ORGANIC LETTERS

2011
Vol. 13, No. 24
6468–6471

Amino Triazolo Diazepines (Ata) as Constrained Histidine Mimics

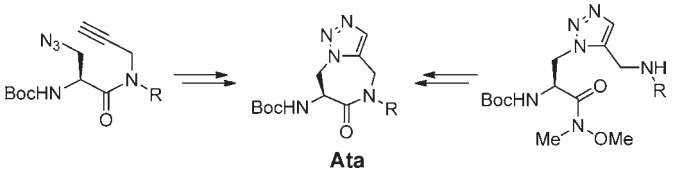
Koen Buysse,[†] Julien Farard,[†] Alexandros Nikolaou,[‡] Patrick Vanderheyden,[‡] Georges Vauquelin,[‡] Daniel Sejer Pedersen,[§] Dirk Tourwé,[†] and Steven Ballet*[,†]

Laboratory of Organic Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium, Molecular and Biochemical Pharmacology Laboratory, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium, and Department of Medicinal Chemistry, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

sballet@vub.ac.be

Received October 14, 2011

ABSTRACT



Ata

Two synthetic routes for the synthesis of amino-triazolodiazepine (Ata) scaffolds are presented. The scope of both of these proceeding through key intra- and intermolecular Huisgen cycloaddition reactions is discussed. The replacement of the His-Pro dipeptide segment in angiotensin IV by the dipeptide mimetic Ata-Gly and subsequent biological evaluation in two inhibitory enzyme assays validated the use of the Ata moiety as a His mimic given the equipotency of both peptidic analogs.

The introduction of conformational constraints in bioactive peptides is an efficient strategy to influence receptor selectivity, ligand potency, metabolic stability, and membrane permeation.[1,2] Peptide cyclization induces a global constraint, whereas single amino acid modifications can induce more local or subtle changes. Especially, side chain constrained analogs of the aromatic amino acids Phe, Tyr, Trp, and His were shown to be very successful in peptide medicinal chemistry.[3]

Due to the appealing structural and electronic properties of 1,2,3-triazoles, they are finding an increased use in peptidomimetic chemistry for introducing global and local conformational constraints.[4] 1,2,3-Triazoles have been employed to great effect as replacements of backbone peptide bonds[5] or to stabilize turn[6,7] or helical[8] architectures

[†] Laboratory of Organic Chemistry, Vrije Universiteit Brussel.

[‡] Molecular and Biochemical Pharmacology Laboratory, Vrije Universiteit Brussel.

[§] University of Copenhagen.

(1) (a) Grauer, A.; König, B. *Eur. J. Org. Chem.* **2009**, 5099–5111. (b) Mann, A. Conformational Restrictions and/or Steric Hindrance in Medicinal Chemistry. In *The practice of Medicinal Chemistry*, 2nd ed.; Academic Press: London, 2003; pp 233−250. (c) Boguslavsky, V.; Hruby, V. J.; O'Brien, D. F.; Misicka, A.; Lipkowski, A. J. *J. Peptide Res.* **2003**, *61*, 287–297. (d) Hruby, V. J.; Li, G.; Haskell Luevano, C.; Shenderovich, M. *Biopolymers* **1997**, *43*, 219–266.

(2) Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615–2623.

(3) Gibson, S. E.; Guillo, N.; Tozer, M. J. *Tetrahedron* **1999**, *55* (3), 585–615.

(4) (a) Angell, Y. L.; Burgess, K. *Chem. Soc. Rev.* **2007**, *36*, 1674–1689. (b) Yoo, B.; Shin, S. B. Y.; Huang, M. L.; Kirshenbaum, K. *Chem.—Eur. J.* **2010**, *16* (19), 5528–5537. (c) Pedersen, D. S.; Abell, A. *Eur. J. Org. Chem.* **2011**, *13*, 2399–2411.

(5) (a) Tam, A.; Arnold, U.; Soellner, M. B.; Raines, R. T. *J. Am. Chem. Soc.* **2007**, *129*, 12670–12671. (b) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126* (47), 15366–15367. (c) van Maarseveen, J. H.; Horne, W. S.; Ghadiri, M. R. *Org. Lett.* **2005**, *7* (20), 4503–4506.

(6) (a) Appukkuttan, P.; Dehaen, W.; Fokin, V. V.; Van der Eycken, E. *Org. Lett.* **2004**, *6* (23), 4223–4225. (b) Oh, K.; Guan, Z. B. *Chem. Commun.* **2006**, *29*, 3069–3071. (c) Holland-Nell, K.; Meldal, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 5204–5206.

(7) Pokorski, J. K.; Jenkins, L. M. M.; Feng, H. Q.; Durell, S. R.; Bai, Y. W.; Appella, D. H. *Org. Lett.* **2007**, *9* (12), 2381–2383.

(8) (a) Cantel, S.; Isaad, A. L. C.; Scrima, M.; Levy, J. J.; DiMarchi, R. D.; Rovero, P.; Halperin, J. A.; D'Ursi, A. M.; Papini, A. M.; Chorev, M. *J. Org. Chem.* **2008**, *73* (15), 5663–5674. (b) Scrima, M.; Le Chevalier-Isaad, A.; Rovero, P.; Papini, A. M.; Chorev, M.; D'Ursi, A. M. *Eur. J. Org. Chem.* **2010**, *3*, 446–457. (c) Ingale, S.; Dawson, P. E. *Org. Lett.* **2011**, *13* (11), 2822–2825.

(9) Pedersen, D. S.; Abell, A. The Huisgen Cycloaddition in Peptidomimetic Chemistry. In *Protection Reactions, Medicinal Chemistry, Combinatorial Synthesis*; Hughes, A. B., Ed.; Wiley-VCH: Weinheim, 2011; pp 101−124.

10.1021/ol202767k
Published on Web 11/16/2011

by cyclization between side chain or backbone modified amino acid residues.[4a,9]

We have previously reported the synthesis and successful incorporation of conformationally constrained amino-azapinone analogs of phenylalanine (**1**, Aba (amino-benzo-azepinone), Figure 1) and tryptophane (**2**, Aia (amino-indolo-azepinone)), in bioactive peptides.[10,11] To complement this we envisaged that an amino-triazolodiazepinone such as **3** (Ata) could potentially serve as a conformationally constrained amino acid analog to replace histidine residues in peptide sequences. Triazole histidine mimics such as aza-His **4** and **5** have previously been described,[12] but the synthesis of small ring cyclic structures such as **3** has not yet been reported. Herein we present the synthesis of histidine analogs Ata **3**, based on two different synthetic strategies employing intermolecular and intramolecular Huisgen cycloaddition reactions. The scope of both strategies in terms of yields, stereocontrol, and synthetic drawbacks is discussed.

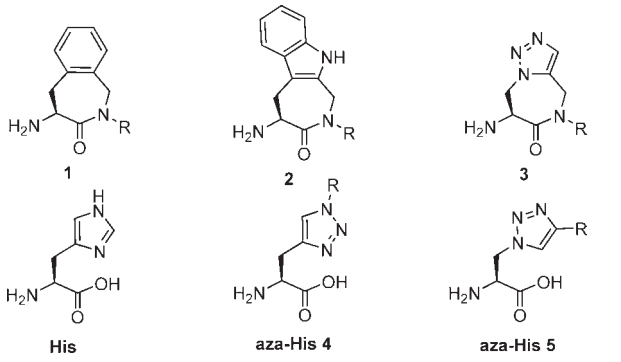


Figure 1. Conformationally constrained amino acids Aba **1**, Aia **2**, Ata **3**, and structures of reported His mimicks **4** and **5**.[12].

In order to validate **3** as a histidine mimic, it was introduced in the angiotensin IV (AT IV) peptide sequence and evaluated as an inhibitor of insulin regulated aminopeptidase (IRAP) and aminopeptidase-N (AP-N) activity.

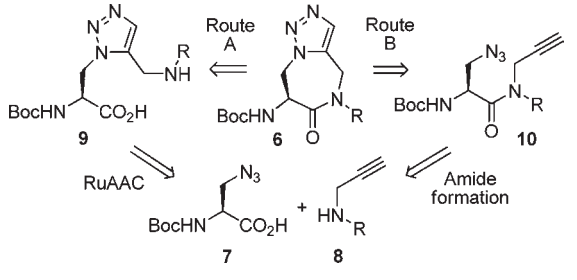
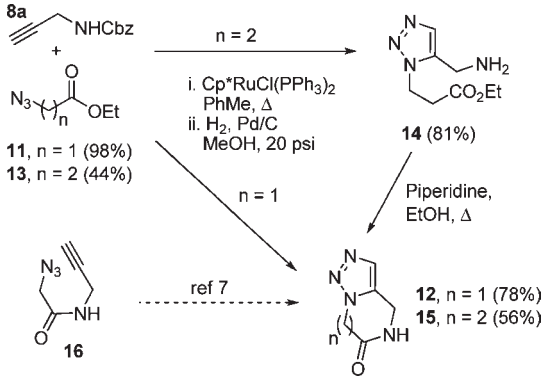


Figure 2. Retrosynthetic strategies A and B for the preparation of Ata scaffold **6** from common precursors.

The key reaction for the construction of Boc-protected scaffold **6** suitable for peptide synthesis (Figure 2) is the regioselective cycloaddition reaction to produce 1,5-substituted triazoles.[13,14] The intermolecular route (Route A, Figure 2) uses a ruthenium catalyst to form the 1,5-substituted triazole **9**, followed by a lactamization to generate the desired **6**. The intramolecular approach on the other hand (Route B, Figure 2) consists of a thermal Huisgen cycloaddition reaction of **10**, which is only able to form the desired 1,5-substituted triazole, during which both cycles, the triazole as well as the lactam ring, are formed simultaneously. The latter strategy was used by Pokorski et al.[7] to prepare the six-membered lactam **12** from **16** (Scheme 1).

Prior to the synthesis of Ata compounds **6**, the feasibility of pathway **A** was studied in models lacking the exocyclic amino substituent (Scheme 1). As an alternative to the intramolecular cyclization used by Pokorski et al.[7] to obtain 1,5-triazolo-pyrazinone **12** from **16** (Scheme 1), the intermolecular RuAAC reaction, followed by lactamization, was investigated. The cycloaddition of Cbz-protected propargylamine **8a** and ethyl 2-azidoacetate **11** was found to give satisfactory yields with both Cp*RuCl-$(PPh_3)_2$ and Cp*RuCl(COD)[15] in refluxing dioxane or toluene, but only poor yields were obtained in THF (Table S1 in Supporting Information (SI)).

Scheme 1. A Model Study of Intramolecular RuAAc vs Intramolecular Thermal Huisgen Cyclization



Surprisingly, the reaction of azide **13** with acetylene **8a** required longer refluxing in toluene and gave a lower yield. After removal of the Cbz protecting group, pyrazinone **12**

(10) Ballet, S.; Frycia, A.; Piron, J.; Chung, N. N.; Schiller, P. W.; Kosson, P.; Lipkowski, A. W.; Tourwe, D. *J. Pept. Res.* **2005**, *66* (5), 222–230.

(11) Feytens, D.; De Vlaeminck, M.; Cescato, R.; Tourwe, D.; Reubi, J. C. *J. Med. Chem.* **2009**, *52* (1), 95–104.

(12) (a) Kee, J. M.; Villani, B.; Carpenter, L. R.; Muir, T. W. *J. Am. Chem. Soc.* **2010**, *132* (41), 14327–14329. (b) Nadler, A.; Hain, C.; Diederichsen, U. *Eur. J. Org. Chem.* **2009**, 4593–4599.

(13) Zhang, L.; Chen, X. G.; Xue, P.; Sun, H. H. Y.; Williams, I. D.; Sharpless, K. B.; Fokin, V. V.; Jia, G. C. *J. Am. Chem. Soc.* **2005**, *127* (46), 15998–15999.

(14) Stanley, N. J.; Pedersen, D. S.; Nielsen, B.; Kvist, T.; Bräuner-Osborne, H.; Taylor, D. K.; Abell, A. D. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7512–7515.

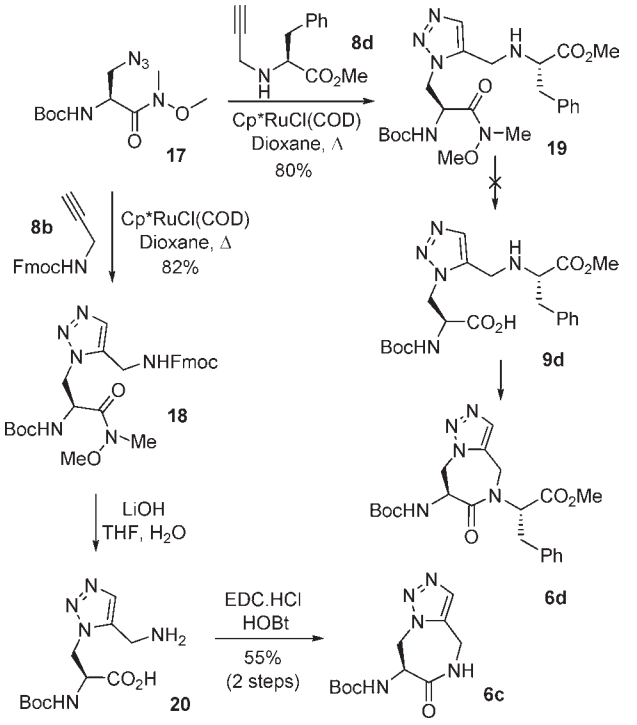
(15) Boren, B. C.; Narayan, S.; Rasmussen, L. K.; Zhang, L.; Zhao, H.; Lin, Z.; Jia, G.; Fokin, V. V. *J. Am. Chem. Soc.* **2008**, *130* (28), 8923–8930.

formed spontaneously. In contrast the seven-membered lactam in **15** did not form spontaneously and required refluxing of **14** in ethanol in the presence of a catalytic amount of piperidine.

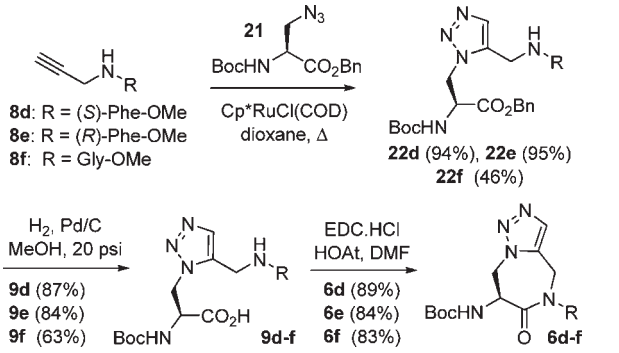
Based on these results, the synthesis of **6c** using the intermolecular cycloaddition reaction (Scheme 2) was started from azide **17** which was prepared in two steps from Boc protected serine according to the method of Panda et al.[16] The RuAAC reaction of **17** with Fmoc-protected *N*-propargyl amine **8b** yielded triazole **18** in satisfying yields. Simultaneous base-promoted deprotection of the fluorenylmethyloxycarbonyl and hydroxamate groups was performed prior to the carbodiimid-induced cyclization and provided the desired Boc-aminotriazoloazepinone (Boc-Ata) **6c** in 55% yield over the two steps. The preparation of an Ata scaffold **6** bearing an amino acid based substituent on *N*-2, using *N*-propargyl-Phe methyl ester **8d**, was not successful by this approach.[17,18] Whereas the cycloaddition reaction did yield triazole **19** in 80% yield, no conditions were found to selectively hydrolyze the Weinreb amide in the presence of the methyl ester. The free carboxylic acid in **9d** was needed for subsequent lacamization.

Therefore, Boc-β-azido-Ala **7**, obtained from Boc-β-amino-Ala,[7,14,19] was converted to its benzyl ester **21**, since free carboxylic acids have been reported to be poorly compatible with RuAAC catalysts (Scheme 3). The RuAAC reaction was carried out by refluxing azide **21** and *N*-propargyl amino acid methyl esters **8d**–**f** in dioxane to give triazolyl amino acid esters **22d**–**f** in moderate to excellent yields. Hydrogenolysis of **22d**–**f** afforded **9d**–**f** ready for cyclization to give the desired triazolodiazepinones **6d**–**f.**

Scheme 2. Preparation of Boc-Ata-H **6c** via Route A



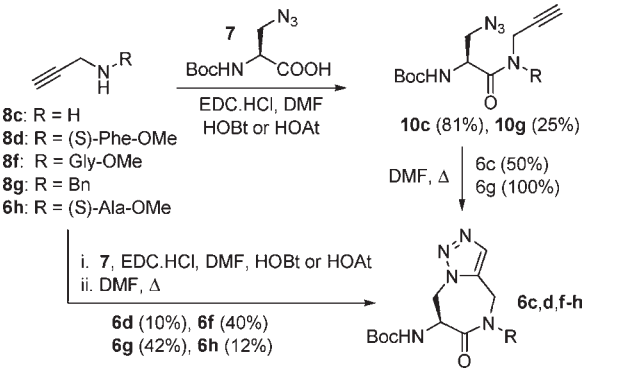
Scheme 3. Intermolecular RuAAC synthesis of Boc-Ata **6d**–**f**



A variety of cyclization conditions were evaluated on amino acid **9d** (see Table S2 in SI). The use of DCC gave the desired **6d**, however contaminated with the *N*-acylurea adduct. The application of EDC or TBTU resulted in partial epimerization (80/20), which could be suppressed using a combination of EDC and HOAt in DMF.[20] Using these optimized conditions Boc-Ata **6d**–**f** were obtained in high yields.

In addition to the successful intermolecular RuAAC route (Scheme 3), the intramolecular cycloaddition reaction was also investigated. This pathway eliminates two reaction steps (ester protection and deprotection) that are required in Route A (Figure 2). Ata scaffold **6c** (R = H) served as a test case. Boc-β-azido-Ala[19] **7** was coupled to propargyl amine **8c** to produce **10c** (Scheme 4). In the following intramolecular cycloaddition, the only possible regiochemical outcome is the 1,5-substituted triazole **6c** in principle making the use of a catalyst for intramolecular RuAAC[21] unnecessary.

Scheme 4. Intramolecular Thermal Huisgen Cycloaddition Synthesis of Boc-Ata **6c,d,f**–**h**



(16) Panda, G.; Rao, N. V. *Synlett* **2004**, *4*, 714–716.
(17) Sudhir, V. S.; Baig, R. B. N.; Chandrasekaran, S. *Eur. J. Org. Chem.* **2008**, 2424–2429.

Triazole precursor **10c** was stirred in refluxing dioxane with and without a Ru-catalyst, and comparable yields (50%) and reaction times confirmed that the intramolecular cycloadditions worked equally well, despite the fact that the amide bond was exclusively in the trans conformation. Since secondary amides exhibit a cis−trans equilibrium, the influence of the amide bond conformation was investigated in the *N*-benzyl-substituted analog **10g**.

The coupling reaction between **8g**[17] and **7** proved to be tedious, probably due to steric encumbrance, as **10g** was only obtained in 25% yield using EDC/HOBt in DMF. The use of phosphonium reagents resulted in even lower yields. Conversely, the subsequent Huisgen cycloaddition went smoothly to give a quantitative yield of Ata **6g**. A study of the reaction conditions of the cylization step (see Table S3 in SI) indicated that the cycloaddition is best carried out catalyst-free, in dioxane at 100 °C for 1 h, to give **6g** quantitatively.

To improve the overall yield of the process we performed the coupling and subsequent cycloaddition in one pot without purification of compound **10g**. After overnight coupling using EDC and HOBt in DMF, refluxing for 1 h gave Ata **6g** in a significantly better 42% yield over two steps (compared to 25% overall for the stepwise process).

Subsequently, various Ata-Xxx dipeptidomimetics were prepared starting from the *N*-propargyl substituted amino acid esters **8d**,**f** and **h**.[17] To obtain **6f** (Boc-Ata-Gly-OMe), the coupling of **7** to **8f** was performed overnight using EDC and HOAt in DMF at room temperature, followed by overnight refluxing to give a 40% yield over the two steps (see Table S4 in SI) showing no epimerization after (*S*)-NIFE[22] derivatization and subsequent LCMS analysis. Likewise, Boc-Ata-Ala-OMe **6h** was prepared using these conditions from **8h**[17] and **7**. Unfortunately, a disappointing yield was obtained for **6h** (12% over two steps), and moreover the ^{1}H NMR spectrum indicated the presence of a mixture of diastereoisomers (dr 75/25), due to epimerization. Similarly, **6d** (Boc-Ata-Phe-OMe) gave a poor yield (10%) showing substantial epimerization (dr 60/40) by HPLC. An intramolecular cycloaddition reaction on *N*-(2-propynyl)amino acid esters under thermal conditions was reported by Sudhir and co-workers,[17] but the enantiopurity of the resulting triazoles was not reported. A comparison of the two pathways indicates that for the preparation of **6c** (Boc-Ata, R = H) and **6f** (Boc-Ata-Gly-OMe) the intermolecular pathway A gives overall yields of 25% and 12% (calculated from Boc-β-amino-Ala) versus 53% and 21% for the intramolecular pathway B. However, for the preparation of the dipeptide mimetics Ata-Ala and Ata-Phe, the intramolecular pathway is clearly inferior with regard to overall yield, but especially because of the substantial epimerization.

To illustrate the potential of the Ata template as a constrained histidine mimic, the His-Pro dipeptide segment in AT IV (H-Val-Tyr-Ile-**His-Pro**-Phe-OH) was replaced by the Ata-Gly dipeptidomimetic. After saponification of the methyl ester in **6f**, this building block was incorporated in the AT IV sequence using standard solid phase peptide synthesis (see SI). In enzyme assays measuring the inhibition of insulin regulated aminopeptidase (IRAP) and aminopeptidase-N (AP-N) activity, the H-Val-Tyr-Ile-**Ata-Gly**-Phe-OH analog was equipotent to the native AT IV (pK_i (IRAP) = 7.093 versus 7.142 for AT IV; Figure S1 in SI). In conclusion, we have developed a versatile synthesis of a new constrained dipeptidomimetic using the azide−alkyne cycloaddition reaction. The best pathway to the dipeptide mimetics is the intermolecular Ru-catalyzed cycloaddition, followed by lactamization. The potential of the new Ata scaffold to replace His in a bioactive peptide was demonstrated in AT IV, where this substitution yielded an analog which was equipotent with the native peptide.

Acknowledgment. This work was supported by the Institute for Innovation through Science and Technology (IWT Vlaanderen), the Research Foundation-Flanders (FWO), and the Queen Elisabeth Research Foundation.

Supporting Information Available. Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(18) Cho, J. H.; Kim, B. M. *Tetrahedron Lett.* **2002**, *43*, 1273–1276.

(19) Brans, L.; Garcia-Garayoa, E.; Schweinsberg, C.; Maes, V.; Struthers, H.; Schibli, R.; Tourwé, D. *ChemMedChem* **2010**, *5*, 1717–1725.

(20) Han, S. Y.; Kim, Y. A. *Tetrahedron* **2004**, *60* (11), 2447–2467.

(21) (a) Isidro-Llobet, A.; Murillo, T.; Bello, P.; Cilibrizzi, A.; J. T. Hodgkinson, J. T.; Galloway, W. R. J. D.; A. Bender, A.; Welch, M.; D. R. Spring, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6793–6798. (b) Kelly, A. R.; Wei, J.; Kesavan, S.; Marie, J.-C.; Windmon, N.; Young, D. W.; Marcaurelle, L. A. *Org. Lett.* **2009**, *11*, 2257–2260.

(22) Péter, A.; Vékes, E.; Torok, G. *Chromatographia* **2000**, *52* 821–826.