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ARTICLE *in* ORGANIC LETTERS · JANUARY 2005

Impact Factor: 6.36 · DOI: 10.1021/ol048084u · Source: PubMed

ORGANIC LETTERS

2004
Vol. 6, No. 26
4857−4860

Design and Synthesis of a Bimodal Target-Specific Contrast Agent for Angiogenesis

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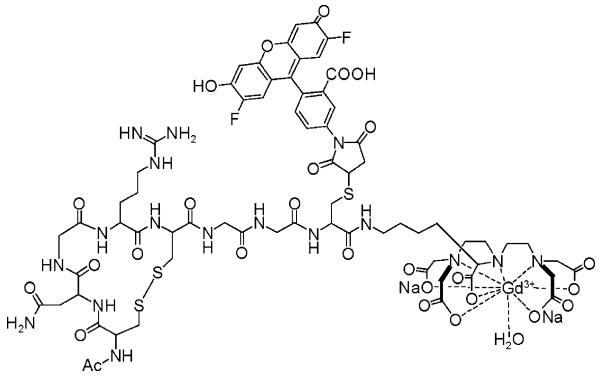
Received September 20, 2004

ABSTRACT



A bimodal target-specific contrast agent based on a cyclic peptide containing the target-specific NGR sequence, gadolinium(III) diethylenetriaminepentaacetic acid (Gd(III)DTPA), and Oregon Green 488 (OG488) suitable for both MR imaging and optical imaging of angiogenesis is developed. The synthetic strategy for this target-specific contrast agent exploits the use of highly efficient, chemoselective reactions, such as native chemical ligation, and gives a straightforward approach for double labeling of peptides in general.

Magnetic resonance imaging (MRI) is a powerful, noninvasive technique for the visualization of soft tissue anatomy and for the diagnosis of diseases. Some phenomena of interest, such as angiogenesis (the formation of new blood vessels), are difficult to image with MRI. Therefore, target-specific MRI contrast agents[1] designed to bind to proteins, expressed by the cells involved in angiogenesis, could improve the imaging of this process drastically due to the accumulation of contrast agents around these cells. The cyclic peptide containing the asparagine glycine arginine (NGR) sequence (cNGR) was reported to bind to CD13, a protein expressed by cells involved in angiogenesis.[2,3] To gain more insight into the targeting process of cNGR a bimodal target specific probe, based on Oregon Green 488 (OG488, a fluorescent label), gadolinium(III) diethylenetriaminepentaacetic acid (Gd(III)DTPA, an MRI label), and cNGR, has been synthesized. This will allow imaging with both MRI and optical methods. In this paper, we describe the synthesis of this bimodal target-specific contrast agent utilizing highly efficient, chemoselective reaction procedures.

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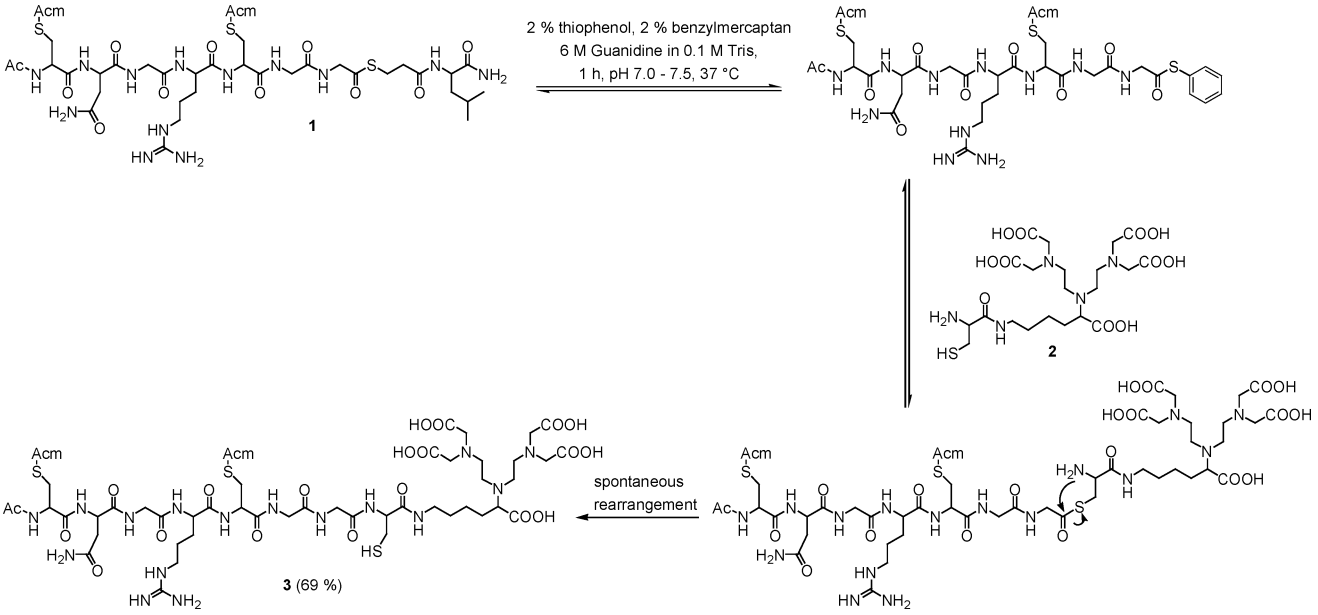
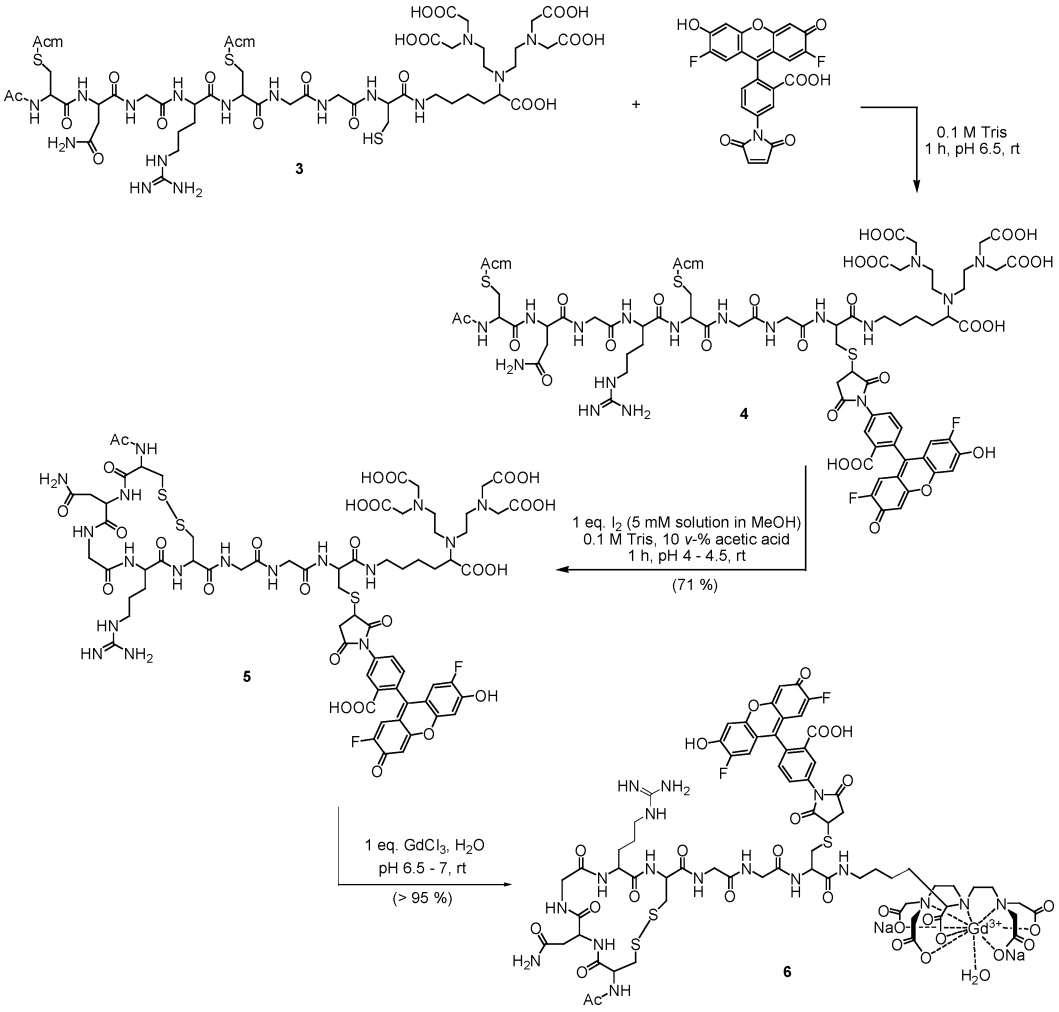
10.1021/ol048084u CCC: $27.50
Published on Web 12/01/2004

Scheme 1



Scheme 2



In 1994, Dawson et al. introduced native chemical ligation as a tool to facilitate the synthesis of proteins of moderate size. The ligation process involves the chemoselective reaction between a C-terminal thioester and an N-terminal cysteine residue, resulting in a native peptide bond at the site of ligation.[4,5] Recently, Tolbert and Wong exploited this approach to tag labels to proteins by reacting a cysteine-functionalized label with a thioester-functionalized protein.[6] In a similar fashion, we use this procedure to tag the cNGR peptide with a Gd(III)DTPA label.

For this, manual solid-phase peptide synthesis (SPPS) using the in situ neutralization/HBTU activation procedure for Boc chemistry on an MBHA resin, as described earlier by Schnölzer et al.,[7] was applied to synthesize a peptide containing the target-specific NGR sequence with a C-terminal thioester **1**. At this point it is important to notice that the thiol groups of the two cysteine residues of the peptide are protected with acetamidomethyl (Acm) groups. This will ensure the correct "folding" of the peptide into its cyclic form later on (vide infra).

Reaction of **1** with a small excess of cysteine-functionalized DTPA synthon **2** under ligation conditions, i.e., in the presence of thiols for exchange while the pH is maintained at 7.0−7.5, rendered the DTPA-functionalized peptide **3** (Scheme 1). The reaction was monitored employing analytical reversed-phase HPLC (RP HPLC) using a C18 column for separation coupled to UV−vis ($\lambda_{probe} = 214$ nm). After 2 h, the ligation reaction was completed and the thioester-functionalized peptide **1** reacted quantitatively with **2**. The ligation product was purified using preparative RP HPLC on a C18 column, obtaining **3** in 69% yield. The free thiol of the cysteine residue of **3**, which took part in the ligation reaction, was used to introduce the fluorescent label Oregon Green 488 (OG488). This was achieved through the reaction of **3** with the maleimide of OG488 rendering **4** (Scheme 2). Again the reaction was monitored employing analytical RP HPLC coupled to UV−vis. Once the reaction went to completion, the reaction mixture was diluted ~30 times with 0.1 M Tris (aq, pH 6.92) containing 10 vol % of acetic acid. Under these conditions the Acm protecting groups of **4** were removed through the addition of 1 equiv of I_2 to give **5**. The removal of the Acm groups resulted instantaneously in the correct "folding" of the peptide unit into its cyclic form (Scheme 2) as confirmed with ES-MS, which shows a loss in molecular weight of 144 corresponding to the weight of the two Acm groups (Figure 1). Purification with RP HPLC using a C18 column gave **5** in 71% yield. Upon addition of 1 equiv of $GdCl_3$ to **5**, the corresponding Gd(III) complex **6** was obtained in a nearly quantitative yield (>95%) as an orange powder (Scheme 2). This was confirmed by ES-MS (Figure 1) and inductively coupled plasma analysis (ICP-AES), via which a gadolinium content of 94% was determined.

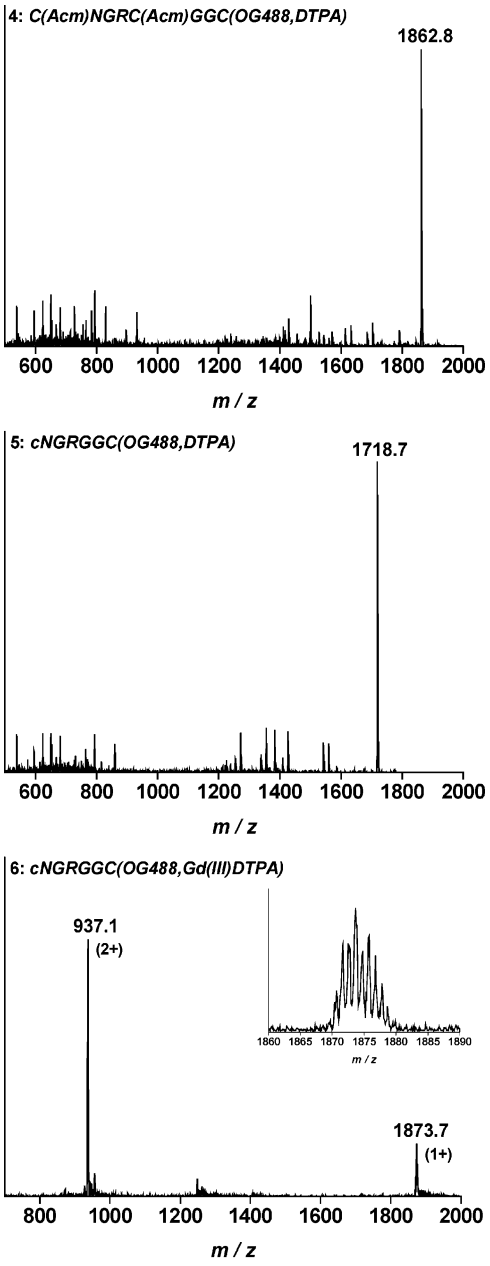


Figure 1. ES-MS spectra of **4**−**6**.

MRI contrast agents are used to improve the contrast in MR images based on the longitudinal (T_1) and transverse (T_2) relaxation times of the protons of water molecules. The ability of Gd(III)DTPA-based MRI contrast agents to lower the T_1 and T_2 is expressed in terms of longitudinal (r_1) and transverse (r_2) relaxivity, respectively ($r_{1,2} = ((1/T_{1,2})_{observed} - (1/T_{1,2})_{diamagnetic})/[Gd(III)]$).[8] To determine the r_1 and r_2 of the bimodal target-specific contrast agent **6**, concentration-dependent measurements of the relaxation times were performed in H_2O, giving an r_1 of 7.5 mM^{-1} s^{-1} and an r_2 of 8.3 mM^{-1} s^{-1}. The longitudinal and transverse relaxivities

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of **6** are substantially higher than the values for the parent Gd-DTPA complex ($r_1 = 4.3\ mM^{-1}\ s^{-1}$ and $r_2 = 4.7\ mM^{-1}\ s^{-1}$ at 1.5 T and 20 °C), presumably due to the slower molecular tumbling of the gadolinium complex as a result of the higher molecular weight.

In conclusion, a straightforward methodology for the double labeling of cNGR, complying the tagging of the cyclic peptide with both Gd(III)DTPA and Oregon Green 488, is developed and can be regarded as a general strategy for the multilabeling of peptides. This bimodal target-specific contrast agent will be investigated in vivo and ex vivo with MRI and optical imaging methods to gain more insight into the targeting of angiogenesis with cNGR.

Acknowledgment. W. Adriaens (University of Maastricht, CARIM) is kindly acknowledged for her support during peptide synthesis and purification. This work was financially supported by the Council for Chemical Sciences of The Netherlands Organization for Scientific Research.

Supporting Information Available: Experimental procedures, ES-MS data, and the HPLC traces of compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL048084U