α-Amino acid Tröger base derivatives, possible conformationally restricted scaffolds?†‡

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The first synthesis of innovative α-amino acid conjugates of Tröger base is reported; their potential application as conformationally restricted scaffolds is proposed and has been investigated using high level *ab initio* calculations.

We report studies on 2,8-*bis*-α-amino acid Tröger base adducts (**1**, Fig. 1) and propose them as scaffolds for use as peptide chain directors *i.e.* **2**. Tröger base[1] is a C_2-symmetric heterocycle with a relatively rigid backbone, hydrophobic cavity and concave conformation. Due to its sharply folded geometry the aryl rings reside in a near perpendicular arrangement (generally 90–100°).[2]

Gaining insights into the biological mode of action of natural/non-natural proteins, polypeptides and enzymes is critical if a comprehensive understanding of protein action is to be acquired. In this respect many studies have been undertaken on the application/development of innovative mimics of β-turns and hairpins.[3] The synthesis of an α-amino acid derived Tröger base scaffold[4] and its appendage with additional α-amino acid derivatives affords a new opportunity to investigate the loop and hinge regions of proteins. α-Amino acid Tröger base derivatives similar to **3** (Fig. 2) appear not only to be able to act as scaffolds, but also to have the capacity to direct appended peptides within a 90–100° range. Small peptide chains have been identified that contain 90–100° bends. For example, the polypeptide TNYLFSPNGPIARAW that binds to EphB4 (IC_{50} 15 nM) embodies a 90° turn induced by the GP dipeptide. This turn within the pentadecapeptide is *critical* for high affinity binding of the polypeptide into the hydrophobic upper convex portion of the active site within EphB4.[5]

The transition-metal mediated synthesis of adducts based on **3** requires a practical, efficient and convenient synthesis of building block **4** (Scheme 1).[6] The *bis*-2,8-dibromo analog of **4** was not considered due to its recalcitrant nature towards Sonogashira

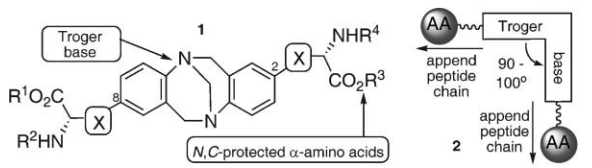

Fig. 1 α-Amino acid Tröger base scaffolds as peptide chain directors.

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‡ The HTML version of this article has been enhanced with colour images.

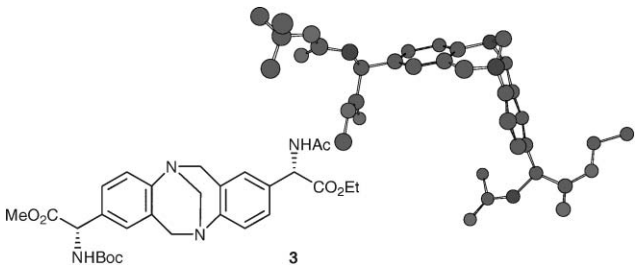
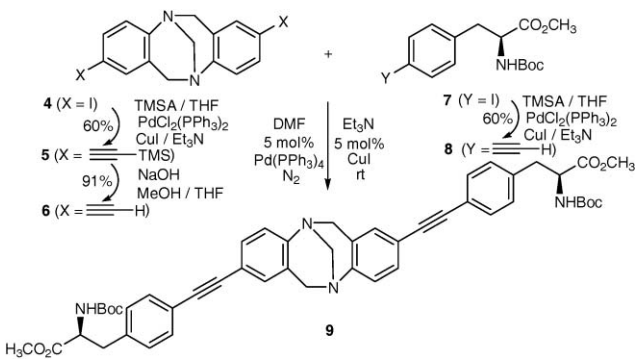


Fig. 2 Chem 3D representation of *bis*-2,8-(*N*,*C*-protected α-amino acid) Tröger base adduct **3**.

couplings.[8] However, the synthesis of milligram quantities of racemic *bis*-2,8-diiodo **4** has been reported by Wärnmark *et al.*[7] Translating this protocol directly to the production of multigram quantities of **4** was problematic with low yields (~10%) resulting. After careful optimisation of the experimental and purification procedures we were able to accrue multigram quantities of racemic **4** in ~46% yield. Employing a single enantiomer of **4** would significantly simplify the analysis of the resulting diastereomeric α-amino acid Tröger base conjugates. That said, all our attempts at resolving **4** using literature protocols failed.[8] Undeterred, we transformed racemic **4** into *bis*-2,8-dialkyne **5** *via* a Sonogashira coupling with TMSA. Desilylation of **5** using TBAF afforded **6** in a poor 23% yield. Switching to sodium hydroxide in a methanol–THF mix negated this problem; an excellent 91% yield of *bis*-2,8-ethynyl **6** resulted. Coupling **6** with 4-iodo-*N*-Boc-(*S*)-phenylalanine methyl ester **7** was attempted. Utilising standard Sonogashira coupling conditions either no reaction took place or poor yields of **9** resulted (~15%). Gratifyingly, when DMF was employed, and freshly synthesised *tetrakis*(triphenylphosphine)palladium(0) and 2.2 equiv. of **7** were utilised the desired Tröger base adduct **9** was returned in an unoptimised 60% yield (Scheme 1).



Scheme 1 Synthesis of 2,8-difunctionalised Tröger base adducts.

Curiously, attempting to couple **4** and *N*-Boc-4-ethynyl-(*S*)-phenylalanine methyl ester (**8**) using the previously successful Sonogashira reaction conditions for **6** and **7** *i.e.* DMF, Et_3N, CuI, $Pd(PPh_3)_4$, failed to return any **9**.

We considered the possibility that the transition-metal mediated coupling reaction between (*S*)-**7** and racemic **4** may result in a chiral resolution of racemic **4** affording diastereomerically enriched **9**. Subjecting **9** to chiral HPLC analysis (Chiralpack AD, 24 × 0.46 cm) clearly showed the (*S*,*S*,*S*)- and (*S*,*R*,*S*)-**9** adducts in an equal ratio.[9] Our attempts at separating, using flash chromatography, (*S*,*S*,*S*)- and (*S*,*R*,*S*)-**9** failed. Similar separation problems with different Tröger base adducts have been reported by Maitra *et al.*[10]

The synthesis of an alicyclic, *bis*-2,8-(*N*,*C*-protected)-α-amino acid Tröger base was attempted. Incorporating **4**, *N*-Boc-(*S*)-propargylglycine ethyl ester (2.5 equivalents) and the reaction conditions/catalysts employed to couple **6** and **7** (Scheme 1), we were surprised to find that from the myriad of by- and decomposition products the desired adduct **10** (Fig. 3) was afforded in a disappointing 10% yield; furthermore we also isolated, albeit in mediocre 28% yield, the corresponding 2-(*N*-Boc-(*S*)-propargylglycine ethyl ester)-8-iodo Tröger base.

Hydrogenation (5% Pd on C, H_2, MeOH, 1 atmosphere) of the rigid *bis*-2,8-ethynyl linkers within **9** and **10** afforded, in quantitative yields, the corresponding alkane linked adducts **12** and **11** respectively. The reported sensitivity of Tröger base to acidic conditions[2a] compelled us to check the feasibility of performing an acid mediated *N*-Boc deprotection. Gratifyingly, reacting **11** or **12** with TFA cleanly removed all the *N*-Boc groups (79% and 82% yields respectively) affording the corresponding TFA salts. Liberation of the free amine (Et_3N) from the TFA salt of *N*-deprotected **12** followed by ^{1}H-NMR analysis confirmed the Tröger base heterocycle to be intact. Testing the concept that additional α-amino acids can be readily appended, tetrapeptide **13** was synthesised in an excellent 89% yield (Scheme 2).

As part of our wider strategy towards utilising Tröger base as a scaffold we sought to append *two* differentially *N*,*C*-protected-α-amino acids onto *one* Tröger base. The possibility of chemoselectively cleaving, when desired, one of the four *N*- or *C*-protecting groups off one of the α-amino acids would significantly enhance the potential of the Tröger base scaffolds.

Disappointingly, utilising **6**, *one* equivalent of **7** and the catalyst/reaction conditions outlined in Scheme 1 failed to afford any significant amounts of the desired *mono*-α-amino acid appended adduct. A complex mixture of products comprising: starting material **6**, homocoupled *bis*-alkynyls, *bis*-2,8-alkynyl Tröger base adduct *i.e.* **9** as well as unidentifiable by-products resulted.

Buchwald *et al.*[11] and Wärnmark *et al.*[7] have reported on an unusually reactive Sonogashira protocol for the efficient coupling of electron-rich aryl halides and alkynes that employs catalytic amounts of tri-*tert*-butylphosphine (10%). Utilising Wärnmark's

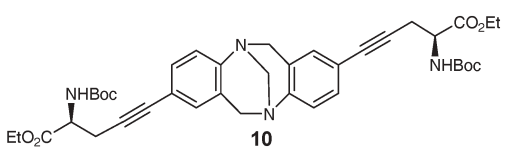
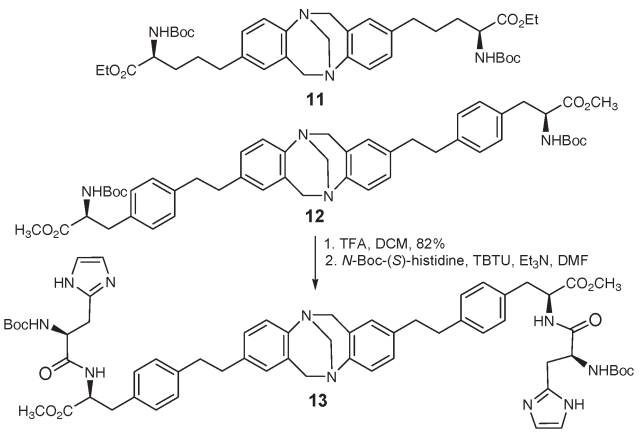
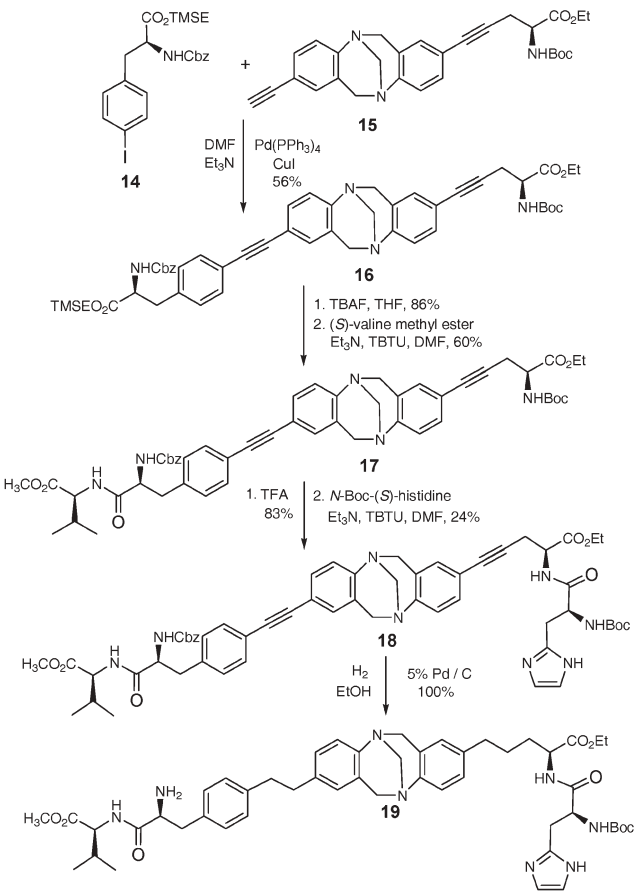


Fig. 3 *Bis*-2,8-[*N*-Boc-(*S*)-propargylglycine ethyl ester] Tröger base.



Scheme 2 Synthesis of Tröger base **13**.

procedure, **4** and *N*-Boc-(*S*)-propargylglycine ethyl ester (1 equivalent each) we isolated the desired *mono* α-amino acid coupled 8-iodo-Tröger base adduct in an unoptimised 56% yield. Subjecting this to a second Sonogashira coupling with TMSA [$PdCl_2(PPh_3)_2$, Et_3N, CuI, THF] and subsequent desilylation afforded **15** which was readily coupled to **14** affording the fully differentially *N*,*C*-protected α-amino acid derived Tröger base **16** (Scheme 3). With this important adduct in hand its chemoselective TMSE *C*-deprotection was undertaken using TBAF; the resulting carboxylic acid was coupled to (*S*)-valine methyl ester affording



Scheme 3 Synthesis of Tröger base derived tetrapeptides **18** and **19**.

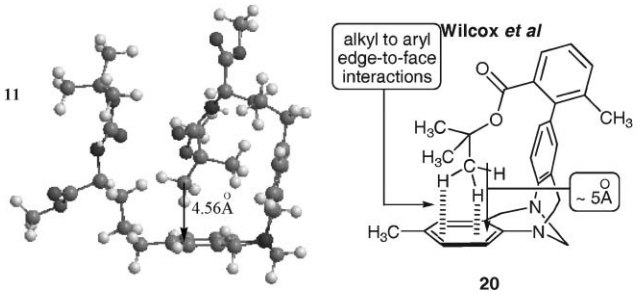


Fig. 4 Calculated (using B3LYP/6-31G) conformation of **11** and *tert*-butyl Tröger base edge-to-face interactions reported by Wilcox *et al.*

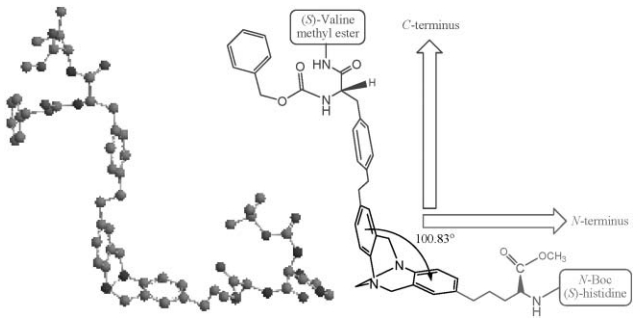


Fig. 5 Calculated conformation using B3LYP/6-31G basis set.

tripeptide **17** in a 60% yield. Chemoselective cleavage of the *N*-Boc group off **17** (TFA, 83%) and subsequent appendage of (*N*)-Boc-(*S*)-histidine afforded Tröger base tetrapeptide **18**, which underwent hydrogenation affording **19**.

With the potential application of the α-amino acid Tröger base conjugates as scaffolds in mind we sought corroboration of their conformation. Despite an intensive effort we have been unable to grow crystals of **11–13** or **19** suitable for X-ray analysis, furthermore NOE experiments were unproductive.

Employing *ab initio* DFT calculations Stephens *et al.*[12] predicted the conformations of a series of Tröger base adducts and compared their results with X-ray crystal structures deposited in the CSD. The agreement of theory and experiment was excellent. Using Gaussian 98[13] (B3LYP functional level and 6-31G basis set) an energy minimisation performed on the relatively simple adduct **11** revealed an unusual 'bite back' of *one* of the *N*-Boc groups such that the *tert*-butyl moiety undergoes edge-to-face aryl–alkyl interactions (Fig. 4). Interestingly, and in agreement with our calculations, Wilcox *et al.* observed similar molecular recognition forces in 'Tilted-T' Tröger base derivatives. Indeed Wilcox *et al.* reported that alkyl *tert*-butyl ester **20** had a 'strong preference, greater than any *aryl* ester, for the formation of edge-to-face interactions'.[14]

To test our concept that α-amino acid Tröger base conjugates may be capable of acting as conformationally restrictive scaffolds we subjected *N*-Cbz derived **19** to *ab initio* DFT calculations (B3LYP and 6-31G). Gratifyingly, the calculations indicate (Fig. 5) that the Tröger base scaffold does indeed constrain appended α-amino acids, holding them to a near perpendicular angle (calculated aryl plane angle of 100.83°). Furthermore, and of importance for application as a scaffold, the DFT calculation suggests that the *N*- and *C*-terminated α-amino acid 'arms' are projected along the aryl planes of the Tröger base.

In summary, we have developed a novel strategy for the synthesis of structurally unique *bis*-(*N*,*C*-protected-α-amino acid) derived Tröger base adducts. Using chemoselective *N*- or *C*-α-amino acid deprotection strategies we have demonstrated the feasibility of synthesising non-symmetric Tröger base tetrapeptides. Our hypothesis that Tröger base adducts can be employed as new conformationally restricted scaffolds has been reinforced by high level *ab initio* calculations.

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