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The *tert*-Butyl Side Chain: A Powerful Means to Lock Peptoid Amide Bonds in the Cis Conformation

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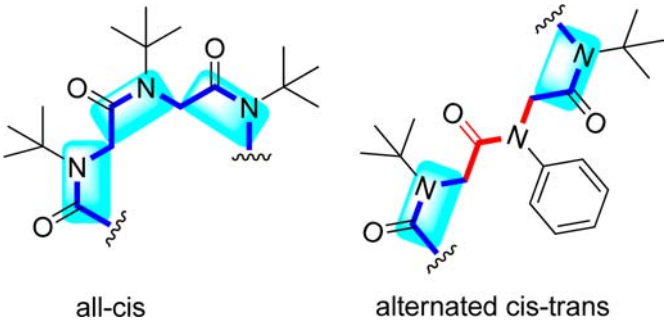
ABSTRACT



The very simple sterically hindered *tert*-butyl side chain exerts complete control over the peptoid amide geometry which only exists in the cis conformation. It is exemplified in *Nt*Bu glycine homo-oligomers and in linear oligopeptoids designed with an alternating cis−trans backbone amide pattern.

Peptoids (*N*-substituted glycines) are formally achieved by shifting the side chains of a peptide from the Cα carbons to the adjacent amide nitrogen atoms.[1] The location of side chains on the amides has a great impact on the conformational behavior of peptoids. Peptoid backbones which are deprived of free NH amides have ineluctably reduced H-bonding capabilities. In addition their *N*,*N*-disubstituted amides are prone to cis/trans isomerization, which explains to a large extent substantial conformational heterogeneity of oligopeptoids.[2] On the other hand, cis/trans isomerization should be regarded as a source of conformational diversity at the peptoid backbone level. While amide bonds in peptides and proteins are mainly in the transoid form,[3] the polyproline type I (PPI) peptide helix featuring only cis amides is a typical peptoid secondary structure.[4,5] Cis/trans amide isomerism is also responsible for the unique threaded loop conformation adopted by some linear peptoid nonamers.[6] Recently, great efforts

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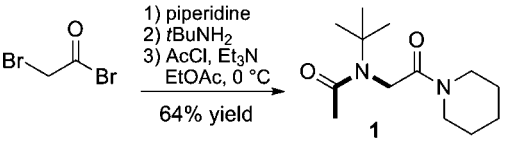
have been devoted to controlling peptoid amide bond geometry in order to minimize backbone conformational heterogeneity. For example, it was demonstrated that $n\rightarrow\pi^*_{Ar}$ donation from the oxygen of a carbonyl (O_{i-1}) to the antibonding orbital (π^*) of the aromatic ring of an α-branched benzylic-type side chain (residue $i+1$) stabilizes the cis-amide conformation.[7,8] The triazolium-containing side chain is a prominent example of a side chain which utilizes the $n\rightarrow\pi^*_{Ar}$ effect to enforce the cis-geometry, independently of any steric contributions.[9] The 1-naphthylethyl side chain (1npe) has also been proposed in this context and has allowed for the first homogeneous and robust PPI-type peptoid helices to be prepared.[5] From X-ray analysis of the shorter *N*s1npe oligomers it was however concluded that steric interactions are certainly the primary cause for conformational restriction in this family. The role of side chain steric hindrance in promoting cis amides is actually well-known.[7b] So, for example, *N*-Cα-branched side chains are commonly used for promoting PPI-type helices. However, with the exception of the 1npe side chain, no other sterically hindered side chain capable of ensuring complete control of α-peptoid amide geometry has been described to date. The highly bulky *tert*-butyl side chain has been shown to produce exclusively the cis amide conformation in *N*-substituted aminomethyl benzamides, termed arylopeptoids,[10] but insertion of *tert*-butyl groups in α-peptoids had never been reported.[11] In this communication we demonstrate the full cis-directing effect of the *t*Bu side chain in α-peptoids and also report its use for the design and construction of peptoids displaying alternated cis and trans amides in a controlled manner.

The effect of a *t*Bu side chain on cis/trans amide isomerism was first studied in the monomeric model **1** capped with acetyl and piperidinyl groups (Scheme 1).[12] Whatever the solvent used for NMR analysis including deuterated water, only one rotamer was observed ($K_{cis/trans} > 19$), whose cis geometry was firmly established by 2D-NOESY experiments. The *t*Bu effect on the backbone amide is remarkable. By comparison, the 1npe side chain exhibits a $K_{cis/trans}$ value of 6.3 in acetonitrile,[7b] as determined from the same monomeric model, and triazolium-type side chains have $K_{cis/trans}$ values around 11.[9]

Scheme 1. Preparation of Model **1** and Cis/Trans Ratios ($K_{cis/trans}$) Determined by NMR in Various Solvents ($CDCl_3$, CD_3CN, $(CD_3)_2CO$, MeOD, D_2O)



$K_{cis/trans}$ > 19 (100% cis based on NMR analysis)

Next, we turned our attention to the solution-phase synthesis of *Nt*Bu homo-oligomers by the submonomer method. The initial conditions for the acylation−substitution iterations from *N*-*tert*-butyl glycine **3** were as follows: bromoacetyl bromide in THF/Et_3N at 0 °C for the first step followed by the reaction of bromoacetamide intermediates with *t*BuNH$_2$ (4 equiv) in THF/Et_3N at rt. These conditions furnished only modest yields in bromoacetamides, around 60%. We then found that replacing bromoacetyl bromide by freshly prepared bromoacetic anhydride produced better yields.[13] With the optimized conditions (Scheme 2), chromatography is needed only once by iteration, after the acylation; the excess of *t*BuNH$_2$ used in the substitution is just evaporated off.[14] The crude secondary amines were directly used in the next iteration or capped with an acetyl group to yield compounds **4**–**7**. Although tedious it might be possible to synthesize longer *Nt*Bu glycine oligomers in solution, following our bromoacetic anhydride based protocol. A block approach consisting of coupling short oligomers would, however, be more straightforward to access longer oligomers. With this in mind several conditions were tested for coupling the amine **3** and the monomer acid Ac−N*t*Bu−OH. Whatever the conditions used (HATU/DIEA, PyBrOP/DIEA, EDCI/DMAP, and DIC/DMAP), the expected dimer **4** was not observed or produced in a very poor yield, not exceeding 10%. Accordingly, the very sterically demanding *t*Bu side chain prevents any peptide coupling reaction, even in solution.

^{1}H and ^{13}C NMR analysis of the *Nt*Bu series in various deuterated solvents showed that these peptoids display conformational homogeneity in solution. For example, dimer **4** displays a unique set of resonances in $CDCl_3$, C_6D_6, CD_3CN, CD_3OD, $(CD_3)_2CO$, and $(CD_3)_2SO$, (see SI

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(12) This model is well suited for predicting the cis/trans ratio ($K_{cis/trans}$) in oligomers: see ref 7b.

(13) Bromoacetic anhydride is scarcely used in peptoid synthesis; see: (a) Saha, U. K.; Roy, R. *J. Chem. Soc., Chem. Commun.* **1995**, 2571. (b) Hara, T.; Durell, S. R.; Myers, M. C.; Appella, D. H. *J. Am. Chem. Soc.* **2006**, *128*, 1995. (c) Mas-Moruno, C.; Cruz, L. J.; Mora, P.; Francesch, A.; Messeguer, A.; Pérez-Payá, E.; Albericio, F. *J. Med. Chem.* **2007**, *50*, 2443. (d) Jagasia, R.; Holub, J. M.; Bollinger, M.; Kirshenbaum, K.; Finn, M. G. *J. Org. Chem.* **2009**, *74*, 2964.

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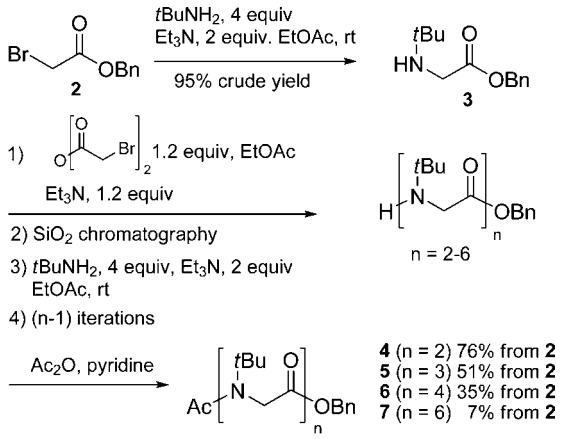
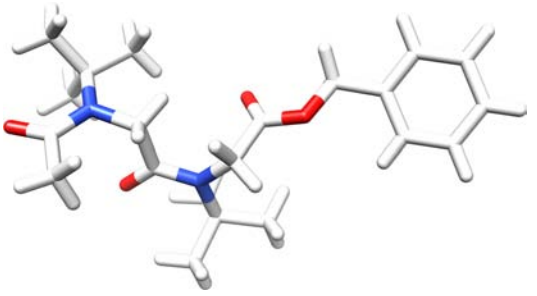
Scheme 2. Optimized Solution-Phase Synthesis of *Nt*Bu Glycine Oligomers



for details). 2D-NOESY data established a cis−cis amide arrangement for dimer **4**, and the cis-amide geometry could be determined for three amides of four in the case of tetramer **6**. Overlapping of the methylene signals of the backbone in the ^{1}H NMR spectrum precluded amide geometry determination of the fourth amide, and for the same reason, amide geometry of longer oligomers could not be established. Nevertheless, based on the observation that the amides of the first members of the *Nt*Bu glycine homo-oligomers exist exclusively in the cis conformation, together with the observation that all the compounds provide a unique set of ^{1}H NMR signals, it can be assumed that all the *Nt*Bu peptoids display an all-cis arrangement. We were pleased to obtain crystals of dimer **4** (by slow evaporation in Et_2O) suitable for X-ray analysis (Table 1). The crystal structure of dimer **4** confirmed that the two amides were in the cis geometry. The centrosymmetric unit cell contains obviously two enantiomeric forms of **4**. The dihedral angle (Table 1) values are very similar to those observed in the crystal structure of the dimer Ac-(*s1npe*)$_2$-CO_2*t*Bu[5] and in PPI-type peptoid helices.[4f] Therefore, it is reasonable to assume that the *Nt*Bu homo-oligomers adopt well-defined helical secondary structures similar to those observed for peptoids bearing *N*-Cα-branched side chains. A model of the *Nt*Bu glycine peptoid hexamer **7** was constructed from the dihedral angles of the dimer **4** crystal structure and minimized at the B3LYP/6-31G(d) level. This structure resembles a PPI-type helix and can be superposed with great accuracy to the crystal structure of pentamer *N*rch$_5$[4f] (see SI for details).

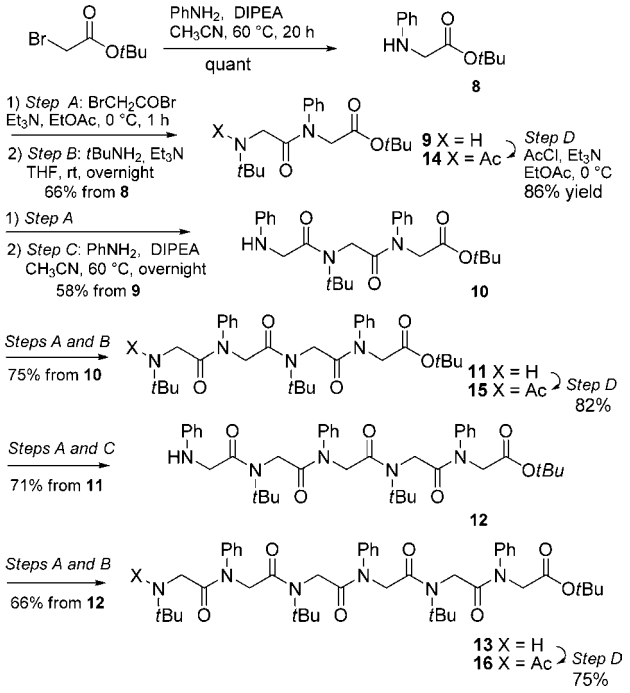
Having demonstrated the applicability of the submonomer protocol for synthesizing *Nt*Bu homo-oligomers featuring all-cis amide bonds, we next envisioned preparing (*Nt*Bu-*N*Ph) oligomers that display alternating cis−trans

Table 1. Dihedral Angles of Dimer **4** Crystal Structure



monomer	ω	φ	ψ
Ac-(*Nt*Bu)	13.3	77.8	170.3
(*Nt*Bu)-COOBn	3.5	−97.4	−151.8

Scheme 3. Solution-Phase Synthesis of (*Nt*Bu-*N*Ph) Oligomers



backbone amide patterns. Indeed *N*-aryl side chains have proven to be powerful side chains for enforcing trans amides in peptoids;[15] *N*-aryl amide bonds usually display a >95% preference for the trans amide geometry in small peptoid models.[15a] Repeating cis−trans amide arrangements were conceptually imagined more than half a century ago by L. Pauling an R. Corey,[16] but to date very little work has yet been undertaken to explore the synthesis and conformation of oligoamides involving cis and trans amides in alternation.[17] The expected peptoid series was synthesized using a solution-phase submonomer protocol

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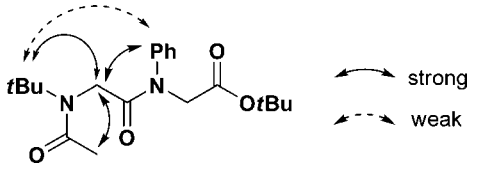

Figure 1. Conformation of peptoid **14** in solution. Observed NOEs between backbone and side chains.

from *tert*-butyl bromoacetate (Scheme 3). Typically the monomers were created by bromoacetylation (bromoacetylbromide in EtOAc at 0 °C in presence of Et_3N) followed by amine displacement with alternatively aniline and tBuNH$_2$. Reactions times of the substitutions were approximately the same for both amines (15 h), but heating (60 °C) was required in the case of substitution by aniline, due to its deactivated character. All peptoids (**9**–**13**) were readily synthesized in yields ranging from 58% to 75% by iteration, with only one SiO_2 chromatography every two steps. Compounds **9**, **11**, and **13** were further acetylated to yield peptoids **14**–**16**.

1D NMR analysis indicated that this series of peptoids involving cis and trans directing side chains in alternation display conformational homogeneity in solution. Despite great effort in obtaining suitable crystals for X-ray diffraction, we were not able to grow crystals of sufficient size. The cis–trans arrangement was however firmly established for dimer **14** from NOESY cross peaks (Figure 1 and SI for details), and the observation of a single set of resonances for all the peptoids of this series evokes a repeating cis–trans pattern along the backbone of all the members of the (*Nt*Bu-*N*Ph) oligomers.

In conclusion, we have demonstrated that incorporation of tBu side chains into peptoids locks the amide sites in the cis conformation. Full suppression of the trans conformers, whatever the solvent, organic or water and the oligomer length is without precedent. We have further shown the applicability of solution-phase submonomer protocols for synthesizing all-cis *Nt*Bu glycine homo-oligomers and a series of oligopeptoids displaying repeating cis–trans amide patterns. Solid-phase synthesis of peptoids containing *Nt*Bu side chains is currently investigated in our group, and tBu functionalized side chains bearing water-solubilizing groups and/or recognition elements will be developed in due course.[18]

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Note Added after ASAP Publication. The author addresses were corrected on April 17, 2013.

Supporting Information Available. Experimental procedures; NMR and mass spectrometry characterization for compounds **1**, **4**–**8**, and **14**–**16**; ^{1}H and ^{13}C spectra for compounds **1**, **4**–**7**, and **14**–**16**; NOESY spectra for compounds **4** and **14**; analytical RP-HPLC for compounds **4**–**7** and **14**–**16**; computational-based model structure of hexamer **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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