Solid-Supported Hydrazine Substrate For Labeling Estradiol Ligands with Rhenium**

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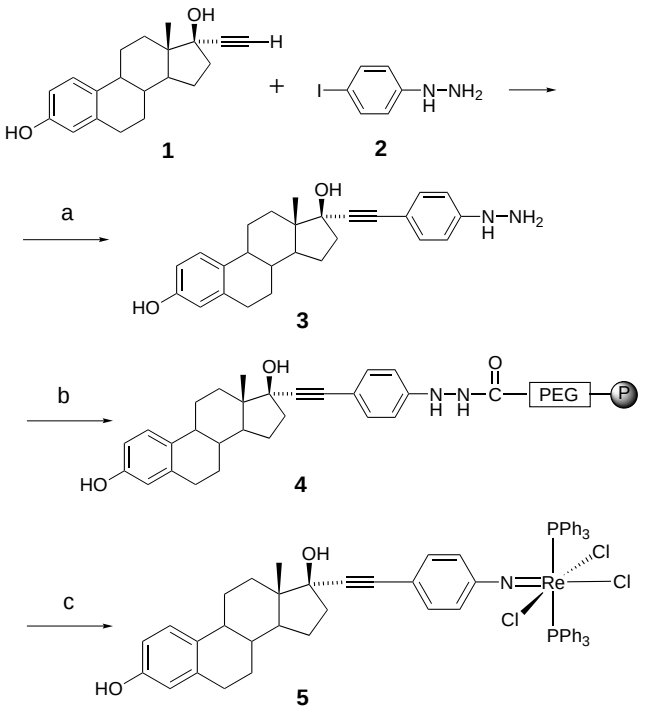
Receptor-targeted radiopharmaceuticals offer great promise for the diagnostic imaging and therapy of tumors and other disease sites. Technetium-99m is readily available in nuclear medicine clinics throughout the world for diagnostic imaging applications, and the β-emitting radioisotopes of its congener, rhenium-186/188, are suitable for irradiating small to medium-sized tumors.[1] Radiolabeled bioligands such as steroids, peptides, and antibodies are capable of binding to receptors expressed by cancer cells, providing the selectivity needed for diagnostic and therapeutic applications.[2–4] The estrogen and progesterone steroid hormone receptors found in approximately two-thirds of breast tumors are suitable targets for steroid-based radiopharmaceuticals.[5] Radiopharmaceuticals with high specific activity are required, and the removal of all excess *unlabeled* ligand is essential to avoid competitive saturation of the binding sites of the ligand receptor. Herein we demonstrate a new strategy for labeling with rhenium using an organoimido-forming reaction of a polymer-supported hydrazine, which simultaneously establishes the steroid–radioisotope linkage and releases the labeled steroid product into solution, thereby facilitating complete removal of all unlabeled ligand by simple filtration. The approach outlined here is uniquely amenable to the specific problem of developing "instant kits" for labeling low-capacity receptor ligands, and the technology is suitable for adaptation to a wide variety of different structural classes of ligands.

The 17α position of estradiol was selected as the site for appending the linking organoimido group, following the examples of organometallic steroid derivatives which exhibit high receptor binding affinities.[6–8] We have previously synthesized highly functionalized organoimido complexes from substituted 1-acetyl 2-phenyldiazane (hydrazine derivatives) using carrier free trichlorooxobis(triphenylphosphane) rhenium(v), $[ReOCl_3(PPh_3)_2]$.[9, 10] Our approach required a convenient method for attaching pendant phenylhydrazine moieties to ethynylestradiol (**1**). The desired hydrazine **3** was obtained directly using a palladium-catalyzed coupling[11] of ethynylestradiol (**1**) with 4-iodophenyl hydrazine (**2**) in diethylamine at ambient temperature in 87% yield (Scheme 1). The free hydrazine **3** was attached to Tentagel carboxy resin (loading capacity = 0.26 mmol g^{-1}) using (1-benzotriazolyl)oxy tris(pyrrolidino) phosphonium hexafluorophosphate (PyBOP) and diisopropylethylamine in dichloromethane to give the corresponding solid-supported acetyl hydrazine derivative **4**. This reaction was monitored using FT-IR spectroscopy to follow the change in the carbonyl stretch from the free carboxylic acid (1737 cm^{-1}) to the carbohydrazide (1660 cm^{-1}), and the appearance of the characteristic aryl C–H bend at 1611 cm^{-1} from the estradiol.



Scheme 1. a) 5 mol% $Pd(OAc)_2$, 10 mol% CuI, PPh_3, $NHEt_2$, 25 °C, 3 h, 87%; b) Tentagel carboxy resin, PyBOP, $NEt{i}Pr_2$, CH_2Cl_2, 25 °C, 20 h, 100% (based on loading capacity = 0.26 mmol g^{-1}); c) $[ReOCl_3(PPh_3)_2]$, PPh_3, CH_2Cl_2, 40 °C, 3 h, 82%.

The organoimido-forming labeling reaction of solid-supported acetyl hydrazine **4** with $[ReOCl_3(PPh_3)_2]$ (2.86 mM in CH_2Cl_2) and triphenylphosphane occurred readily in solution to produce the air- and moisture-stable complex **5** as an olive-colored solid in 82% yield (Scheme 1). The product exhibited a single ^{31}P NMR signal due to the coordinated triphenylphosphane ligands at $\delta = -20.4$ and displayed a characteristic UV/Vis absorption spectrum with maximum at $\lambda = 370$ nm ($\varepsilon = 16\,200$, CH_2Cl_2).

The previous example demonstrates the efficient reactivity of the polymer-supported hydrazines. The specific requirements for radiolabeling with rhenium and technetium involve highly dilute conditions, therefore a series of labeling reactions were carried out using the polymer-supported hydrazine **4** and dilute solutions of $[ReOCl_3(PPh_3)_2]$ from 10^{-5} to 10^{-6} M (Table 1). The formation of the organoimido complex **5** was followed spectroscopically by observing the absorption maximum at $\lambda = 370$ nm. The half lives for the labeling reactions ($t_{1/2} = 2$ h) were unchanged over a 100- to 1000-fold excess of the support **4** relative to rhenium concentration. The yields of the reaction were similar when a solution of $[ReOCl_3(PPh_3)_2]$ prepared in situ from potassium perrhenate was used (entry 3, Table 1).[10] The organoimido

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Table 1. Rhenium labeling using the solid-supported hydrazine **4**.

Entry	Concentration [10^{-5} M]	Conditions[a]	Time [h]	Yield [%]
1	2	A	2	53
			5	72
2	0.2	B	2	50
			5	70
3	9.3	C	2	54
			5	70
4	2	D	2	55
			5	65

[a] Reactions carried out in CH_2Cl_2 (10 mL) at 40 °C. Reactant ratios used: A: **4**/PPh_3/[$ReOCl_3(PPh_3)_2$] = 100/100/1; B: **4**/PPh_3/[$ReOCl_3(PPh_3)_2$] = 1000/1000/1; C: **4**/PPh_3/$KReO_4$ = 100/100/1; D: **4**/$HPPh_3Cl$/Bu_4NReO_4 = 100/100/1.

complex **5** was also prepared using a one-pot procedure starting with tetrabutylammonium perrhenate and triphenylphosphane hydrochloride in dichloromethane (entry 4, Table 1).

These examples illustrate a new strategy for labeling estradiol ligands with rhenium using a solid-supported hydrazine substrate, and this chemistry should also be successful for preparing technetium analogs.[12] The ability to use perrhenate and pertechnetate salts for labeling is particularly advantageous, since these species are obtained directly from the radionuclide generators. The efficiency and convenience of this approach can be extrapolated to a new generation of rhenium and technetium complexes for diagnostic and therapeutic applications in nuclear medicine. Further studies that are currently in progress involve evaluation of the receptor binding affinity and in vivo stability of these estradiol derivatives, and the extension of this technology to other low-capacity receptor systems.

Experimental Section

4: To a suspension of Tentagel Carboxy resin (1.0 g, 0.26 mmol) in dichloromethane (50 mL) was added PyBOP (405 mg, 0.78 mmol) followed by hydrazine **3** and diisopropylethylamine (0.3 mL). The resulting suspension was stirred at 25 °C for 20 h. The reaction product was filtered to give the yellow polymer support **4**. FT-IR (KBr): $\tilde{\nu}$ = 3443, 2869, 1652, 1611, 1104 cm^{-1}.

5: A suspension of **4** (110 mg, 0.0286 mmol), triphenylphosphane (7.5 mg, 0.0286 mmol), and [$ReOCl_3(PPh_3)_2$] (23.8 mg, 0.0286 mmol) in dichloromethane (10 mL) was heated at 40 °C for 3 h. The reaction mixture was filtered, and washed thoroughly with dichloromethane. The combined organic layers were concentrated, and the product was precipitated from dichloromethane/hexanes and recrystallized to provide the complex **5** (28 mg, 82 %) as olive-green crystals containing CH_2Cl_2. Elemental analysis for $C_{62}H_{57}Cl_3NO_2P_2Re \cdot 0.5\,CH_2Cl_2$: calcd: C 60.23, H 4.61, N 1.12; found: C 60.29, H 4.66, N 1.50. Spectral data is provided in the Supporting Information.

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Regioselective Lactonization of Tetrasialic Acid**

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Polysialic acids (PSAs) are polymers of *N*-acetylneuraminic acid. Depending on their glycosidic linkages, these sugar polymers exist in nature as α-2,8-, α-2,9-, and α-2,8/2,9-linked polysaccharides.[1] PSAs have been reported to demonstrate many important biological functions.[2] For example, α-2,8-PSA is mainly linked to the neural cell adhesion molecule (N-CAM). This homopolymer of sialic acid has been implicated in reducing N-CAM adhesion; removal of the PSA increases the adhesive capability of N-CAM.[3] In addition, α-2,8- and α-2,9-PSAs are the capsular polysaccharides of, respectively, serogroups B and C of *Neisseria meningitidis*, a leading worldwide cause of meningitis and rapidly fatal sepsis in otherwise healthy individuals.[4]

Structural diversities of PSAs are even more complicated with the possibility of PSA lactonizations. For α-2,8-PSA, the C-2 carboxylic acid of one residue can condense with the C-9 hydroxyl group of an adjacent residue to generate a δ-lactone under acidic conditions. Such δ-lactonizations have also been

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