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**Research Article**

# Labelling, purification, and receptor affinity of radioactive iododiethylstilbestrol ($^{*}$I-DES) with high specific activity and first structure analysis with $^{nat}$I-DES

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## Summary

Diethylstilbestrol **1** is a well known, non-steroidal estrogen with high affinity for the estrogen receptor (ER). When labelled with an Auger-electron emitter such as $^{123}$I, $^{125}$I or $^{77}$Br **1** would be an useful radiopharmaceutical for therapy of ER-positive mamma tumours. In the present work **1** was labelled easily and quickly with iodine by a modified chloramin T method (methanolic instead of aqueous solution), which leads to the desired product **2** in good yields (50–74%), with high radiochemical purity (> 98%) and – in opposite to former attempts – very high specific activities ($^{123}$I-DES: 8800 TBq/mmol, $^{125}$I-DES: 80 TBq/mmol, $^{131}$I-DES: 870 TBq/mmol). After separation by RP-HPLC all products were characterized by NMR-techniques ($^{1}$H-, $^{13}$C-NMR, H,H-COSY, and HMBC) for the very first time and the main product 3′-Iodo-DES **2** as well as some by-products like the *p*-toluol-sulfonic acid amide **3**, **1**, and 1-chloro-1-(4′-hydroxy-phenyl)-propene-1 **4** could be identified. The complex dissociation constant for $^{125}$I-DES with ER was determined by Scatchard plot and is comparable with that of 16α-[$^{125}$I]-iodoestradiol ($^{125}$I-DES: $K_D = (2.67 \pm 1.02) \times 10^{-9}$ mol/l, 16α-[$^{125}$I]-iodoestradiol: $K_D = (3.92 \pm 2.27) \times 10^{-9}$ mol/l). Copyright © 2004 John Wiley & Sons, Ltd.

**Key Words:** diethylstilbestrol; radioiodine; estrogen; high specific activity; NMR; mamma carcinoma

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## Introduction

Radioactive estrogens, labelled with Auger-electron emitters are excellent candidates to achieve high specific cytotoxicity in combination with low degree of side effects.[1–4] Numerous attempts have been made to find suitable compounds for this purpose. Some famous candidates were 16α-[$^{*}$I]-iodoestradiol,[5] hexestrol,[6] 4-iodotamoxifen,[7] and *E*/*Z*-11β-methoxy-17α-iodo-vinyl-estradiol,[8] but they never were commonly accepted for clinical routine.

Diethylstilbestrol (3,4-Di-(4′-hydroxyphenyl)-hexene-3) **1** is a well-known, synthetic, non-steroidal estrogen-analogue[9] with very high affinity for ER.[10–15] In nearly all former attempts, except one[16] the water-soluble derivative tetra-sodium-diethylstilbestrol-diphosphate **5**[17–22] was labelled instead of **1**. The specific activities reached with these synthesis methods were between 0.3 and 1.2 GBq/mmol[21] leading to non-advantageous biodistributions.[19–22] Besides low specific activities, other difficulties occurred, like small and not reproducible yields or time-consuming purification processes.[17,18] In all previous labelling attempts no structure analysis of the desired product or by-products were carried out.

Therefore, a simple labelling method for **1** was developed in the present work and the labelling products were characterized regarding the structure and binding affinity.

## Experimental

### *General*

All chemicals used were purchased from Fluka or Merck. HPLC eluents were water G (CHROMASOLV®) and methanol G (CHROMASOLV®) from RiedeldeHaen. The most important properties of radiochemicals used are shown in Table 1. HPLC equipment from Pharmacia (analytical HPLC) and Knauer (preparative) was used.

Reaction products were analysed by NMR at the Institute of Organic Chemistry, University of Cologne, with a Bruker DPX 300. Radioactivity measurements were carried out with an isotope calibrator Veenstra VDC-303 (Netherlands) or in a well counter type LB 2044 from Berthold (Germany).

**Table 1. Properties of the radiochemicals used in this work**

| Isotope | Chemical form | Radiochemical concentration | Specific activity | Manufacturer |
|---|---|---|---|---|
| $^{123}$I | NaI | 2.96 GBq/ml | n.c.a. | Mallinckrodt |
| $^{125}$I | NaI | 3.70 GBq/ml | n.c.a. | CIS bio international |
| $^{131}$I | NaI | 12.3 GBq/ml | n.c.a. | CIS bio international |
| $^{125}$I | 16α-iodoestradiol | 5.83 MBq/ml | 81.4 TBq/mmol | DuPont (NEN) |

 

For the Lowry method of protein determination an UV-vis-Spectrometer Pharmacia LKB Novaspec II was used.

*Radiolabelling of DES*

For radiolabelling a stock solution containing 1 mg **1** per ml methanol was used. Stored at 4°C the solution kept stable for a couple of month. Five microlitre of the stock solution of **1**, diluted with 95 µl methanol (= 50 µg/ml), about 40 MBq $Na^*I$ solution (10–20 µl) and 50 µl chloramin T solution (4 mg/ml methanol) were added. After 10 min incubation at room temperature the reaction was stopped by adding 50 µl sodium bisulfite solution (15 mg/ml water), followed by HPLC for purification and quality control.

*Synthesis of $^{nat}$I-DES for NMR experiments*

1 ml methanolic DES solution (50 mg/ml), 50 µl KI solution (300 mg/ml water), and 300 µl chloramin T-solution (100 mg/ml methanol) were mixed. After 10 min incubation at room temperature the reaction was stopped by adding 100 µl sodium bisulfite solution (450 mg/ml water) and the products were separated by preparative HPLC.

*Purification and quality control*

Purification and quality control were carried out by means of reversed phase HPLC under the following conditions: Hypersil ODS column, 250 × 4 mm (analytic) or 250 × 20 mm (preparative), 10 µm, 20% methanol G in water G to 70% within 5 min, flow 1 ml/min (analytic) 20 ml/min (preparative), UV detection at 254 nm, injection of 20 µl (analytic) or 200 µl (preparative), respectively.

*NMR-experiments*

All fractions received by HPLC were evaporated to dryness at 40°C under vacuum and 20–50 mg of each re-dissolved in 1 ml $CD_3OD$. Afterwards $^1$H-NMR, $^{13}$C-NMR, H,H-COSY (two-dimensional H,H-Correlation Spectroscopy), and HMBC (Heteronuclear Multiple Bond Correlation) were carried out.

*Determination of the estrogen-receptor-complex dissociation constant*

For preparation of tumour cytosol the method of McGuire *et al.* was used.[23] After determination of protein content according to Lowry's method[24] the sample was diluted with *Tris*-HCl-EDTA-DTT buffer (10 mM *Tris*-HCl; 1.5 mM EDTA; 0.5 mM dithiothreitol; pH 7.4) to a final concentration of 1–2 mg/ml. The dissociation constants for the $^{125}$I-DES- and 16α-[$^{125}$I]-iodo-estradiol-ER-complex were determined by DCC (dextran coated charcoal)

 

saturation analysis.[23] All samples were tested in triplicate. Dissociation constants were determined by Scatchard plots.[25]

## Results

### *Radiolabelling of DES and stability of *I-DES*

Radiochemical yields of $^{123}$I-DES, $^{125}$I-DES, and $^{131}$I-DES and the percentage of free iodide are shown in Table 2.

The best yields were obtained with $^{131}$I, the lowest with $^{123}$I. The labelled product is stable over 48 h; only small amounts ($\leqslant 5\%$) of free iodine could be detected after that time. Specific activities were for $^{123}$I-DES: 8800 TBq/mmol, for $^{125}$I-DES: 80 TBq/mmol, and for $^{131}$I-DES: 870 TBq/mmol.

### *Purification and quality control*

A typical HPLC-plot of the reaction mixture after labelling of **1** with $^{125}$I is presented in Figure 1. With the other isotopes the chromatograms look very similar.

**Table 2. Radiochemical yields (referred to iodine) measured by HPLC in % ($\pm$ standard deviation). Differences to 100% are caused by unidentified iodine species**

| | $^{123}$I ($n=13$) (%) | $^{125}$I ($n=42$) (%) | $^{131}$I ($n=12$) (%) |
|---|---|---|---|
| *I-DES | 50.7 $\pm$ 14.0 | 64.0 $\pm$ 13.1 | 74.0 $\pm$ 9.0 |
| *I-iodide | 20.6 $\pm$ 13.9 | 8.9 $\pm$ 6.7 | 7.9 $\pm$ 4.1 |

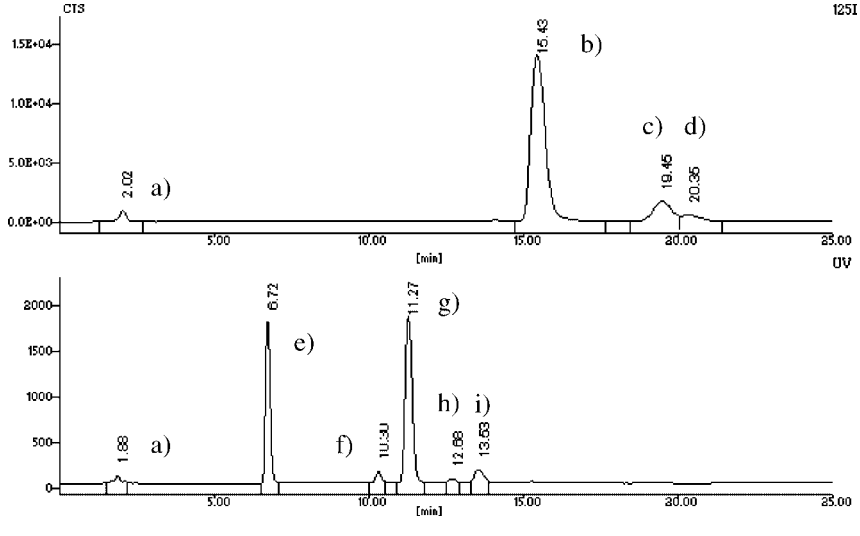


**Figure 1. Typical HPLC-chromatogram of a reaction mixture of $^{125}$I-DES (upper: radioactivity detection, lower: UV-detection). (a) iodide, (b) $^{125}$I-DES, (c,d) not identified, (e) *p*-toluene sulfonic acid amide, (f) not identified, (g) DES, (h) not identified, (i) 1-chloro-1-(4-hydroxy-phenyl)-propene-1**

 

Figure 2 shows the quality control HPLC-chromatogram of purified $^{125}$I-DES fraction. Radiochemical purity is $>98\%$.

*Synthesis and purification of $^{nat}$I-DES for NMR-experiments*

After separation by preparative HPLC, five fractions were collected (Figure 3):

The yield of I-DES (= substance D) was 20 mg (0.05 mmol), that means 28.2% related to DES. The substance eluted at 17–18 min could not be collected and identified, because of its very low amount in relation to that eluted just before and afterwards. In the HPLC-profile of a reaction mixture for synthesis of stable I-DES peaks were found at the same time points as in the case of radioactive synthesis (Figure 3).

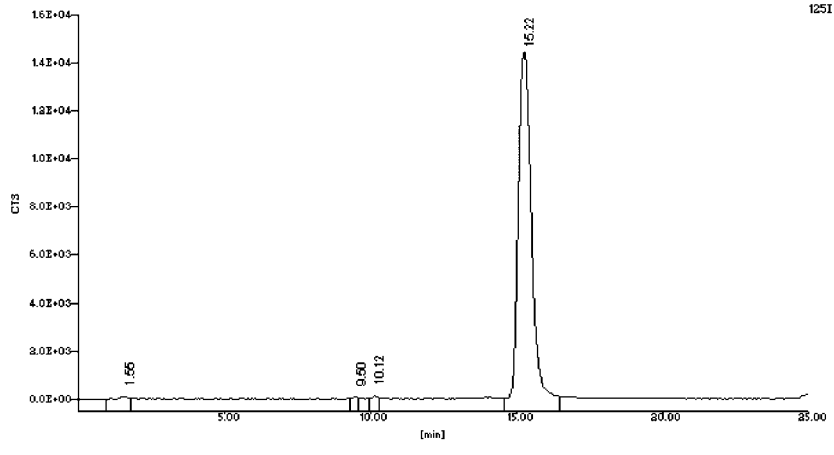


**Figure 2. HPLC-chromatogram of purified $^{125}$I-DES (radioactivity detection)**

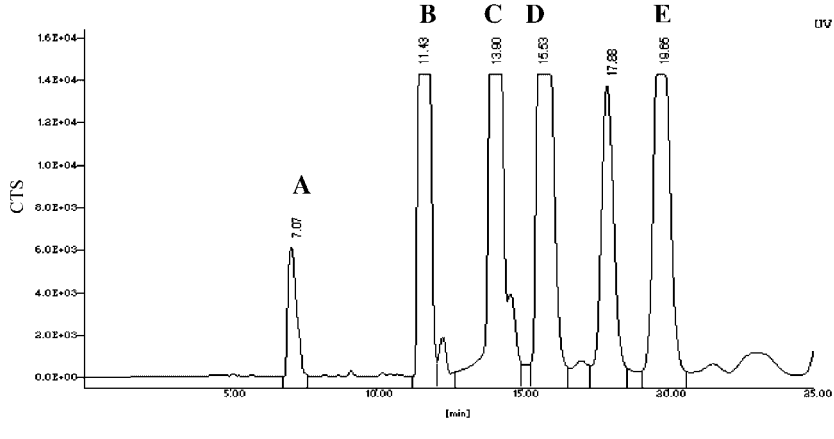


**Figure 3. HPLC-chromatogram of a reaction mixture after synthesis of $^{nat}$I-DES**

 

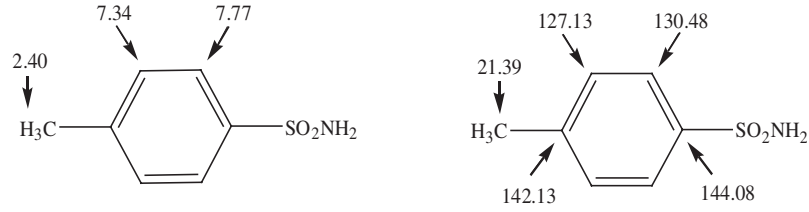


**Figure 4. Chemical shifts (in ppm) of 3 (substance A), left: $^{1}$H-NMR, right: $^{13}$C-NMR**

*NMR-experiments*

Experimental determined chemical shifts and related chemical structures of the substances A, B, C, and D are shown in Figures 4–7. For exact analysis H, H-COSY and HMBC were necessary in some cases.

A small signal at 7.23 ppm was found with substance D, which belongs to an isomer of **2**: the 2′I-DES **6** (iodine meta to OH). Integration of the typical peaks for **2** and **6** showed a ratio of 8.5 : 1 (ortho/meta).

Identification of substance E was not possible, because the fraction seemed to contain several compounds. The mixture itself was not stable, indicated by change of colour from white to brown within a short time even at low temperature, therefore further purification made no sense.

*Determination of the estrogen-receptor-complex dissociation constant*

The value of the $^{125}$I-DES-ER-complex dissociation constant was determined by Scatchard analysis: $K_D = (2.67 \pm 1.02) \times 10^{-9}$ mol/l. For 16α-[$^{125}$I]-iodoestradiol the complex dissociation constant with ER was $K_D = (3.92 \pm 2.27) \times 10^{-9}$ mol/l.

## Discussion

*Radiolabelling of DES*

With the labelling method presented in this work it is possible to radioiodinate **1** quickly and in a simple manner. The radiochemical yields (see Table 2) obtained were higher than for **5** as described in literature.[17,18,21] Also the specific activities (80–8800 TBq/mmol) and radiochemical purity were much higher than obtained with other methods (0.3–1.2 GBq/mmol).[17–22] Differences between the three iodine isotopes regarding the radiochemical yields were caused by small differences in pH during labelling procedure. The sensitivity of radiochemical yield to pH is well known for radioiodination reactions with chloramin T.[26]

Consequently, from the practical point of view **1** should be preferred instead of **5** for labelling with radioiodine. Radioiodinated **5** was favoured earlier because of its better solubility in aqueous media but this advantage can be neglected in

 

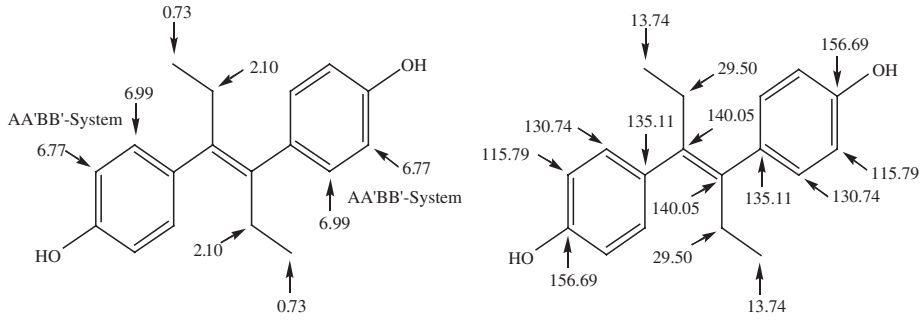


**Figure 5. Chemical shifts (in ppm) of 1 (substance B), left: $^{1}$H-NMR right: $^{13}$C-NMR**

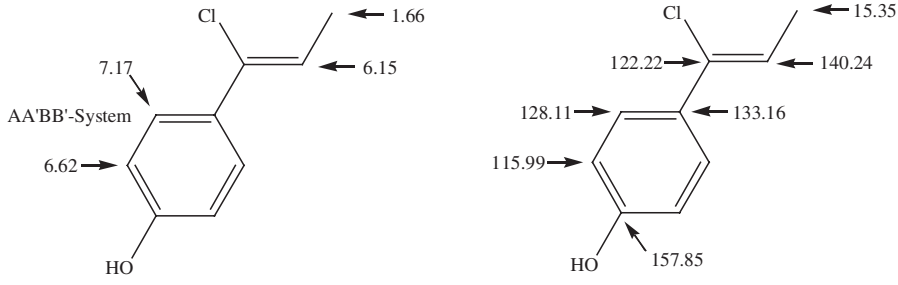


**Figure 6. Chemical shifts (in ppm) of 4 (substance C), left $^{1}$H-NMR, right: $^{13}$C-NMR**

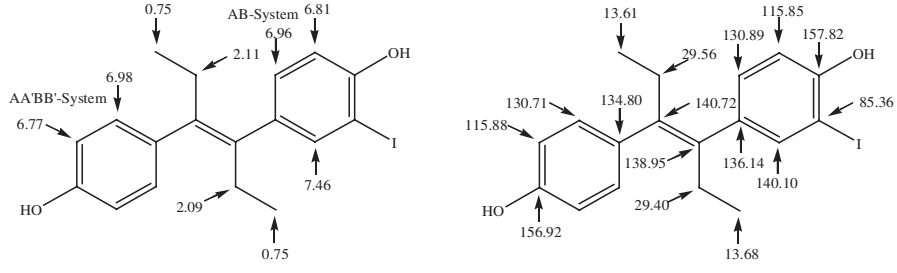


**Figure 7. Chemical shifts (in ppm) of 2 (substance D), left: $^{1}$H-NMR, right: $^{13}$C-NMR**

the case of very low mass concentrations used for the synthesis of radiopharmaceuticals. **5** could only be radioiodinated with chloramin T at pH of 1–2.[17,18] Under such acidic conditions the intermediately formed oxidizing reagent HOCl is not very stable, which leads to the low yields observed with this method.

*Purification and quality control*

The HPLC-system used was able to separate starting material, desired product and by-products very well (Figure 1) and can be used as well for

 

purification and quality control of radioiodinated **1**. Specific activities after labelling with the three radioiodines were in the range of the theoretical obtainable values. Such high specific activities have not been obtained with all methods described in literature before. The used MCF-7 cells have only a limited number of about 14 000 receptors per cell,[27] therefore the highest receivable specific activity is necessary to get as much activity into the cell as possible, especially for therapeutic uses.

*NMR-experiments*

All experimental NMR-data were in accordance with the shown structures (Figures 4–7). It was shown for the very first time that 90% of **1** is labelled at the ortho-position and 10% at the meta-position referred to the OH-group.

*Determination of complex dissociation constant $K_D$*

Comparison of the determined complex dissociation constants for $^{125}$I-DES and 16α-[$^{125}$I]-iodoestradiol points to a very similar affinity for ER. For the latter a constant of $3.6 \times 10^{-10}$ mol/l with MCF-7 cells has been published.[28] For tritium-labelled estradiol the value is lower: $2.8 \times 10^{-10}$ mol/l[28] and $2.2 \times 10^{-10}$ mol/l,[29] respectively. These small differences in comparison to the experimental data presented here are probably caused by differences in kind of tumour cells used and in the experimental design.

## Conclusion

For the first time a simple and quick labelling and purification method for $^{*}$I-DES is now available, which leads to the desired product with reproducible high yield and higher specific activity than with all the other methods described in literature. The structure of the main product and some by-products were well characterized also for the first time now. The radioactive receptor-affine compound binds reversibly with high affinity to its receptor. Auger-emitting **2** would impose radiocytotoxicity restricted to ER-positive tissues and would therefore inflict only minimal side effects. Thus, a receptor-specific radiopharmaceutical is available, which can deposite radionuclides with high cytotoxicity effectively and selectively into the cell nucleus of ER-positive tumor cells. First results about the cytotoxic effects and biodistribution of this promising compounds have been published already.[30–33] Detailed reports will be published recently.

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## References


1. Adelstein SJ. *Am J Roentgen* 1993; **160**: 707–713.
2. Burki HJ, Roots R, Feinendegen LE, Bond VP. *Int J Radiat Biol* 1973; **24**: 363–375.
3. Chan PC, Lisco E, Lisco H, Adelstein SJ. *Radiat Res* 1976; **67**: 332–343.
4. O'Donoghue JA, Wheldon TE. *Phys Med Biol* 1996; **41**: 1973–1992.
5. Hochberg RB, *Endocr Rev* 1998; **19**: 331–348.
6. Heiman DF, Senderoff SG, Katzenellenbogen JA, Neeley RJ. *J Med Chem* 1980; **23**: 994–1002.
7. Hardcastle IR, Horton NM, Osborne MR, Hewer A, Jarman M, Phillips DH. *Chem Res Toxicol* 1998; **11**: 369–374.
8. Rijks LJM, Boer GJ, Endert E, De Bruin K, Van den Bos JC, Janssen AGM, Van Royen EA. *Nucl Med Biol* 1997; **24**: 65–75.
9. Dodds EC, Goldberg L, Lawson W, Robinson R. *Nature* (*London*) 1938; **141**: 247–248.
10. Lieberman ME, Gorski J, Jordan VC. *J Biol Chem* 1983; **258**: 4741–4745.
11. Korach KS, Chae K, Gibson M, Curtis S. *Steroids* 1991; **56**: 263–270.
12. Hardcastle IR, Rowlands MG, Houghton J, Parr IB, Potter GA, Jarman M, Edwards KJ, Laughton CA, Trent JO, Neidle S. *J Med Chem* 1995; **38**: 241–248.
13. Goswami R, Harsy SG, Heiman DF, Katzenellenbogen JA. *J Med Chem* 1980; **23**: 1002–1008.
14. Hochberg RB, Rosner W. *Proc Natl Acad Sci USA* 1980; **77**: 328–332.
15. Rijks LJM, van den Bos JC, van Doremalen PAPM, Boer GJ, de Bruin K, Doornbos T, Vekemans JAJM, Posthumus MA, Janssen AGM, van Royen EA. *Nucl Med Biol* 1998; **25**: 411–421.
16. Maysinger D, Marcus CS, Wolf W, Tarle M, Casanova J. *J Chromatogr* 1977; **130**: 129–138.
17. Meller-Rehbein B, Schomäcker K, Bastian C, Schicha H, Bähre M, Richter E. *Nuklearmedizin* 1995; **34**: A140.
18. Meller-Rehbein B, Schomäcker K, Schicha H, Bähre M, Richter E. *Eur J Nucl Med* 1994; **21**: 878.
19. Mende T, Hennig K, Wollny G, Gens J. *Z Urol Nephrol* 1978; **71**: 529–534.
20. Mende T, Hennig K, Wollny G, Gens J, Zotter S. *Gynäk Rdsch* 1979; **19**: 30–36.
21. Tubis M, Endow JS, Blahd WH. *Nucl Med* 1967; **5**: 1–15.
22. Tubis M, Endow JS, Blahd WH. *Nucl Med* 1967; **5**: 184–202.
23. McGuire WL, De La Garza M, Chamness GC. *Cancer Res* 1977; **37**: 637–639.
24. Lowry OH, Rosebrough NJ, Lewis Farr A, Randall RJ. *J Biol Chem* 1951; **193**: 265–275.
25. Scatchard G. *Ann NY Acad Sci* 1949; **51**: 660–672.
26. Dewanjee MK. *Radioiodination*: *Theory, Practice*, *and Biomedical Application*. Kluwer Academic Publishers: Boston/Dordrecht/London, 1992; 133–137.
27. Schomäcker K, Fischer T, Böttcher B, Mraheel SA, Scharl A, Gabruk-Szostak B, Scheidhauer K, Göhring U-J, Meller-Rehbein B, Shukla SK, Schicha H. *Nuklearmedizin* 1998; **37**: 134–140.


 

28. Pieslor PC, Gibson RE, Eckelman WC, Oates KK, Cook B, Reba RC, *Clin Chem* 1982; **28**: 532–537.
29. Rijks LJM, Boer GJ, Endert E, De Bruin K, Van den Bos JC, Van Doremalen PAPM, Schoonen WGEJ, Janssen AGM, Van Royen EA. *Eur J Nucl Med* 1996; **23**: 295–307.
30. Schomäcker K, Fischer T, Meller B, Moka D, Schicha H. *Eur J Nucl Med* 1998; **25**: 843.
31. Schomäcker K, Fischer T, Löhr H, Moka D, Scheidhauer K, Schicha H. *J Nucl Med* 1999; **40**(5): 100P.
32. Fischer T, Schomäcker K, Gabruk-Szostak B, Moka D, Schicha H. *Nuklearmedizin* 2000; **39**: A44.
33. Fischer T, Schomäcker K, Moka D, Gabruk-Szostak B, Schicha H. *Nuklearmedizin* 2000; **39**: A88.

 