Bioorganic & Medicinal Chemistry Letters 20 (2010) 5521–5526

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of MRI contrast agents derived from DOTAM-Gly-L-Phe-OH incorporating a disulfide bridge: Conjugation to a cell penetrating peptide and preparation of a dimeric agent

Mojmír Suchý [a,b], Alex X. Li [b], Robert Bartha [b], Robert H. E. Hudson [a,*]

[a] Department of Chemistry, The University of Western Ontario, London, ON, Canada N6A 5B7
[b] Centre for Functional and Metabolic Mapping, Robarts Research Institute, The University of Western Ontario, London, ON, Canada N6A 5K8

ARTICLE INFO

Article history:
Received 6 May 2010
Revised 15 July 2010
Accepted 16 July 2010
Available online 23 July 2010

Keywords:
PARACEST
Europium
Contrast agent
TAT
Bimetallic

ABSTRACT

A cell penetrating peptide conjugate and dimeric PARACEST MRI contrast agents, based on the DOTAM-Gly-L-Phe-OH scaffold have been prepared in moderate yields using diethyl azodicarboxylate (DEAD) or iodine-mediated disulfide bridge formation as a key step. Magnetic (PARACEST) properties of these agents have been evaluated.

Magnetic resonance imaging (MRI) represents an important diagnostic tool widely employed to obtain anatomical images of soft tissues based upon the detection of protons in the body.[1] Gd^{3+}-based T_1-shortening MRI contrast agents (CAs) are prominently used in clinical diagnostics and decrease bulk water relaxation time constants through the rapid exchange of agent-bound water with bulk water. However, there are several limitations associated with their current use. Firstly, their presence is always detected in an MR image; and secondly, designing Gd^{3+}-compounds sensitive to metabolic or environmental conditions that maintain rapid water exchange and consequently high relaxivity is difficult.

Recently, an alternative method of generating contrast in MRI was introduced based on chemical exchange saturation transfer (CEST).[2] Subsequently, a large number of paramagnetic ion-containing lanthanide(III) complexes, mainly involving Eu^{3+} and Tm^{3+}, have been prepared which are capable of inducing large hyperfine shifts of coordinated water protons as well as other exchangeable protons present in close proximity to the metal center.[3] The term PARACEST MRI CAs has been coined to describe these molecules.[4] When compared to classical Gd^{3+}-derived MRI CAs, the spectrum of information obtained using PARACEST MRI CAs is potentially broader as the CEST effect is sensitive to the environment. PARACEST CAs have been previously used to detect physiological conditions such as tissue temperature[5] and pH.[6] Primary metabolites such as glucose,[7] polyarginine[8] and lactate[9] can also be detected, along with biologically important phosphate anions[10] and Zn^{2+} cations.[11] PARACEST MRI CAs have been successfully applied toward the detection of enzymatic activities, for example: cathepsin D;[12] β-galactosidase;[13] caspase-3;[14] or urokinase plasminogen activator.[15]

Despite the number of PARACEST MRI contrast agents recently described, their detection in vivo[16] remains a challenging task. This can be attributed to rather low sensitivity as well as limited bioavailability of these agents. To address these issues, we turned our attention to a sulfanyl (thiol, SH) modified ligand **1** (Fig. 1),[17] based on Eu^{3+} DOTAM-Gly-Phe-OH (**2b**, Fig. 1),[18] a DOTAM-polyamide based PARACEST MRI CA exhibiting a high level of sensitivity within the physiological temperature range (36–40 °C). To facilitate the vectorization of **2b** and increase its bioavailability, thiol **1** was conjugated to a cell penetrating TAT peptide[19] by diethylazodicarboxylate (DEAD)-promoted disulfide bond formation[20] followed by metalation with $EuCl_3 \cdot 6H_2O$. The resulting conjugate (**3**, Fig. 1) is expected to undergo reductive disulfide cleavage by endogenous thiols, such as glutathione, within the intracellular compartment leaving the Eu^{3+} containing reporter group trapped inside the cells for an extended period of time. Alternatively, treatment with an exogenous thiol may facilitate excretion after a period of MR imaging.[21]

Disulfide linkages have been employed previously in the preparation of macromolecular complexes and peptide conjugates,

* Corresponding author. Fax: +1 519 661 3022.
E-mail address: rhhudson@uwo.ca (R.H.E. Hudson).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.070

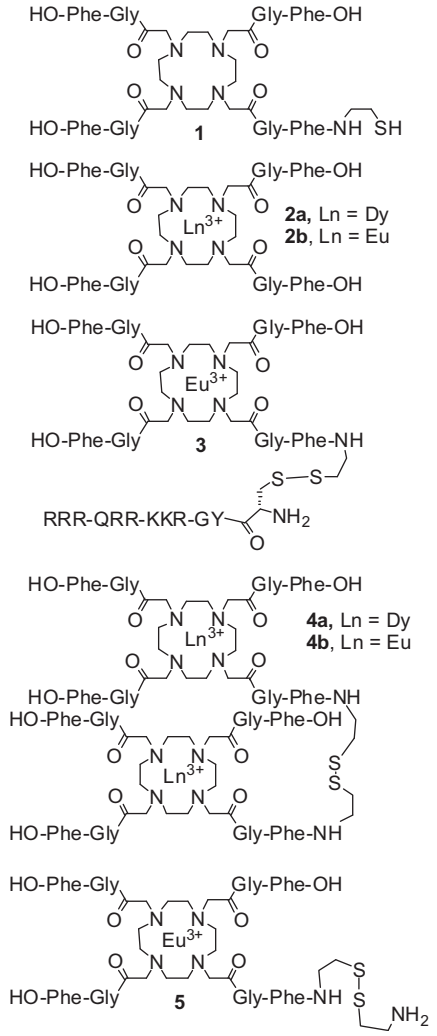


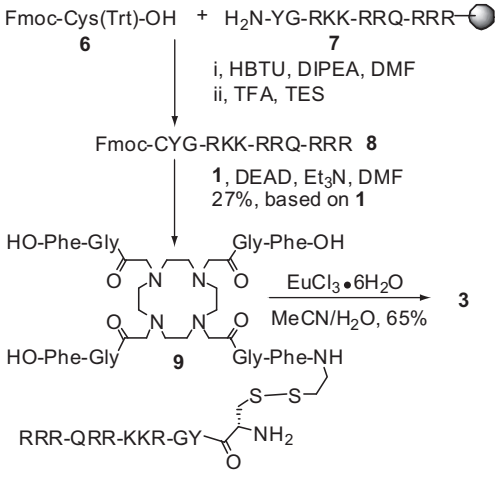
Figure 1. Structures of ligands and complexes **1**–**5.**

although disulfide bond formation was not always the key step. For instance, an approach using cystamine as a linker wherein the β-amino group was used for amide bond formation has been described for the conjugation of Gd^{3+}-derived MRI CAs with poly(L-glutamic acid)[21] and poly(L-arginine).[22] A Gd^{3+}-based DOTA has been conjugated to reduced bovine serum albumin (BSA) or silica nanoparticles by disufides formed through an intermediate methanethiosulfonate derivative.[23] Also carbon–sulfur bond formation was exploited when a sulfanyl modified DOTA ligand was attached to phosphopeptides by Michael addition to dehydroalaninyl or β-methyldehydroalaninyl residues.[24]

The presence of a free SH group in **1** also prompted us to explore the preparation of a homodimeric ligand via symmetrical disulfide bond formation. The resulting bifunctional disulfide-bridged ligand was envisioned to undergo a double metalation with Dy^{3+} or Eu^{3+} salts to form a bimetallic PARACEST MRI CAs **4a**,**b** (Fig. 1) expected to possess higher sensitivity, compared to monometallic CAs **2**, based on literature precedent. Sherry and co-workers used free-radical polymerization and prepared a series of DOTAM-based linear polymers which upon metalation with Eu^{3+} salts afforded PARACEST MRI CAs with enhanced sensitivities.[25] A large number of PARACEST CAs have also been shown to be conjugated to adenovirus, although diminished viral bioactivity was observed when the number of ligands attached to the virus exceeded ~1000.[26] Several dendritic pH responsive multimeric PARACEST MRI CAs have been synthesized and have been shown to retain sensitivity on a per-lanthanide basis.[27] PANAM-based dendritic PARACEST CAs have been prepared and used to detect flank tumor in vivo.[16a,28] Morrow and co-workers have evaluated Eu^{3+}- and Nd^{3+}-based bimetallic complexes consisting of two DOTAM units joined by *p*-dibenzyl linkage as potential carbonate or DNA responsive PARACEST MRI CAs.[29] We have recently prepared and evaluated both homo- (containing two Eu^{3+}) and heterobimetallic (containing Eu^{3+} and Tm^{3+}) complexes featuring two different chelator subunits.[30]

In the present study, we report the synthesis and magnetic properties associated with cell penetrating peptide conjugated PARACEST MRI CA **3** and bimetallic complexes **4** (Fig. 1). The synthetic methodology represents an alternative approach to conjugation of DOTA- and DOTAM-derived ligands with peptides that rely mainly on amide bond formation between DOTA- or DOTAM-derived carboxylic acids and N-terminus unprotected peptides attached to a solid support.[31]

Sulfanyl-modified ligand **1** has been prepared following the experimental protocol recently established in our laboratory.[17] We initially attempted to conjugate ligand **1** to the cell penetrating TAT peptide ('*N*'-YG-RKK-RRQ-RRR-'*C*') by solid phase peptide synthesis. The fully protected peptide (for the protecting groups see ref.32) attached to Wang's resin was derivatized with commercially available Fmoc-Cys(Mmt)-OH. The acid labile Mmt protecting group was selectively removed (1% TFA in CH_2Cl_2), followed by treatment with ligand **1** preactivated with diethylazodicarboxylate (DEAD).[20] Cleavage of the peptide from the resin (5% TES in wet TFA) followed by HPLC purification afforded the desired disulfide-bridged conjugate in unacceptable (<5%, based on ligand **1**) yield, despite many attempts to modify the reaction conditions. To overcome this problem we turned to solution phase conjugation chemistry as indicated in Scheme 1.[33] Thus, Fmoc-Cys(Trt)-OH (**6**) was coupled (HBTU, DIPEA, and DMF) to the resin bound, fully protected peptide **7**. Peptide **8** was obtained after cleavage from the resin and HPLC purification (Scheme 1). Treatment of peptide **8** with ligand **1** under the conditions of DEAD-promoted asymmetrical disulfide bond formation afforded conjugate **9** in 27% yield (based on **1**) after HPLC purification. With ligand **9** in hand we performed the metalation with $EuCl_3{\cdot}6H_2O$ (Scheme 1). This reaction was found to be surprisingly slow, requiring large excess of the Eu^{3+} salt (20 equiv), elevated reaction temperature (60 °C) and prolonged reaction time (2–3 days). A time course study of the metalation of **9** with $EuCl_3{\cdot}6H_2O$ can be found in Supplementary data. HPLC purification afforded desired Eu^{3+} DOTAM-Gly-L-Phe-OH-TAT peptide conjugate **3** in 65% yield (Scheme 1). It is worth



Scheme 1. Synthesis of Eu^{3+} DOTAM-Gly-L-Phe-OH peptide conjugate **3**.