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Click chemistry to functionalise peptidomimetics

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Abstract—Sharpless modified Huisgen's [2+3] cycloaddition of azide and acetylenic derivatives was employed as an efficient and simple method to conjugate azabicycloalkane amino acids, mimics of a homoSer-Pro dipeptide, with biologically relevant partners.

1. Introduction

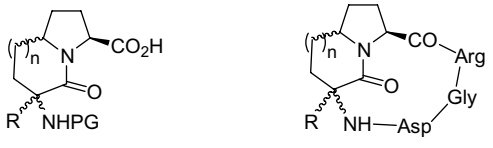
Biologically active peptidomimetics have often turned out to be good drug candidates. These molecules are usually characterised by high affinity for specific receptors and, in contrast to their natural analogues, can be metabolically stable towards endogenous protease, as well as possess greater oral bio-availability and more rapid excretion.[1]

The possibility to functionalise peptidomimetics with molecules of biological interest makes these substances even more attractive. The functionalisation of peptidomimetics with lipophilic or hydrophobic appendages may improve peptide-receptor affinity by interacting with hydrophobic or hydrophilic pockets. Moreover it is also possible to conjugate peptidomimetics with other biologically active molecules,[2] with small molecular probes (a fluorescent dye or an affinity tag) or with radiolabelled molecules.[3]

In the course of our studies on peptide secondary structure mimics, we have synthesised several 6,5- and 7,5-fused-2-oxo-1-azabicyclo[*X*.3.0]alkane amino acids.[4] These structures can be regarded as conformationally restricted substitutes for Ala-Pro and Phe-Pro dipeptide units and, if their conformations meet certain criteria, they can be used to replace the central ($i+1$ and $i+2$) residue of β-turns.[5] In particular some of these molecules have been employed in the synthesis of a small library of cyclic pseudopentapeptides containing the RGD sequence (Fig. 1). Many members of this library revealed themselves to be active and selective ligands for αvβ3 and αvβ5 integrins.[6]

Recently our research group has reported an efficient and simple synthesis of functionalised azabicycloalkane amino acids, mimics of a homoSer-Pro dipeptide (Fig. 2), where the key step is an intramolecular nitrone cycloaddition reaction on 5-allyl- or 5-homoallylproline that was found to be completely regio- and stereoselective.[7] These mimics present heteroalkylic side chain ending with hydroxy group that can be easily transformed into other suitable functional groups (i.e., azide, amine, etc.) or directly used for conjugation.

Our approach to the synthesis of bio-conjugates is based on the so called 'click chemistry'.[8] In particular we adopted the Sharpless modified Huisgen's [2+3] cycloaddition of azide and acetylene to give 1,2,3-triazoles.[9] This reaction presents many advantages: it is



n = 1, 2
R = H, Alk., Bn

Figure 1. Azabicyclo[*X*.*Y*.0]alkane amino acids and cyclic pseudopentapeptides containing the RGD sequence.

Keywords: Peptidomimetics; Conjugates; Click chemistry; [2+3] Cycloaddition.
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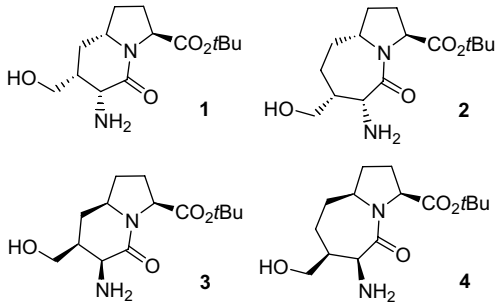


Figure 2. Functionalised azabicycloalkane amino acids mimics of a homoSer-Pro dipeptide.

chemo- and regioselective, it is performed in mild conditions and is usually characterised by high yields.

In this letter, we report the studies towards the conjugation of the pseudodipeptide homoSer-Pro with entities of biological interest.

2. Results and discussion

To apply the click chemistry 1,3-dipolar cycloaddition reaction, it is first necessary to introduce either an azide or an acetylenic moiety on the azabicycloalkane scaffolds. It proved to be synthetically convenient to introduce an azide group onto the scaffold and to functionalise the biological molecules to be conjugated with an alkyne. As proof of concept, we chose in particular to conjugate a sugar (glucose), a fluorescent dye (fluorescein) and an affinity probe (biotin).

After protection of the free amine with CbzCl, the hydroxy group was first treated with methansulfonylchloride to give the corresponding mesylate, which was subsequently converted to the azide with NaN_3 in DMF at 80 °C (Scheme 1).

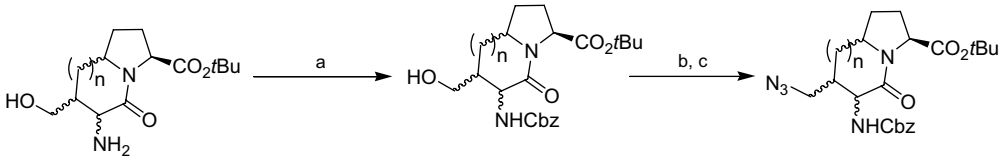
The 1,3-dipolar cycloaddition between pseudopeptides **6** or **8** and the 1-*O*-propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucose **9**[10] was performed using $Cu(OAc)_2$ and Na ascorbate as catalysts in *t*-BuOH/H_2O 1:1[11,12] (Scheme 2). The reaction proceeded overnight at room temperature and the desired products were isolated, after purification by flash chromatography, in good yields.

To increase the solubility of the sugar in the reaction solvents and to avoid a deprotection step, the reaction was also performed in the same conditions, using the unprotected glucose **10**[13] (Scheme 2). The cycloaddition products were isolated, after purification by flash chromatography, in slightly lower yields.

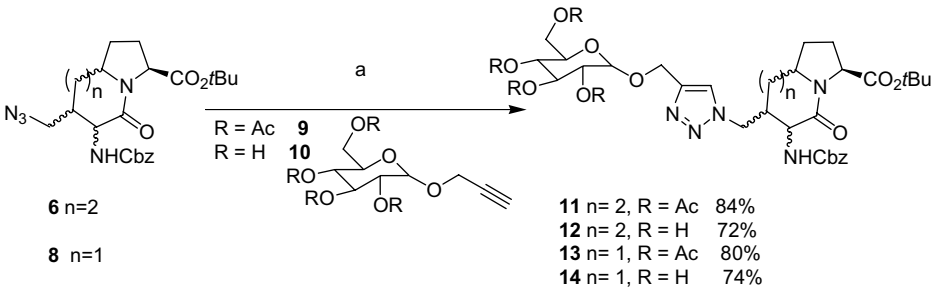
The conjugation of biotin and fluorescein were also attempted. In both cases, a spacer was introduced between the pseudodipeptide and the molecular tag. For this purpose, spacer **15**[14] was coupled with propargylamine and the Boc protecting group was removed (Scheme 3) to give **17**, which was finally conjugated to biotin using HBTU and DIPEA.

Conjugation to fluorescein, to give **20**, was performed by adding commercially available 5(6)-carboxyfluorescein-*N*-hydroxysuccinimide ester **18** to a basic solution of **17** (Scheme 3).

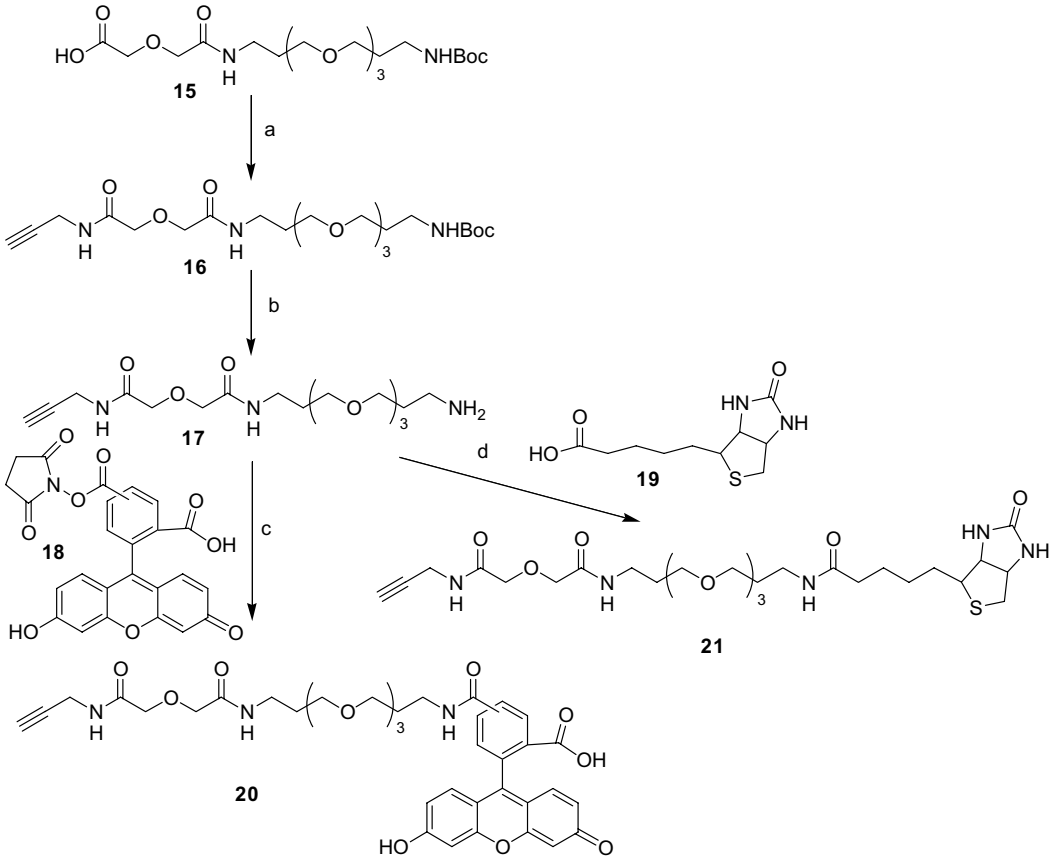
The click reaction between biotin conjugated with the functionalised spacer **21** and pseudopeptides **6** or **8** was performed under the same conditions reported above, yielding the conjugation products **22** and **24**, respectively, in 50–72% yield (Scheme 4).



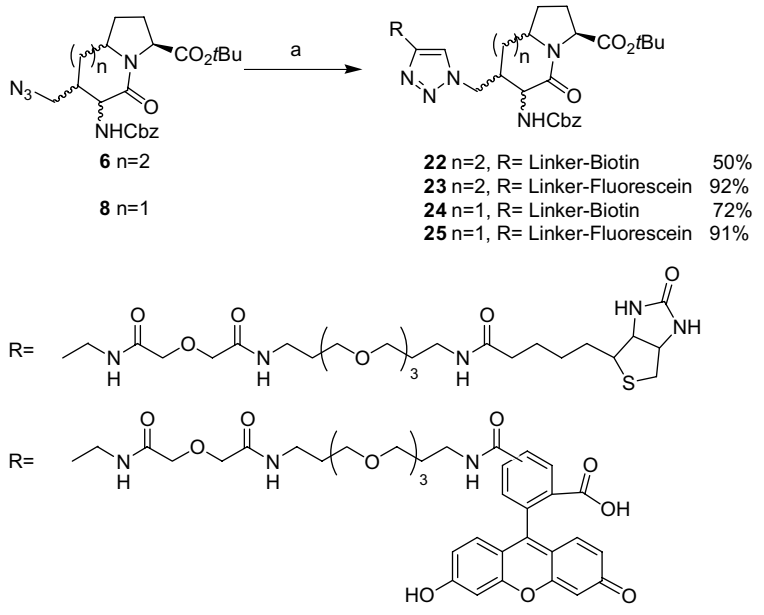
Scheme 1. Introduction of the azide group on to the azabicycloalkane scaffolds. Reagents and conditions: (a) CbzCl, TEA, CH_2Cl_2, rt, 18 h; (b) MsCl, TEA, CH_2Cl_2, rt, 45 min; (c) NaN_3, DMF, 80 °C, 18 h.



Scheme 2. 1,3-Dipolar cycloaddition between pseudopeptides **6** or **8** and the 1-*O*-propargyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucose. Reagents and conditions: (a) **9** or **10**, Na-ascorbate, $Cu(OAc)_2$, *t*-BuOH/H_2O 1:1, rt, 18 h.



Scheme 3. Conjugation of fluorescein and biotin to the linker. Reagents and conditions: (a) propargylamine, HBTU, DIPEA, CH_2Cl_2, rt, 18 h (97%); (b) TFA (50%), CH_2Cl_2, rt, 1 h; (c) **18**, TEA, THF, rt, 22 h (78% over two steps); (d) **19**, HBTU, DIPEA, CH_2Cl_2, rt, 20 h (60% over two steps).



Scheme 4. 1,3-Dipolar cycloaddition between pseudopeptides **6** or **8** and the functionalised tags. Reagents and conditions: (a) **20** or **21**, Na-ascorbate, $Cu(OAc)_2$, *t*-BuOH/H_2O 1:1, rt, 20 h.

The same reaction performed between **6** or **8** and the fluorescein tag **20**, gave the final compounds **23** and **25** in higher yields (91–92%).

The click reactions between **6** or **8** and the biotinylated tag **21** (Scheme 4) were also attempted using $CuSO_4$ as source of copper, but no increase in yield was observed.

3. Conclusions

In conclusion, click chemistry has been established as an efficient and simple method for the functionalisation of azabicycloalkane amino acids, mimics of a homoSer-Pro dipeptide with biologically relevant partners. The compatibility of Cu(I)-catalysed azide–alkyne coupling with many functional groups allows for the versatile modular synthesis of pseudopeptide conjugates using a variety of suitable functionalised molecule (drugs, fluorophores, affinity tags, etc.). Moreover conjugation occurs through the formation of a 1,2,3-triazole, the presence of which could impart greater stability to enzymatic degradation.

The pseudopeptide conjugates will be investigated as potential bioactive molecules (to be reported in due course), but can also be employed to substitute natural peptides in a more complex structure of biological interest.

Studies are in progress to apply this methodology to a small oligopeptide containing the homoSer-Pro dipeptide mimic.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.03.149.

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11. General procedure for click reaction: To a solution of compound **6** or **8** (0.1 mmol) and an appropriate alkynyl derivative (**9**, **10**, **18**, **19**) (0.1 mmol) in H_2O/*t*-BuOH 1:1 (500 μL), a solution of sodium ascorbate 0.9 M (44 μL, 0.04 mmol, 0.4 mol equiv) and a solution of $Cu(OAc)_2$ 0.3 M (67 μL, 0.02 mmol, 0.2 mol equiv) were added. The reaction mixture was stirred at rt for ca. 18 h with TLC monitoring. After the completion of the reaction, compounds **11**–**14** were extracted with CH_2Cl_2 (2 mL × 3) and the organic phase was washed with a saturated solution of $NaHCO_3$ (5 mL × 1) and subsequently with brine (5 mL × 1). The organic phase, dried with Na_2SO_4, was evaporated under reduced pressure. For compounds **22**–**25**, after the completion of the reaction, the solvent was evaporated under reduced pressure. The crudes were purified by flash chromatography on silica gel affording the desired products.
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