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# Communications to the Editor

## Technetium-99m-Labeled HOE 140: A Potential Bradykinin $B_2$ Receptor Imaging Agent

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Bradykinin is a potent vasodilator and inflammatory nonapeptide that produces its effects through specific Bradykinin receptors.[1] It is released from inactive precursor molecules as a consequence of tissue trauma and injury, ischemia, and low pH and participates in a variety of physiological responses:[2] Bradykinin causes contraction of smooth muscle, vasodilatation, microvascular leakage, and pain through stimulation of sensory nerve endings. It also contributes to the inflammatory response by producing pain, swelling, redness, and heat. On the basis of these potent biological actions, Bradykinin may play a role in the pathogenesis of a variety of different, seemingly unrelated diseases. Due to their therapeutic potential, great effort was devoted to the development of Bradykinin antagonists.[3] The most potent and selective antagonist known so far is HOE 140 (proposed international nonproprietary name: Icatibant),[4] a decapeptide (D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg) containing several unusual amino acids.

Its high affinity and selectivity make HOE 140 an appropriate candidate for the development of a Bradykinin receptor imaging agent. A Bradykinin receptor imaging agent could be very useful for the diagnosis of diseases where Bradykinin and Bradykinin receptors are involved (for example, rheumatoid arthritis, where the number of Bradykinin receptors in the affected tissue was found to be increased). Furthermore it could also serve as a very useful tool for the investigation of the participation of Bradykinin receptors in physiology and pathophysiology. At present there is little knowledge on the expression and changes of Bradykinin receptors in physiology and diseases.

In order to develop such an agent, HOE 140 had to be labeled so that it could be visualized in a receptor-bound stage using a well-established imaging technique.

Scintigraphy is a highly sensitive $\gamma$-radiation-based technique, which focuses on the visualization of physiological changes and is used in most hospitals.[5] The favorite $\gamma$-emitter for clinical applications is technetium-99m, which emits only $\gamma$-radiation, is available from a generator and has a low radiation energy and a short half-life of only 6 h.

As the direct $^{99m}$Tc labeling of HOE 140 is impossible, an appropriate chelator had to be connected to HOE 140 as a vehicle for $^{99m}$Tc. The polyazamacrocycle cyclam 1,4,8,11-tetraazacyclotetradecane is known to form thermodynamically and kinetically stable complexes with $^{99m}$Tc[6] which is required for medical applications. It has been successfully applied to the labeling of antibodies.[7,8]

From structure–activity data obtained during the development of Bradykinin antagonists, it is known that derivatization at the N-terminus of these compounds even with bulky substituents does not significantly reduce their activity. Therefore, the N-terminus of HOE 140 was used as the attachment site for the spacer-equipped cyclam.

For our study, cyclam **1** (CPTA)[10] was selected, which carries a rigid benzyl spacer and has been successfully used for antibody labeling.[11] The amino groups of **1** were protected by treatment with Boc-anhydride to generate **2**, which was used for the synthesis of the HOE 140 conjugate (Scheme 1). HOE 140 was synthesized applying solid phase technology. A standard Wang resin was chosen as solid support, and the HOE 140 derivative **3** was synthesized as reported.[12] The cyclam **2** was coupled manually onto **3** utilizing the well-established TOTU reagent.[13] A mixture of *m*-cresol and bromotrimethylsilane in trifluoroacetic acid was used to cleave the peptide off the resin and remove the acid labile protecting groups in a single step. The crude product was purified by preparative HPLC.[14]

The antagonistic activity of CPTA–HOE 140, **4**, was assessed in the guinea pig ileal membrane receptor

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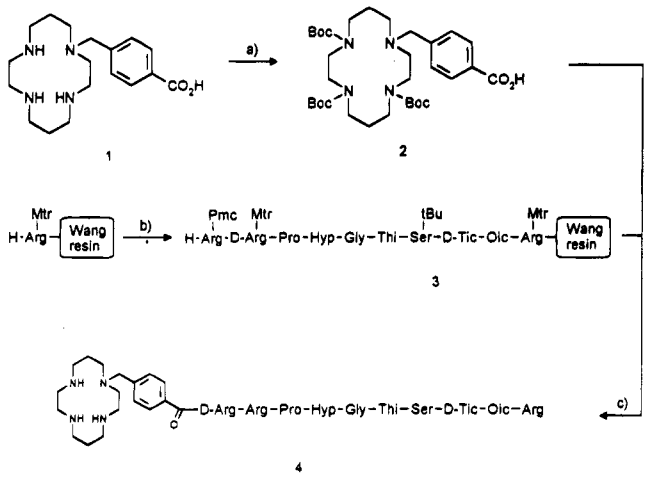
**Scheme 1**[a]




[a] (a) $(BOC)_2O$ (6 equiv), $NEt_3$ (5 equiv), DMAP (1 equiv), $CH_2Cl_2$, 25 °C, 74%; (b) see ref 12; (c) (1) **3** (815 mg of HOE 140 containing resin), **2** (45 mM); TOTU (45 mM), $NEt_3$ (45 mM), 25 °C; (2) 20% piperidine in DMF; (3) *m*-cresol:$CH_2Cl_2$:trifluoroacetic acid:bromotrimethylsilane (0.8:1:10:1), 25 °C.

**Table 1.** Comparison of the in Vitro Data of HOE 140 and CPTA–HOE 140, **4**

| | $IC_{50}$ (nM) | |
|---|---|---|
| | HOE 140 | CPTA–HOE140, **4** |
| guinea pig ileal membrane receptor assay | 1.07 | 8.03 |
| guinea pig pulmonary artery assay | 5.4 | 14 |

assay and the guinea pig pulmonary artery assay[15] and compared to the potency of HOE 140.

The data in Table 1 reveal that the conjugation of the cyclam **1** to HOE 140 hardly changes the antagonistic activity of this compound as the activity is still within the same order of magnitude. This proves that **4** a highly potent Bradykinin antagonist and tightly binds to the Bradykinin receptor, which is required to become a Bradykinin receptor imaging agent.

With these encouraging results in hand, the labeling experiments were conducted. At physiological pH the complexation of **4** with $^{99m}Tc$ was quite slow and yielded only 81% after 10 min. With increasing pH, higher yields could be obtained. At pH 10, greater than 95% complexation was achieved after 10 min using tin(II) citrate and sodium [$^{99m}Tc$]pertechnetate(VII).[16] After the complexation was completed, the pH was readjusted to physiological conditions (for the organ distribution study) without altering the complexation yield. The results accomplished with **4** meet the requirements of fast complexation kinetics that have to be fulfilled by an imaging agent in order to guarantee a convenient application in a hospital.

The next step on the way to a Bradykinin receptor imaging agent is the investigation of the organ distribution of **4**. This is an important step, because an undesired enrichment of **4** in certain parts of the body, e.g., in specific organs, blood etc., would result in an increased background activity and could make the detection of a receptor enrichment in a specific tissue impossible. In such a case **4** would be useless as an imaging agent. The investigation of the organ distribution was conducted in healthy rats by intravenous injection of $^{99m}Tc$-labeled **4** into the femoral vein.[17] The organ distribution was examined after 5 min, 15 min, 30 min, 4 h, and 24 h by measuring the organs radioactivity. The resultant uptake values represent the percentage of injected dose (%ID) corrected for the physical decay.

**Table 2.** Organ Distribution of $^{99m}Tc$-Labeled CPTA–HOE 140, **4**, in Healthy Rats after Intravenous Injection into the Femoral Vein

| | organ distribution (%) | | | | |
|---|---|---|---|---|---|
| organs | 5 min | 15 min | 30 min | 4 h | 24 h |
| liver | 5.25 | 4.48 | 4.31 | 5.03 | 2.72 |
| spleen | 0.27 | 0.19 | 0.19 | 0.12 | 0.08 |
| lung | 1.20 | 0.82 | 0.55 | 0.10 | 0.04 |
| kidney | 8.46 | 7.00 | 8.86 | 4.33 | 3.07 |
| bladder and urine | 3.83 | 20.3 | 33.0 | 74.9 | 79.9 |
| small intestine | 2.92 | 3.74 | 1.67 | 0.71 | 0.19 |
| content of small intestine | 1.83 | 2.79 | 4.84 | 1.85 | 0.06 |
| colon | 1.00 | 0.65 | 0.48 | 0.44 | 0.09 |
| content of colon | 0.22 | 0.20 | 0.17 | 6.96 | 0.56 |
| feces | | | | | 8.40 |
| blood | 13.2 | 8.62 | 5.50 | 0.50 | 0.50 |
| thyroid gland | 0.12 | 0.11 | 0.11 | 0.02 | 0.01 |
| stomach | 1.55 | 1.13 | 0.91 | 0.46 | 0.06 |
| total | 101.7 | 102.5 | 102.6 | 100.6 | 97.7 |

The organ distribution data listed in Table 2 show no enrichment in a specific organ except liver and kidney, which are important for the excretion of the **4**. The labeled **4** is rapidly cleared from the blood. The main path of excretion runs through the kidney and bladder into the urine. The excretion via liver clearly is of less significance. Without any unspecific organ enrichment, CPTA–HOE 140 has the potential to become a scintigraphic imaging agent for Bradykinin receptors.

In conclusion, CPTA–HOE 140, **4**, carrying a spacer-equipped cyclam at the N-terminus of HOE 140, showed binding to the Bradykinin receptor in the same order of magnitude as HOE 140 itself. The $^{99m}Tc$ labeling proceeded with more than 95% yield within 10 min, thus demonstrating sufficiently fast complexation. Labeled CPTA–HOE 140 was not unspecifically enriched in any organ of the rats. High affinity and lack of background activity enables the visualization of Bradykinin receptors in tissues and it can be expected that disease-related changes in the expression of Bradykinin receptors can be detected. The obtained results show **4** fulfills major requirements to become a clinically applicable Bradykinin receptor imaging agent.

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(14) The chromatography was carried out on a Eurochrom C18-RP column (23 cm × 1.6 cm o.d.) using a step gradient: acetonitrile (10 → 20 → 25%), water (90 → 80 → 75%) containing 1% trifluoroacetic acid.

(15) For a description of the assays, see ref 4b.

(16) The labeling experiment was conducted as follows: CPTA–HOE 140, **4** (1 mg), was dissolved in water, and the pH was adjusted to 10 using 0.1 N sodium hydroxide solution. Tin(II) citrate (21.7 nmol) and sodium [$^{99m}$Tc]pertechnetate (159.7 Mbq, eluated from a Tecegen S generator) were added in succession. After 10 min a complexation yield of greater than 95% was detected by TLC and HPLC.

(17) The solution used contained 0.96 mg of CPTA–HOE 140, **4**, and 98.8 Mbq [$^{99m}$Tc]technetium per gram.

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