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In-depth evaluation of the cycloaddition–retro-Diels–Alder reaction for in vivo targeting with [^{111}In]-DTPA-RGD conjugates

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Abstract

Introduction: The spontaneous copper-free tandem 1,3-dipolar cycloaddition–retro-Diels–Alder (tandem crDA) reaction between cyclic Arg-Gly-Asp-D-Phe-Orn(N_3) [*c*(RGDfX)] and oxanorbornadiene-DTPA (o-DTPA) or methyloxanorbornadiene-DTPA (mo-DTPA) into two DTPA-*c*(RGDfX) regioisomers is characterized. Since there is no information on the stability and reaction rate of the tandem crDA reaction in biological media, we set out to characterize these reaction parameters.

Methods: The effects of concentration of the reactants, temperature, pH and reaction environment (serum, blood) on the kinetics of the reaction were determined using ^{111}In-labeled oxanorbornadiene-DTPA analogs. The affinity of the radiolabeled conjugate was determined in a solid-phase $\alpha_v\beta_3$ integrin binding assay. Furthermore, the octanol–water partition coefficient was determined and, finally, the biodistribution of the labeled compounds in mice with subcutaneous $\alpha_v\beta_3$-expressing tumors was determined.

Results: Fifty percent conversion was reached after 26 h. Kinetic experiments furthermore established that the reaction rate of the tandem crDA reaction follows temperature- and concentration-dependent second-order kinetics, but is independent of the pH of the medium. Affinity of the two [^{111}In]DTPA-*c*RGDfX conjugates for $\alpha_v\beta_3$ integrin is 191 nM. Biodistribution studies showed specific ($\alpha_v\beta_3$-mediated) uptake of [^{111}In]DTPA-*c*(RGDfX) in the tumor and in $\alpha_v\beta_3$-expressing tissues.

Conclusion: The tandem crDA reaction using methyl-substituted oxanorbornadiene is a versatile method for a single-step ligation that proceeds independently of pH and also proceeds in serum and blood. Currently, we are further looking into enhancement of reaction kinetics and exploitation of tandem crDA in vivo.

Keywords: Oxanorbornadiene; Cycloadditions; Radiolabeling; Cyclic-RGD; Bioconjugation

1. Introduction

In recent years, single-step straightforward conjugation methods in living cells or systems are gaining more and more interest in the molecular imaging field. Obviously, in vivo application of such so-called bio-orthogonal ligation methods requires high reactivity. The fundamental basis of bio-orthogonal methodologies relies on the principle of specific reaction between two participating functional groups that are tuned such that interference with coexisting functionalities is circumvented. Tools in this field that are already commonly employed are the Diels–Alder [1,2] and Staudinger ligation [3–6]. Probably, the most widely applied ligation technique, however, is the Cu(I)-catalyzed variant of the Huisgen 1,3-dipolar azide-alkyne cycloaddition reaction [7], a prototypical example of a so-called click reaction [8,9]. The strength of this reaction is the use of normally inert azide and alkyne functionalities, but when brought together in the presence of copper(I) leads to the formation of a 1,2,3-triazole in high efficiency and yield. Both the azide and alkyne can bear functionalized tails, and since the cycloaddition is compatible with a large variety of functional groups,

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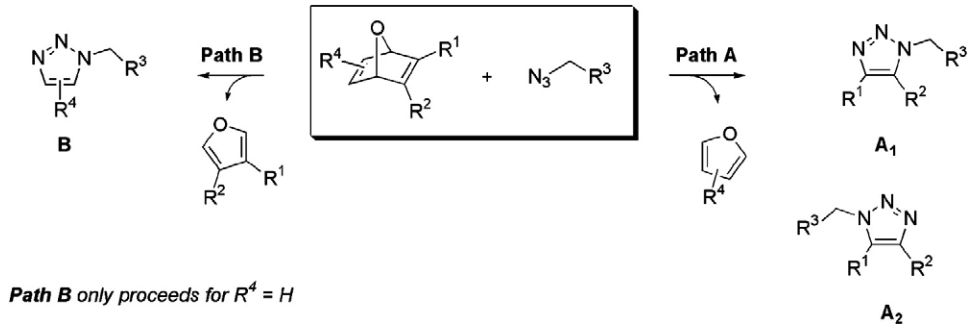


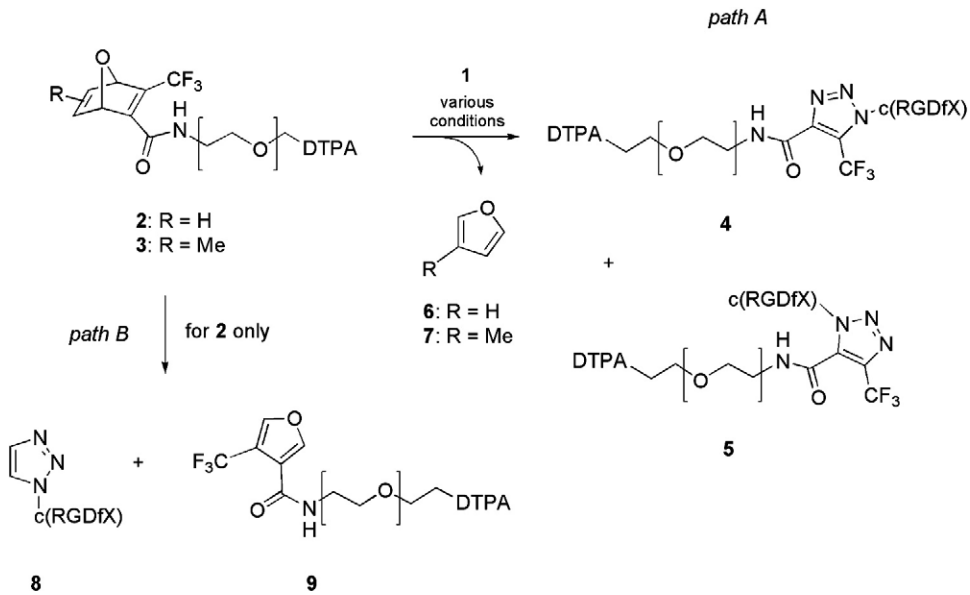
Scheme 1. Reaction mechanism of the tandem crDA reaction.

this reaction represents a generally applicable and relatively mild method to ligate molecules.

The in vivo application of the copper(I)-catalyzed Huisgen azide-alkyne cycloaddition is rather limited since copper ions are toxic for both pro- and eukaryotic cells [10]. To circumvent this difficulty, several copper-free azide-alkyne cycloaddition reactions have been developed. Most prominently, Agard et al. [11,12] reported a strain-promoted 1,3-dipolar cycloaddition of functionalized cyclooctyne derivatives, while Li et al. [13] explored the 1,3-dipolar cycloaddition between azides and electron-deficient alkynes. Very recently, a synthetically more accessible cyclooctyne suited for spontaneous ligation reactions was published by Ning et al. [14].

In search of alternative strategies for metal-free 1,3-dipolar cycloaddition reactions, we previously described the combination of ring strain and electron deficiency in oxa-bridged bicyclic systems such as oxanorbornadiene as a viable ligation approach [15]. When such electron-deficient oxanorbornadienes encounter an organic azide, a spontaneous tandem 1,3-dipolar cycloaddition–retro-Diels–Alder (tandem crDA) reaction occurs, resulting in a 1,2,3-triazole linkage (Scheme 1). Interestingly, the azide may react with the oxanorbornadiene via two distinct pathways: in case the oxanorbornadiene system bears electron-withdrawing R^1 and/or R^2 substituents, e.g., a trifluoromethyl group, Pathway A is favored. Cycloaddition of the azide then occurs predominantly at the most electron-deficient double bond ultimately resulting in the stable 1,4,5-trisubstituted 1,2,3-triazoles A_1 and A_2. This process proceeds via initial triazoline formation, followed by a fast retro-Diels–Alder reaction and concomitant loss of furan. Inversely, in the case of R^4=H, cycloaddition on the other double bond of the oxa-bridged bicyclic compound may occur, eventually leading to the monosubstituted 1,4,5-triazole B and the corresponding 3,4-substituted furan. Recently, we demonstrated that product B can be formed in yields ranging from 3% to 16%, depending on the various substituents R^1 and R^2 [16].

The latter studies served to prepare cyclic Arg-Gly-Asp–diethylenetriamine pentaacetic acid (RGD-DTPA) conjugates, which were then subjected to preliminary in vitro biological evaluation showing a high affinity for $\alpha_v\beta_3$ integrin receptors (IC_{50}=192 nm) and favorable pharmacokinetics. To further



Scheme 2. Tandem crDA reaction of *c*(RGDfX) and (m)o-DTPA.