close to  $-100^\circ$ , confirming the observations made for pentamer **5**. In the packing, the helices pile up in a head to tail manner forming infinite stacks. (Figures S9-S13)

#### 2.4. CD Analysis of peptoids oligomers 1-9, 6' and 8'

Structural investigation was also carried out by Circular Dichroism (CD) to explore in more depth the conformation in solution and to compare with the observations made by NMR and X-Ray crystallography.

**CD Analyses for homooligomers 1-9.** Monomer **1** and dimer **2** displayed only weak CD signals in acetonitrile with no well-defined maxima (Figure 4a). Although of weak intensity, trimer **3** exhibits a CD signal that prefigures the CD signature of the longer oligomers. The CD spectra of peptoids **5**, **6**, **8** and **9** have a similar shape with two minima near 188 and 225 nm and a maximum at  $\sim 209$  nm. The three maxima are red shifted by 3 to 5 nm going from pentamer **5** to nonamer **9**. The CD spectrum of tetramer **4** also resembles those of the longer oligomers but with blue shifted maxima. Interestingly, the peramide molar ellipticity (MRE) intensities of the maximum near 209 nm increase with the oligomer length, with the octamer **8** and nonamer **9** displaying similar intensities. The CD spectra of **8** and **9** are indeed nearly identical in intensity and shape throughout the spectral window. The length-dependent MRE increase does suggest that helix folding of the *Nsitbe* oligomers is a cooperative process, as previously observed for other peptoid systems containing aromatic chiral side chains (*spe*, *sinpe*, Figure 2) and chiral aliphatic side chains (*sch*, Figure 2). The CD spectra of the *Nsitbe* family were compared to the spectra obtained for peptoids containing entirely chiral aliphatic side chains. Homooligomers of (*S*)-*N*-(1-cyclohexylethyl)glycine (*Nsch*) and (*S*)-*N*-(sec-butyl)glycine (*Nssb*) with a free N terminal amino group and a carboxamide at the C-terminus have been analysed by CD at the 6-, 12- and 15-mer length.<sup>16</sup> The CD spectra of the longer oligomers revealed a distinct CD signature with a maximum at 210 nm and two shallow minima at 200 and 225 nm, as described by the authors. Looking carefully at the spectra in this publication, the minima

described near 200 nm appear to be blue shifted around 190-195 nm. With this clarification, the CD maxima of the  $Nsitbe$  oligomers match the previously reported CD data for the  $Nsch$  and  $Nssb$  systems. We observed here that the  $N$ - and  $C$ -termini groups have a great influence on the conformation. Indeed while the  $H-(sch)_6-CONH_2$  CD spectrum is weak and do not resemble those of the longer  $Nsch_{12}$  and  $Nsch_{15}$  oligomers, peptoid **7** ( $Ac-(sch)_6-COOtBu$ ), exhibits CD spectral features that resemble those of the  $Nsitbe$  oligomers. However, by comparison of the intensities of the CD curves of the two hexamers **6** ( $Nsitbe$ ) and **7** ( $Nsch$ ), it is obvious that peptoid **7** displays a greater extent of backbone flexibility. The short length  $Nsitbe_3$  (**3**) and  $Nsitbe_4$  (**4**) oligomers present a distinct CD signature reminiscent of the CD spectra of longer  $Nsitbe$  peptoids. This suggests that a nascent ordered helical fold may appear at the trimer or tetramer length when a first helical turn has been formed. Regarding the

maxima intensities, the CD spectra of  $(Nsitbe)_n$  more closely resemble those of  $(Nssb)_n$  peptoids. Indeed the CD bands at 225 nm are reduced in intensity among  $Nsitbe$  and  $Nssb$  oligomers relative to the  $Nsch$  peptoids. Furthermore, the CD bands at 225 nm decrease in a length-dependent manner among  $Nsitbe$  peptoids, which can also reflect a cooperative effect. The reduced intensity near 225 nm for peptoids substituted with *sec*-butyl side chains (*ssb*) relative to peptoids with cyclohexylethyl side chains (*sch*) was interpreted as a sign of greater flexibility of  $Nssb$  peptoids. In light of CD data of the  $Nsitbe$  peptoids, together with high  $K_{cis/trans}$ , we believe, on the contrary, that the reduced CD band at 225 nm is indicative of a strong conformational ordering. We also note that the CD shape and relative intensities of the three CD bands of  $Nsitbe$  peptoids, *i.e.* a weak negative band at 225 nm, and

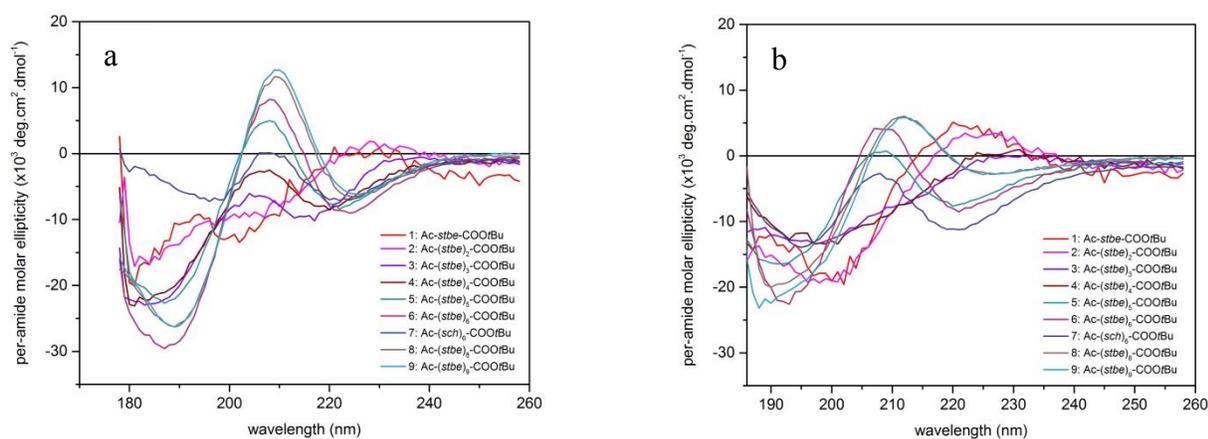


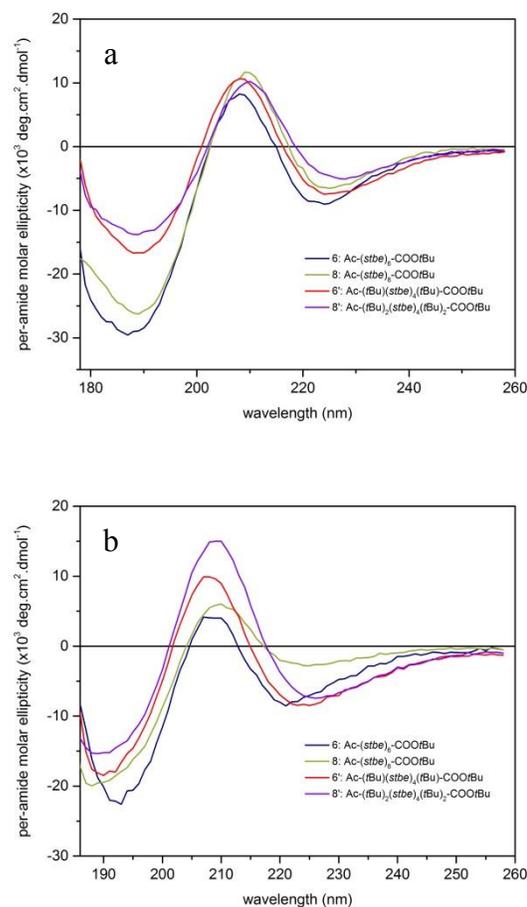
Figure 4. CD spectra of peptoids **1-9** at  $\sim 500 \mu M$ . (a) in acetonitrile. (b) in methanol.

two intense bands, a negative near 190 nm and a positive near 210 nm, are in good agreement with the CD of proline-rich polypeptides in the PPI conformation.<sup>21</sup> We next studied sensitivity to solvent effect on changing the solvent from acetonitrile to methanol (Figure 4b). The CD shape of a PPI-type helical conformation was achieved at the pentamer stage and beyond. Indeed, the CD spectra of the longer oligomers **5**, **6**, **8** and **9** resemble those obtained in acetonitrile with the difference that the bands at 210 nm are reduced in intensities despite higher overall backbone amide  $K_{cis/trans}$  in MeOH than in acetonitrile, as measured by NMR. The CD spectra of **1** and **2** are essentially characterized by an intense minimum around 200 nm and a maximum near 220 nm. Trimer **3** and tetramer **4** exhibit a similar CD pattern, which differ from that of the longer oligomers. It is likely that they adopt a specific conformation in solution which differs from a nascently helical structure, despite the high overall ratio of backbone *cis*-amides, 75% for **3** ( $^{CD3OD}K_{cis/trans} = 3.0$ ) and  $\sim 100\%$  for **4** ( $K_{cis/trans} > 19$ ).

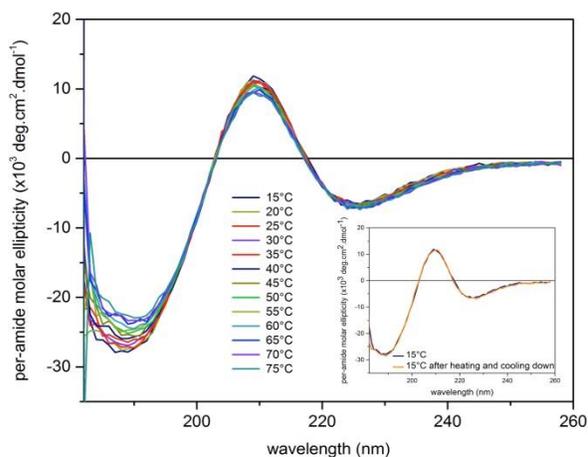
**CD Analyses for heterooligomers **6'** and **8'**.** The CD spectra of heterooligomers  $Ac-tBu-(sitbe)_4-tBu-CO_2tBu$  (**6'**) and  $Ac-(tBu)_2-(sitbe)_4-(tBu)_2-CO_2tBu$  (**8'**) were compared to their related homooligomers **6** and **8** (Figure 5). In acetonitrile a significant decrease in intensity of the minima near 188 nm was observed for **6'** and **8'** relative to **6** and **8** while the rest of the curves remained broadly unchanged. For example, the two hexamers **6** and **6'** have both a positive maximum and a second minimum centred at 208 nm and 224 nm, respectively, the per-amide molar ellipticity of **6'** being slightly increased at 208 nm relative to that of **6**. This increase in ellipticity at about 210 nm for the heterooligomers is much more pronounced in MeOH, which can be attributed to an increased helicity of **6'** and **8'** in this protic solvent. Interestingly, regardless the solvent, acetonitrile or MeOH, no decrease of ellipticity or even an increase in MeOH, was observed despite the presence of achiral *tBu* side chain at the extremities of the sequences of **6'** and **8'**.<sup>13b</sup> This means that the inner tetrameric chiral segment is able to control the overall twist

sense of the backbone, which is in accordance with the X-Ray crystal structure of octamer **8'**. Interestingly, in oligomers **6'** and **8'**, a two-way communication appears to occur between the chiral core and the *t*Bu residues at the extremities. On the one hand, the *Nt*Bu residues help promote a higher conformational homogeneity of the inner chiral segment, on the other hand, the helical handedness of the chiral core is propagated towards the extremities giving rise for the first time to homogeneous and robust helices containing only aliphatic residues. It will be interesting to see how, in the future, the degree of peptoid helicity can vary with the amount of chiral *l*tbe side chains and their site-specific incorporation in mixed oligomers containing  $\alpha$ -chiral *N*s*l*tbe and achiral *Nt*Bu monomers.<sup>22</sup>

Thermal stability of the *Ns**l*tbe system was also probed. For this study, spectra of nonamer **9**, the longest of the series, were acquired over the temperature range 15–75 °C, increasing the temperature by 5 °C (Figure 6). Only slight decreases of intensities were observed as the temperature increased, confirming the exceptional stability of oligo-peptoids adopting the all-cis PPI-type helical conformation. The CD spectrum after cooling down to 15 °C was also shown to be perfectly superposable to the initial spectrum acquired at this temperature. Finally, from compound **9**, it was also verified that the CD spectral shape and intensity were not dependent on the concentration (Figure S14)



**Figure 5.** CD spectra of peptoids **6**, **8**, **6'** and **8'** at ~ 500  $\mu$ M. a) in acetonitrile. b) in methanol



**Figure 6.** CD spectra of peptoid **9** at ~ 500  $\mu$ M in acetonitrile. Data were collected between 15–75 °C in 5° increments. On inset, CD spectra collected at 15 °C before and after the temperature ramp.

## Summary and outlook

The discrete and predictable folding of peptoids largely depends on the capacity of the attached side chains to individually establish local interactions with the backbone, notably to control the *cis*/*trans* equilibria of pep-

toid amides. Chirality which can be introduced through the side chains is also critical to get well-folded secondary structures of defined handedness. This goal was achieved in the past with side chains comprising an aromatic moiety. Hence, homooligomers bearing chiral (*S*) 1-naphthylethyl side chain form well-folded PPI helices with all-*cis* amides. Our objective was to construct for the first time conformationally homogeneous peptoid PPI helices composed exclusively of *N*-alkyl glycine monomers and of defined handedness. Based on the fact that side chain steric hindrance is one of the best means for ensuring conformational restriction of peptoid chains and promote their folding in all-*cis* PPI helices, we have identified a new aliphatic chiral side chain favouring the *cis* conformation. We have discovered that (*S*)-*N*-(1-*tert*-butylethyl)glycine homooligomers are capable of forming conformationally homogeneous and stable PPI helices. This is supported first by high overall amide  $K_{cis/trans}$ , particularly in chloroform and methanol solvents in which all-*cis* structures are observed from the tetramer length. The X-ray crystal structure of pentamer **5** revealed a right-handed helix featuring backbone dihedral angles consistent with those of PPI peptide and peptoid helices. Homopeptoids **1-9** feature length-dependent CD curves suggesting a folding cooperative process as already observed for peptoids with  $\alpha$ -chiral aromatic side chains. The CD signature is reminiscent that of PPI peptide helices, in contrast with that of peptoids with  $\alpha$ -chiral aromatic side chains. The longest solid state structure for any linear peptoid was obtained for octamer **8'** (Ac-(*t*Bu)<sub>2</sub>(*sitbe*)<sub>4</sub>(*t*Bu)<sub>2</sub>-COO*t*Bu). Despite the reduced number of chiral side chains (50%), octamer **8'** forms a very regular right-handed PPI helical structure in the solid state. The all-*cis* arrangement characterizing PPI helices was also verified in solution (CDCl<sub>3</sub> and CD<sub>3</sub>CN) for **8'** and also for hexamer **6'** (Ac-(*t*Bu)(*sitbe*)<sub>4</sub>(*t*Bu)-COO*t*Bu). All together these results suggest that the *Nt*Bu residues strengthen the conformational stability of the inner chiral segment, on the other hand, the helical handedness of the chiral core is propagated towards the extremities giving rise for the first time to homogeneous and robust helices comprising only aliphatic residues. Site sequence specific incorporation of achiral *tert*-butyl side chains in *Nsitbe* oligomers will be studied to delineate boundaries of this new system.

This work opens up excellent prospects for new peptoid design based foldamers with chemical and biological applications, including asymmetric catalysis,<sup>2a</sup> selective recognition of metal ions<sup>4a</sup> and targeting helix-mediated protein-protein interactions.<sup>23</sup> Helices contribute more than 60% to protein-protein interfaces<sup>24</sup> and modulators of protein-protein interactions are now considered promising candidates in drug development. Peptoid helices have undeniably a major role to play in this regard. Their conformation is not stabilized by intramolecular hydro-

gen bonds; and consequently is less likely to be influenced by the environment. The *itbe* side chains could participate to hydrophobic contacts which are crucial in a number of protein-protein interactions. The future design of inhibitors of protein-protein interactions based on the *itbe* side chain will need to introduce solubilizing groups, for example at non interacting positions, to improve their aqueous solubility.

## ASSOCIATED CONTENT

**Supporting Information.** Experimental procedures for peptoid synthesis, characterization data for peptoid monomer **1**. HPLC and MS data for peptoids **1-9**, **6'** and **8'**. <sup>1</sup>H and COSY spectra for peptoid oligomers. X-ray crystallographic data for peptoids **5** and **8'** and CD data for peptoid oligomers. This material is available free of charge via the Internet at <http://pubs.acs.org>

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The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript. / ‡These authors contributed equally. (match statement to author names with a symbol)

### Funding Sources

This work was supported by the ANR ARCHIPEP project, grant ANR Blanc - SIMI 7 2013 of the French Agence Nationale de la Recherche.

## ACKNOWLEDGMENT

We would like to acknowledge Aurélie Job (HPLC) and Martin Leremboure (mass spectrometry) for their assistance. Circular dichroism experiments were performed thanks to the SCBIM (Service Commun de Biophysique Interactions Moléculaires, Fédération de Recherche 3209 BMCT) of Université de Lorraine.

CCDC 1561294-1561295 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/structures](http://www.ccdc.cam.ac.uk/structures).

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