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Synthesis of novel multivalent fluorescent inhibitors with high affinity to prostate cancer and their biological evaluation

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ABSTRACT

Prostate-specific membrane antigen (PSMA) is an important biological target for therapy and diagnosis of prostate cancer. In this study, novel multivalent PSMA inhibitors with glutamate-urea-lysine structures were designed to improve inhibition characteristics. Precursors of the novel inhibitors were prepared from glutamic acid with di-*tert*-butyl ester. A near-infrared molecular dye, sulfo-Cy5.5, was introduced into the precursors to generate the final PSMA fluorescent inhibitors, compounds **12**–**14**, to visualize prostate cancer. Biological behaviors of the inhibitors were evaluated using *in vitro* inhibition assays, *in vivo* fluorescent imaging, and *ex vivo* biodistribution assays. K_i values from inhibition studies indicated that dimeric inhibitor **13** with a glutamine linker showed approximately 3-fold more inhibitory activity than monomeric inhibitor **12**. According to other biological studies using a mouse model of prostate cancer, dimeric inhibitor compounds **13** and **14** had higher tumor accumulation than the monomer. However, glutamine-based dimeric inhibitor **13** showed lower liver uptake than dimeric inhibitor **14**, which had a benzene structure. Thus, these studies suggest that glutamine-based dimeric inhibitor **13** can be a promising optical inhibitor of prostate cancer.

Prostate cancer is one of the most common cancers in men and the third leading cause of cancer-related deaths in American men.[1] Many patients with prostate cancer are treated with radical prostatectomy which has the drawback of positive surgical margins (PSM), meaning incomplete cancer resection.[2] Avoiding PSM is taxing for surgeons because of difficulty identifying local invasion by prostate cancer during surgery.[3] Therefore, a technical means of visualizing tumor invasion would help surgeons improve prostatectomy outcomes. An optical imaging agent with a fluorescent moiety emitting light in wavelengths in the near-infrared (NIR) region between 650 and 900 nm would be ideal because NIR light efficiently penetrates biological tissues, which emit low autofluorescence in this wavelength range.[4]

Prostate-specific membrane antigen (PSMA), known as glutamate carboxypeptidase II, is a type II membrane glycoprotein.[5] PSMA is a zinc metalloenzyme catalyzing the hydrolysis of *N*-acetylaspartylglutamate to glutamate and *N*-acetylaspartate.[6] Since it is distinctly overexpressed in most prostate cancers including metastatic tumors,[5–7] PSMA is an attractive biological target for imaging prostate cancer.[5–9] Although a variety of imaging tracers with antibodies targeting PSMA have been developed and demonstrated positive results,[10–12] these agents have drawbacks for image-guided surgery such as slow clearance from non-target sites, which delays target recognition.[13] Thus, many PSMA-targeting small molecular agents have been developed.[13–24] Among them, many promising inhibitors that target PSMA have a key moiety, a glutamate-urea-lysine (GUL) structure.[15,18–24]

Cancer-targeting fluorescence imaging systems are considered useful tools for cancer diagnosis and image-guided surgery, so several research groups have focused on the development of effective fluorescent PSMA probes with the GUL structure for targeting prostate cancer.[19,20,23] The groups have widely investigated for linker effects between a fluorescent dye and the GUL structure and the dye's effects, but not for multimerization of the GUL, which can be an attractive approach for increasing binding affinity and

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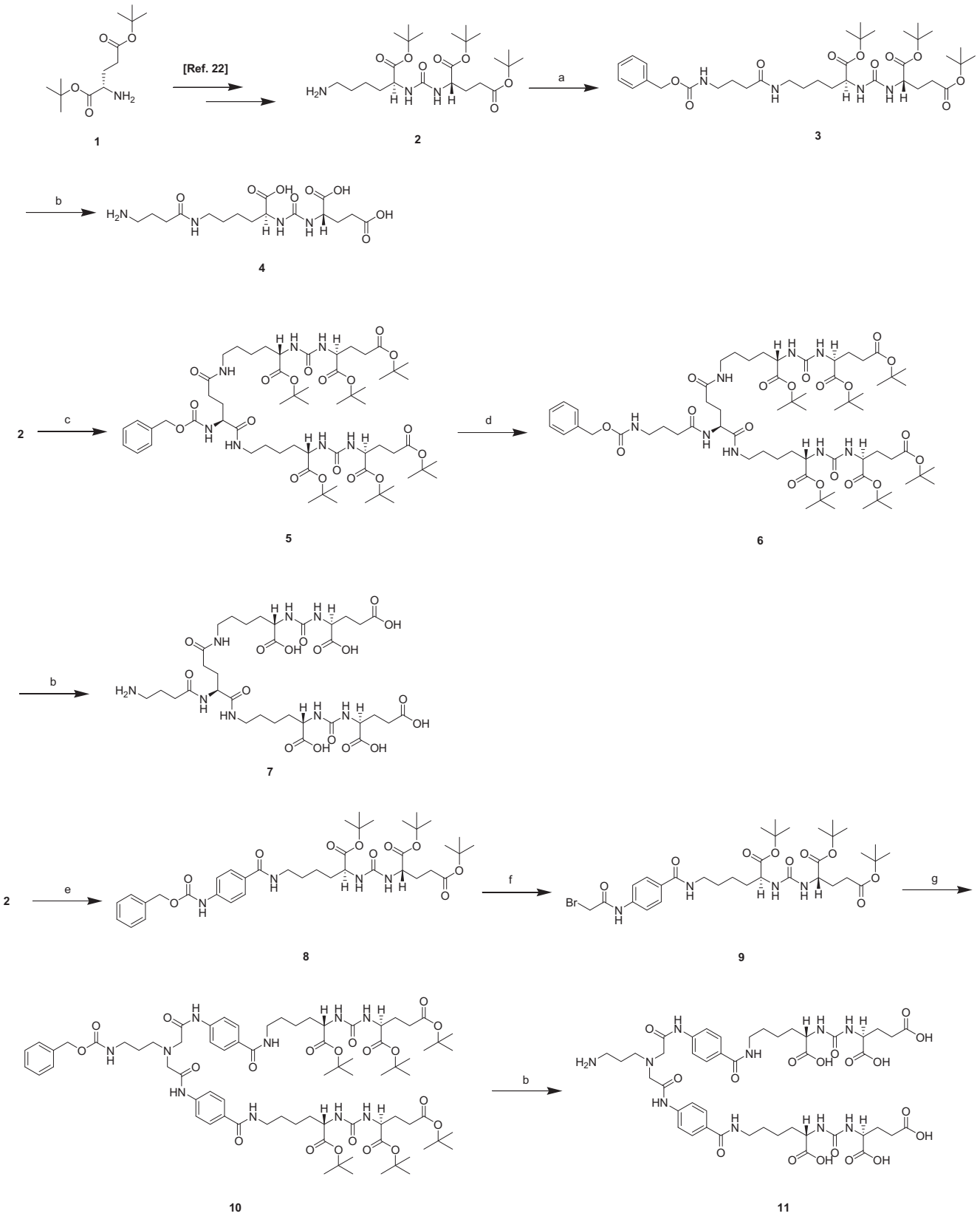

Scheme 1. The synthesis of inhibitor precursors. Reagents and conditions: (a) 4-(benzyloxycarbonylamino)butanoic acid, hydroxybenzotriazole (HOBt), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), triethylamine (Et_3N), dichloromethane, r.t., 9 h; (b) 1. H_2 gas, 10% Pd/C, methanol, r.t., 12 h, 2. Trifluoroacetic acid, dichloromethane, r.t., 20 or 22 h; (c) (*S*)-2-(benzyloxycarbonylamino)pentanedioic acid, HOBt, EDC, Et_3N, *N*,*N*-dimethylformamide (DMF), r.t., 14 h; (d) 1. H_2 gas, 10% Pd/C, methanol, r.t., 16 h, 2. 4-(benzyloxycarbonylamino)butanoic acid, HOBt, EDC, Et_3N, dichloromethane, r.t., 12 h; (e) 4-(benzyloxycarbonylamino)benzoic acid, HOBt, EDC, Et_3N, DMF, r.t., 15 h; (f) 1. H_2 gas, 10% Pd/C, methanol, r.t., 15 h, 2. bromoacetyl bromide, *N*,*N*-diisopropylethylamine (DIPEA), dichloromethane, r.t., 3 h; and (g) benzyl 3-aminopropylcarbamate, DIPEA, acetonitrile, 75 °C, 24 h.