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# Synthesis of a pteroic-based conjugate with ibuprofen moiety and macrocyclic chelator DOTA-GA

**Natalia S. Volkova,[a] Anton A. Larenkov,[b] Nina S. Boutakova,[a] Elena K. Beloglazkina[a] and Aleksei E. Machulkin*[a]**

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. E-mail: alekseymachulkin@rambler.ru*

[b] *Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency, 123098 Moscow, Russian Federation*

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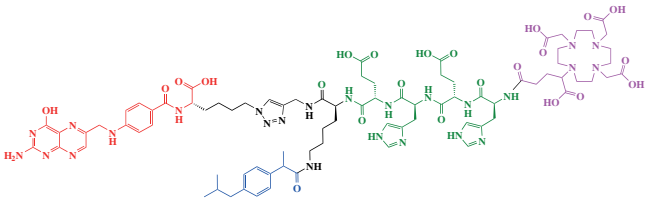
**Conjugate containing fragments of pteroic acid, anti-inflammatory drug ibuprofen, macrocyclic chelator DOTA-GA and (His-Glu)$_2$ moiety has been synthesized. Key structural motifs of this compound were introduced to optimize the pharmacokinetic characteristics of the pteroic acid-based conjugate. The complexation of the conjugate with radioactive lutetium-177 provided radiochemical yield of >99% and radiochemical purity of 97.6%.**



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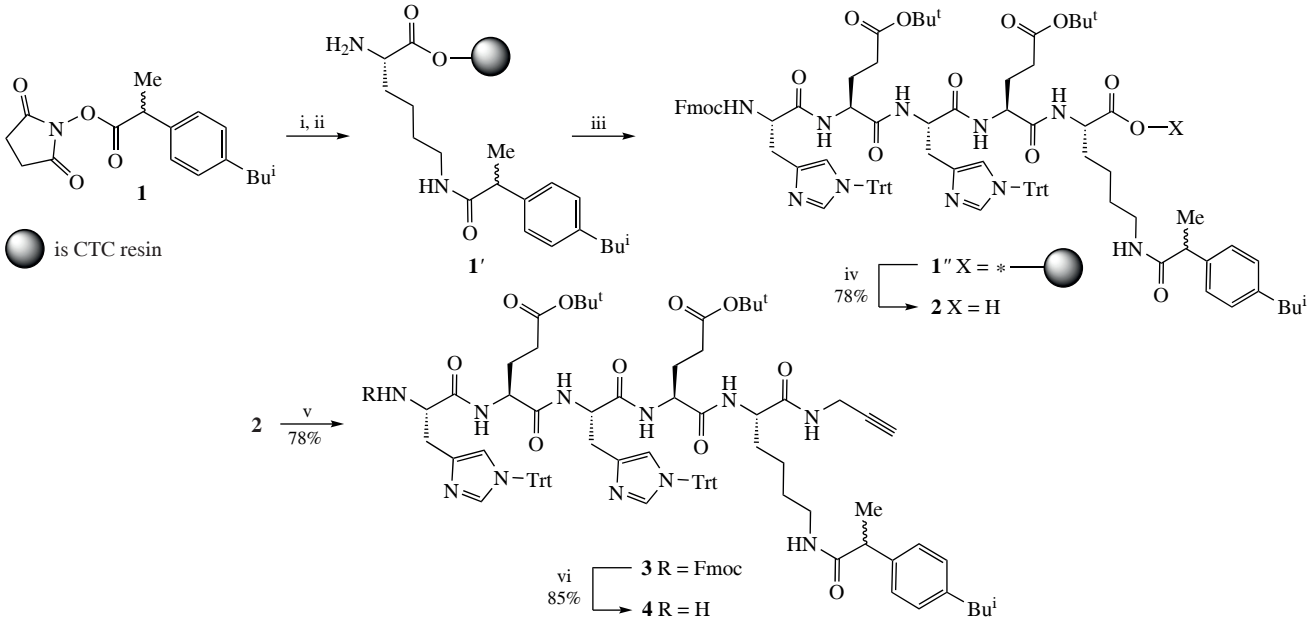
Oncological pathologies are among the most socially significant diseases, ranking second among all causes of death in the world.[1] Among positron-emitting radionuclides, $^{18}$F and $^{68}$Ga currently occupy a leading position in terms of the number of studies.[2] Together with β-emitting $^{177}$Lu, $^{68}$Ga make up a promising theranostic pair: conjugates with $^{68}$Ga are used for the diagnosis of malignant neoplasms, and conjugates with $^{177}$Lu are used for therapy.[3]

One of the most perspective tools in oncology is receptor-specific molecular therapy and imaging.[4,5] A relatively new and promising target for nuclear medicine is folate receptors (FR), a group of proteins that provide unidirectional transport of folates into the cells. There are four FR isoforms: FRα, FRβ, FRγ, and FRδ, of which FRα and FRβ are of greatest interest for medicine.[6] FRα is overexpressed in a number of tumors, while is practically not expressed in healthy tissues.[7] FRβ is overexpressed predominantly on hematopoietic cells of the myeloid lineage and in 70% of cases of acute myeloid leukemia,[8,9] as well as in activated macrophages, which is very promising for imaging autoimmune processes (*e.g.*, rheumatoid arthritis).[10,11]

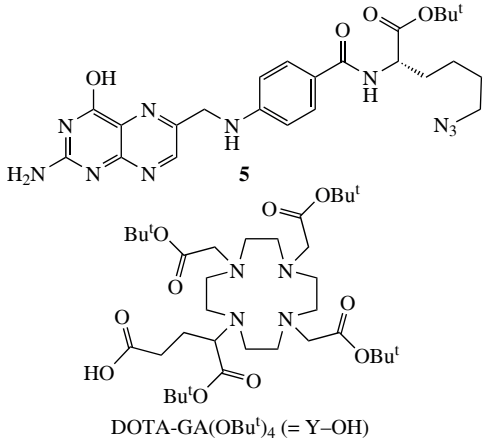
The aim of this work was to synthesize new pteroic acid-based conjugate intended for radionuclide therapy of malignant neoplasms. Its design is based on the structural modification of previously[12] obtained conjugate of folic acid with the (HE)$_2$ (His-Glu-His-Glu) peptide (for the structure, see Online Supplementary Materials, Scheme S1). In this work, the key structural modification was the introduction of ibuprofen, a non-steroidal anti-inflammatory drug, to increase the conjugate accumulation in tumor tissues and to reduce its accumulation in non-target organs, such as kidneys.[13] A key feature of this conjugate is the presence of certain structural fragments: (His-Glu)$_2$, which does not affect the binding capacity with FRs but allows for a 2–4-fold decrease in the accumulation in non-target tissues (mainly in the kidneys);[12,14] a fragment of ibuprofen as an albumin-binding entity to increase the accumulation of the radiopharmaceutical in the tumor focus;[15–17] and applied in medical imaging chelating fragment DOTA-GA for subsequent binding to $^{68}$Ga or $^{177}$Lu.

Important points of the synthesis (Scheme 1) was the preparation of modified peptide **4** comprising Lys-(His-Glu)$_2$ sequence and 'azidolysine'-containing pteroic acid **5** (see inset) which, similarly to folic acid, had an affinity for FRs.[18,19] The trityl-, *tert*-butyl-, and Boc-protected Lys-(His-Glu)$_2$ peptide was synthesized according to Fmoc protocols using 2-chlorotrityl chloride (CTC) resin and the appropriate trityl- and *tert*-butyl-protected amino acids.[20] For this purpose, *O*-CTC-tethered $N^2$-Fmoc-lysine (for synthetic details, see Online Supplementary Materials, Scheme S2) was coupled with *N*-hydroxysuccinimide ester of *rac*-ibuprofen **1** to afford amino amide **1′** (see Scheme 1). Next, peptide sequence was assembled on the modified resin by sequentially attaching Fmoc-Gly(OBu$^t$)-OH and Fmoc-His(Trt)-OH and removing the Fmoc protections, with the exception of the terminal Fmoc group, to produce resin-tethered material **1″**. Removal of the peptide from CTC resin was performed under acidic conditions to afford compound **2** whose further reactions with propargylamine and removal of the Fmoc protection led to compound **4** (see Scheme 1). The synthesis of pteroic acid modified with an azidolysine fragment, compound **5**, was performed traditionally (see Online Supplementary Materials, Scheme S3).

The final steps of the synthesis of the target conjugate **8** are shown in Scheme 2. Alkyne **4** and azide **5** entered the 1,3-dipolar cycloaddition reaction to form triazole derivative **6**. Its reaction with DOTA-GA(OBu$^t$)$_4$ under peptide synthesis conditions and subsequent removal of all protective groups led to the target conjugate **8**. When removing the protective groups in the last step using standard methods, LCMS data revealed that in addition to the target compound **8**, the reaction mixture contained the corresponding mono-, di-, and tri-*tert*-butylated derivatives, which, due to the insignificant difference in molecular weight



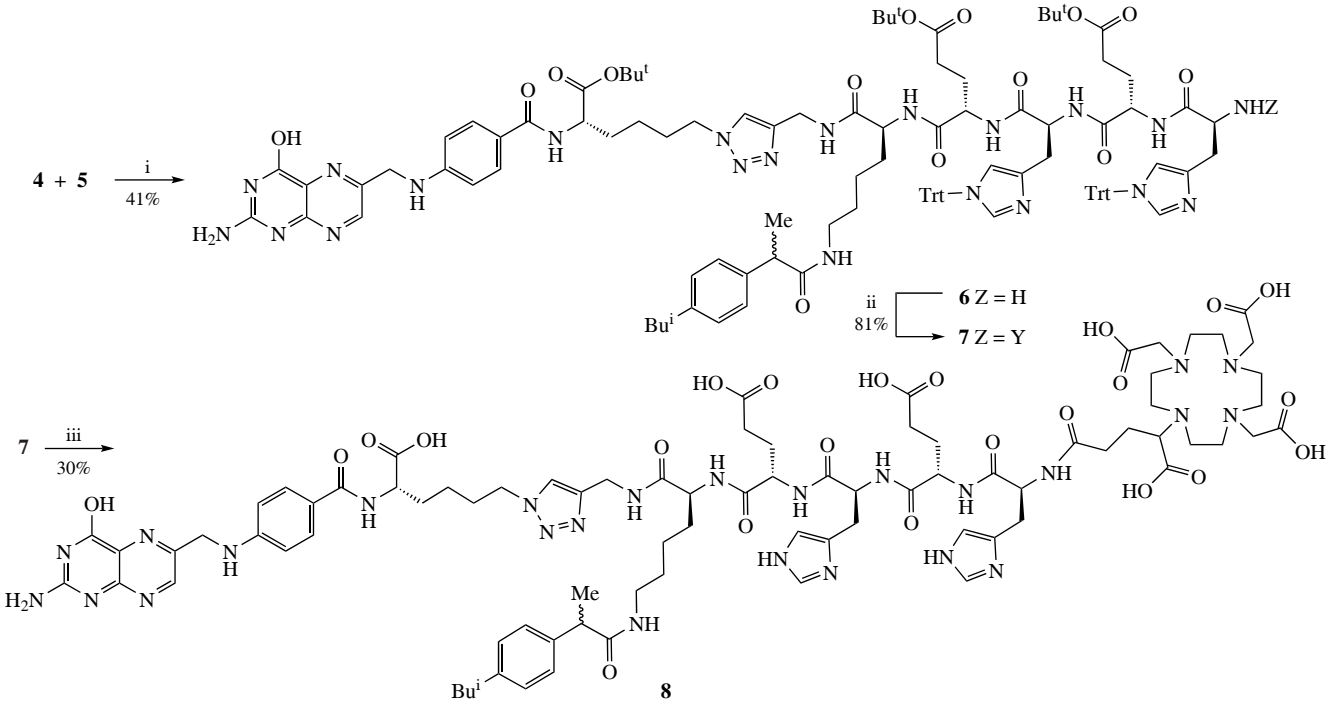

**Scheme 1** *Reagents and conditions*: i, *O*-CTC-tethered $N^2$-Fmoc-lysine, DIPEA (1.25 equiv.), $CH_2Cl_2$; ii, 20% 4-methylpiperidine, DMF; iii, multistep peptide synthesis (see Online Supplementary Materials); iv, 0.75% $CF_3CO_2H$, $CH_2Cl_2$; v, $HC{\equiv}CH_2NH_2$, DIPEA, DMF; vi, $Et_2NH$, MeCN.




and retention on silica gel with the target product, could not be separated using column chromatography. This required optimization of the final step conditions (see Online Supplementary Materials, Table S1). The final product **8** was purified by preparative HPLC.

An evaluation experiment was then conducted on radiolabeling of the purified **8** conjugate with n.c.a. lutetium-177. The results of radio-TLC and radio-HPLC showed that radioconjugate [$^{177}$Lu]Lu-**8** was obtained with a radiochemical yield of >99% and a radiochemical purity of 97.6%, the resulting graph is provided in the Online Supplementary Materials. Thus, at a [Lu]:[**8**] molar ratio of 1:5, a specific activity of 142 MBq nmol$^{-1}$ was achieved for [$^{177}$Lu]Lu-**8** radioconjugate.

To summarize, synthetic approach to conjugate **8** containing the fragment of pteroic acid as well as ibuprofen, chelator




**Scheme 2** *Reagents and conditions*: i, $CuSO_4{\cdot}5H_2O$, AscNa, DMF; ii, DOTA-GA(OBu$^t$)$_4$, HOBt, HBTU, DIPEA, DMF; iii, $CF_3CO_2H$, $Pr^i{}_3SiH$, $H_2O$.

DOTA-GA and $(His\text{-}Glu)_2$ moieties was found suitable for further *in vitro* and *in vivo* biological investigations.

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*Online Supplementary Materials*

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7764.

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