Synthesis, characterization and bioevaluation of technetium-99m labeled N-(2-Hydroxybenzyl)-2-amino-2-deoxy-D-glucose as a tumor imaging agent

Qaisar Nadeem[2], Irfan Ullah Khan[1*], Muhammad Javed[3], Zaid Mahmood[2], Ume-Kalsoom Dar[1], Muhammad Ali[4], Syed Waqar Hyder[1] and Sohail Murad[3]

[1]Radiopharmacy Division, Institute of Nuclear Medicine & Oncology (INMOL), New Campus Road, POB 10068, Lahore, Pakistan
[2]Institute of Chemistry, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan
[3]Gujranwala Institute of Nuclear Medicine (GINUM), Gujranwala, Pakistan
[4]School of Biological Sciences (SBS), University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

Abstract: N-(2-Hydroxybenzyl)-2-amino-2-deoxy-D-glucose (NHADG) was synthesized by conjugation of salicylaldehyde to glucosamine. The obtained compound was well characterized via different analytical techniques. Labeling of the synthesized compound with technetium-99m (^{99m}Tc) in pertechnetate form ($^{99m}TcO_4^-$) was carried out via chelation reaction in the presence of stannous chloride dihydrate. Maximum radiochemical yield of ^{99m}Tc-NHADG complex (99%) was obtained by using 1 mg NHADG, 200 µg $SnCl_2.2H_2O$, at pH 9.5 and reaction time of 15 min. The radiochemical purity of the ^{99m}Tc-NHADG complex was measured by Instant Thin Layer Chromatography (ITLC) and Paper Chromatography (PC), without any notable decomposition at room temperature over a period of 4h. The biological evaluation results show that the ^{99m}Tc labeled NHADG conjugate is able to specifically target mammary carcinoma in mice models, thus highlighting its potential as an effective ^{99m}Tc labeled glucose-derived agent for tumor imaging.

Keywords: Synthesis, Technetium-99m, glucosamine derivative, tumor, imaging

INTRODUCTION

Carbohydrates are essential hydrophilic nutrients whose transport is mediated by a number of specific carriers. Diseases are associated with glucose transport and metabolism defect such as myocardial ischemia diabetes and cancer (Schelbert *et al*., 1988; Garvey *et al*., 1993; Rigo *et al*., 1996; Weber *et al*., 1982). [^{18}F]fluoro-deoxyglucose [^{18}F]FDG is the preeminent radiotracer used to image glucose metabolism and is an important tool in cardiology and neurology. As well, [^{18}F]FDG is used in oncology as a tumor marker, due to elevated level of glucose metabolism in many tumor types (Krohn *et al.*, 2005; Segall, 2002; Beuthien *et al.*, 2000; Czernin, 2002). Unfortunately, the radioisotope [^{18}F] used in production of [^{18}F]FDG, is cyclotron-produced and has a short half life ($t_{1/2}$ = 110 min), thus significantly limiting availability of radiotracer. Hence, there is much interest in developing [^{18}F]FDG-like radiopharmaceuticals that are less expensive and easily accessible. By comparison, ^{99m}Tc is an optimal radioisotope; ^{99m}Tc is already used in over 90% of nuclear medicine scans and is generator-produced, making it inexpensive and widely available (Dilworth *et al*., 1998). The physical properties of ^{99m}Tc ($t_{1/2}$ = 6.01h, gamma radiation energy = 142.7 kev) are ideal for imaging with single photon emission computed tomography (SPECT). Recently, a lot of ^{99m}Tc-labeled glucose derivatives have been synthesized in order to develop one subrogate in SPECT for [^{18}F]FDG in PET (Storr *et al*., 2005a; Bayly *et al.*, 2004; Storr *et al.*, 2005b; Storr *et al*., 2005c; Schibli *et al*., 2005). Developed by Yang, ^{99m}Tc-labeled ethylenedicysteine - deoxyglucose (^{99m}Tc-ECDG) showed similarities with [^{18}F]FDG in tumor uptake (Yang *et al*., 2003). This suggests the feasibility for ^{99m}Tc-labeled deoxyglucose as a tumor metabolic imaging agent. However, [^{99m}Tc]-ECDG still has some drawbacks such as slow clearance from blood, which would cause high blood background and large molecular weight, thus limiting its penetration through blood-brain barrier (BBB). Thus, it would be desirable to develop a smaller ^{99m}Tc based deoxyglucose derivative with rapid blood clearance and still maintaining its high tumor uptake.

On introduction of $-COO^-$ or $-NH_2$ group (or both) into a sugar molecule, its complex forming ability is enhanced by several orders of magnitude, even in acidic or neutral solution. These complexes are usually very stable (Burger *et al*., 1990; Nagy *et al.*, 1992; Gyurcsik *et al.*, 2000). Glucosamine (2-amino-2-deoxy-D-glucose) is a highly attractive scaffold for a glycosyl ligand, because the amine acts both as a potential coordination site and as a useful target for further functionalization. Furthermore, there is much evidence in literature to suggest that N-functionalized glucosamines show activity with GULTs (glucose transporters) and hexokinase - the enzymes that are most closely associated with the metabolism of FDG even when the functional group is large (Schibli *et al.*, 2000). Several glycosylamines are known in literature, but as far as the glycosylamine derived imine molecules

*Corresponding author: e-mail: drirfankhan69@gmail.com

are concerned, the literature is scarce. The purpose of this study is to conjugate glucosamine with salicylaldehyde and to evaluate the feasibility of the ^{99m}Tc-labeled glucosamine derivative as a candidate for tumor diagnosis. In this study, we have synthesized the imine by the reaction of salicylaldehyde with glucosamine and then reduction with $NaBH_4$ to produce secondary amine. Afterwards, the complexation of secondary amine with ^{99m}Tc and its biodistribution were carried out in mice-bearing mammary tumor.

MATERIALS AND METHODS

Materials and Equipment

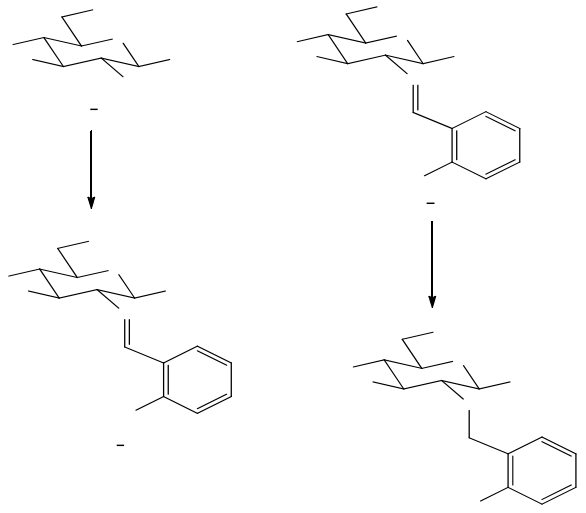
D-glucosamine hydrochloride was purchased from MP Biomedicals; salicylaldehyde was purchased from Fluka, USA. $Na^{99m}TcO_4$ was obtained by elution with saline from a ^{99}Mo/^{99m}Tc PakGen generator supplied by Pakistan Institute of Nuclear Science and Technology (PINSTECH). Other chemicals were purchased from commercial suppliers and used as received. ^{1}H NMR and ^{13}C NMR spectra were recorded on 300 and 75 MHz, respectively, at H.E.J. Research Institute of Chemistry, University of Karachi. The chemical shifts (δ values) are given in ppm. FT-IR spectra were recorded on Perkin-Elmer 882 spectrophotometer at the Institute of Chemistry, University of the Punjab, Lahore, Pakistan. Elemental analyses were performed by the use of Elemental analyzer MOD-1106 Carlo Erba at H.E.J. Research Institute of Chemistry, University of Karachi, Pakistan. Mass spectrum was recorded on LC-ESI-MS 6224 TOF, LC/MS Agilent Technologies at School of Biological Sciences (SBS), University of the Punjab, Lahore, Pakistan. All radioactivity measurements were carried out by using NaI(Tl) Scintillation Counter at Institute of Nuclear Medicine and Oncology (INMOL), Lahore, Pakistan.

Synthesis of N-(2-hydroxybenzyl)-2-amino-2-deoxy-D-glucose

To a solution of glucosamine hydrochloride **1** (2.156 g, 0.01 mole) and sodium bicarbonate **b** (1.008 g, 0.012 mole) in 20 ml of distilled water, 1.06 ml of salicylaldehyde **a** (0.01mole) was added dropwise and the mixture was stirred at room temperature. After 5 min time interval, yellow precipitates were formed. Mixture was further stirred for 4h. The residue was then filtered, washed with cold water and dried in a vacuum dessicator. The residue was redissolved in methanol and recrystallized to get compound **2** with high purity (yield 70.3%). The bands in IR spectrum were exhibited at 3375 cm^{-1} (OH of aromatic ring), and 1626 cm^{-1} (C=N of imine). ^{1}H NMR (300 MHz, CD_3OH): δ = 3.4-3.9 (m, 7H, sugar), 6.7-7.3 (m, 4H, arom), 8.4 (s, 1 H, CH=N). ^{13}C NMR (75 MHz, CD_3OH): δ = 62.95 - 97.07 (sugar carbons), 169.09 (1 CH), 117.66 -168.40 (aromatic carbons). Elemental analysis, calculated for $C_{13}H_{17}NO_6$: C 55.08; H 6.002; N 4.94. Found: C 54.608; H 6.150; N 4.273.

Synthesis of N-(2-hydroxybenzyl)-2-amino-2-deoxy-D-glucose (NHADG) 3

To a suspension of imine **2** (2.83 g, 0.01 mole) in 20 ml methanol, $NaBH_4$ **c** (0.7566 g, 0.02 mol) was slowly added in small portion within 30 min below 10^0C with constant magnetic stirring. After the completion of addition, the reaction mixture was stirred for 2h under the same conditions. The mixture was stirred for 30 min at room temperature. After the evaporation of solvent, distilled water was added in the mixture and extracted with dichloromethane (DCM) to get compound **3** with high yield of 85% and purity. The IR spectrum (ν cm^{-1}) exhibited bands at 3375 showing presence of (OH of aromatic ring) and at 1626.88, showing absence of (-C=N) azomethine bond. ^{1}H NMR (300 MHz, CD_3OH): δ = 2.89-3.80 (m, 7H, sugar), 6.83-7.067 (m, 4H, arom), 3.85 (s, 2 H, CH_2-NH) ppm. ^{13}C NMR (75 MHz, CD_3OH): δ=62.20- 91.2 (sugar carbons), 45.63(1 CH_2), 118.72 - 157.57 (aromatic carbons). Its MS showed the molecular ion peak at *m/z* 286.



Scheme 1: Synthesis of N-(2-Hydroxybenzyl)-2-amino-2-deoxy-D-glucose **3**. Reagents: (a) salicylaldehyde; (b) $NaHCO_3$; (c) $NaBH_4$

Radiolabeling

NHADG **3** was radiolabeled with $Na^{99m}TcO_4$ with high yield. As an experimental procedure, 1 mg of NHADG was dissolved in 1 ml distilled water followed by addition of 1 mg sodium potassium tartrate, 1 mg sodium citrate tribasic and 200 µg of freshly prepared acidic solution of stannous chloride dihydrate in 0.2 N HCl. The final pH was adjusted to 9.5 by sodium hydroxide solution. The kit was radiolabeled by adding (20 mCi/0.5 ml) freshly eluted $Na^{99m}TcO_4$ in saline and passing the radiolabeled product through a 0.22 µm membrane and introduced whole solution in sterile glass vial, closed with a rubber

stopper and an aluminum cap. The mixture was left to react at room temperature (25 ^{0}C) for 15 min.

Analysis of ^{99m}Tc-N-(2-hydroxybenzyl)-2-amino-2-deoxy-D-glucose

Radiochemical purity of ^{99m}Tc-N-(2-Hydroxybenzyl) -2-amino-2-deoxy-D-glucose was assessed by ascending paper chromatography. Free $^{99m}TcO^-_4$ in the labeled solution was determined by using Whatmann No. 3 MM chromatography paper, in acetone free $^{99m}TcO^-_4$ moved with the solvent front while reduced TcO_2 and bound ^{99m}Tc complex remained at the origin which was counted with well type Scintillation counter. Similarly, reduced hydrolyzed TcO_2 in labeled solution was measured by Instant Thin Layer Chromatography (ITLC) in saline. Reduced hydrolyzed TcO_2 remained at the origin while free $^{99m}TcO_4^-$ and bound ^{99m}Tc complex moved with the solvent front.

Stability of ^{99m}Tc complex at room temperature

Stability of the ligand complex with ^{99m}Tc was studied at time intervals of 0, 1, 2, 3, and 4h at room temperature. Change in stability of the radiolabeled complex was analyzed at each time interval by ITLC-SG and PC to determine any dissociation of the complex. The percentage of free pertechnetate (%) at a particular time presented percentage dissociation of the complex at that time. In case of significant loss of metal-complex stability, the radiopharmaceutical is usually not considered suitable for clinical applications.

Biodistribution in mice

Biodistribution of ^{99m}Tc-NHADG complex was performed in Swiss Webstar mice bearing mammary tumor (18-20 g), obtained from School of Biological Sciences, University of the Punjab, Lahore, Pakistan. A 100 µl solution of radiopharmaceutical was injected via a tail vein and the injected radioactivity was measured with a gamma well counter. The mice were sacrificed at 0.5h, 2h and 4h post-injection (p.i.). The tumor, other organs of interest and blood were collected, weighed and measured for radioactivity. The results were expressed as the percent uptake of injected dose per gram of tissue (%ID/g). Based on the results of %ID/g in tissue and tumor, tumor-to-blood ratio (T/B) and tumor-to-lung ratio (T/L) were also calculated. Animal studies were performed at the Institute of Nuclear Medicine and Oncology (INMOL), Lahore, Pakistan, according to the local rules and regulations of the Institute (INMOL 53/07).

Histopathological study

After dissection, the tumor samples from the mice were subjected to HE stains and CK-specific immunostaining. For this purpose, paraffin-embedded blocks were prepared from the tumor tissue and serial sections of 4 µm in thickness were prepared from the blocks. Immunohistochemical staining was performed by the streptavidin-biotin method with a murine monoclonal antibody, CAM 5.2, against low molecular weight CK. In brief, dewaxed and dehydrated sections were heated in a microwave oven for 10 min for retrieval of antigens in the specimens. Endogenous peroxidase was blocked by incubation of samples with 3% hydrogen peroxide in 100% methanol. The tissue sections were then incubated with the primary antibody CAM 5.2 at 25 µg per ml overnight at 4°C. The second antibodies, biotinylated antibodies against mouse immunoglobulin, were applied, and sections were then incubated with peroxidase-labeled streptavidin. Reaction products were visualized with diaminobenzidine as the chromogen, and sections were counted stained with methyl green for visualization of lymphocytes. Tris-buffered saline was used instead of the primary antibody for negative controls.

RESULTS

Synthesis and radiolabeling

The synthesis of NHADG **3** is depicted in Scheme 1. To start the experimental procedure, 2-amino-2-deoxy-D-glucopyranose hydrochloride was reacted with salicylaldehyde in distilled water containing sodium bicarbonate to get imine **2** which was then reduced by sodium borohydrate in methanol to get NHADG 3. The compound **3** was radiolabeled with $^{99m}TcO^-_4$ by stannous reduction method yielding high radiochemical purity. As a result of radiolabeling, three species were formed, the bound ^{99m}Tc complex, free pertechnetate ($^{99m}TcO_4^-$) and reduced or hydrolyzed TcO_2, which were separated by PC and ITLC, respectively. In PC, $^{99m}TcO_4^-$ appeared at *Rf* of 0.8 – 0.9, while the ^{99m}Tc-ligand complex and the reduced or hydrolyzed TcO_2 had an *Rf* = 0.00 - 0.01. The reduced or hydrolyzed fraction was separated from ^{99m}Tc-ligand complex by using saline, in which case the ^{99m}Tc-ligand complex and free $^{99m}TcO_4^-$ had an *Rf* = 0.9 - 1.0, and reduced or hydrolyzed fraction (TcO_2) was detected at *Rf* = 0.00 - 0.01. Radiochemical purity (RCP) determined by instant thin layer chromatography (ITLC) and paper chromatography, after reconstitution of kit, was more than 99 %.

Stability study

As far stability data are concerned, the ligand (NHADG) remained intact with the radiometal in complex form and no significant change was observed in the percentage binding till 4h at room temperature. These data indicate that the radiometal complex should be quite suitable for its further evaluation as a potential imaging agent.

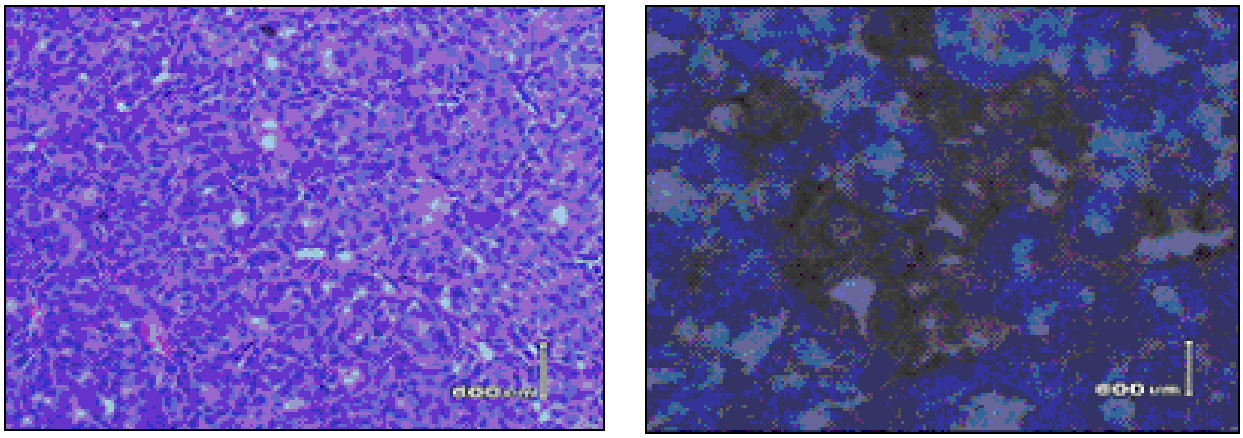
Biodistribution studies in tumor bearing mice

Biodistribution characteristics of ^{99m}Tc-NHADG were evaluated in Swiss Webstar mice bearing mammary tumor. Biodistribution data are summarized in table 1.

Table 1: Biodistribution of ^{99m}Tc-NHADG in Swiss Webstar mice bearing mammary carcinoma (%ID/g)[b]

Tissue	30 min	120 min	240 min
Blood	0.542 ± 0.085	0.321 ± 0.064	0.034 ± 0.053
Lung	1.291 ± 0.145	1.012 ± 0.112	0.224 ± 0.127
Liver	17.64 ± 1.078	6.112 ± 1.723	1.661 ± 1.782
Spleen	0.262 ± 0.065	0.132 ± 0.064	0.087 ± 0.017
Kidney	10.50 ± 1.841	6.634 ± 1.530	2.046 ± 0.876
Tumor	4.670 ± 0.162	4.211 ± 0.112	3.616 ± 0.160
Brain	0.021 ± 0.006	0.002 ± 0.001	0.001 ± 0.001
Heart	0.727 ± 0.091	0.604 ± 0.078	0.401 ± 0.075
Muscle	0.824 ± 0.219	0.336 ± 0.198	0.099 ± 0.183
Tumor/Lung	3.617 ± 0.084	4.161 ± 0.074	16.14 ± 0.063

[b]All data are the mean percentage (n = 3) of the injected dose of ^{99m}Tc NHADG per gram of wet tissue
± Standard deviation of the mean



(A) (B)

Fig. 1: Immunohistochemistry data of tumor tissue samples obtained after dissection of mice, **A)** Mammary carcinoma after staining with hematoxylin-eosin (HE), **B)** Aggregated cancer cells of mammary carcinoma identifiable after immunostaining specific for cytokeratin (CK).

Blood radioactivity was the lowest among all tissues examined. In ^{99m}Tc-NHADG biodistribution studies, liver uptake was the highest among all the organs. Tumor uptake was 4.670 %ID/g at 0.5h p.i., 4.211 %ID/g at 2h p.i., and 3.616 %ID/g at 4h p.i. Heart uptake after 0.5, 2 and 4h p.i., was 0.727, 0.604 and 0.401 %ID/g, respectively.

DISCUSSION

The raising interest in developing [^{18}F]FDG-like ^{99m}Tc-labeled radiopharmaceuticals that are less expensive and easily accessible, to image glucose metabolism in oncology as a tumor marker, stimulated us to develop a novel Tc-99m based radiopharmaceutical N-(2-Hydroxybenzyl)-2-amino-2-deoxy-D-glucose (NHADG) and evaluate the potential of this drug as a diagnostic radiopharmaceutical in tumor-bearing animal models. We have labeled (NHADG) by using the stannous chloride reduction method with technetium-99m (^{99m}Tc).

In vitro stability of the radiolabeled (NHADG) was evaluated as a function of time by determining the amount of free pertechnetate and colloid formed with constant intervals of time up to 4 hr. It was observed that the labeling efficacy remained almost constant till 4 hr with minor fractions of free and colloid formation. The high labeling efficacy shows the validity of labeling technique with the radiometal. These data indicate that the radiometal complex should be quite suitable for its further evaluation as a potential scintigraphic agent.

In biodistribution studies, ^{99m}Tc-N-(2-Hydroxybenzyl)-2-amino-2-deoxy-D-glucose showed maximum uptake in liver among all organs and significantly lower uptake in heart. Though, ^{99m}Tc-N-(2-Hydroxybenzyl)-2-amino-2-deoxy-D-glucose showed little brain and heart uptake, it demonstrated good tumor accumulation, and was excreted rapidly through kidney. The results showed also fast blood clearance. The tumor to lung ratio (T/L), which contributes to image contrast, was also evaluated. Image contrast would be poor if lung uptake is high enough compared with that of the breast lesion (Amano *et al*.,

1998). ^{99m}Tc-N-(2-Hydroxybenzyl)-2-amino-2-deoxy-D-glucose complex showed significantly higher T/L ratio, thus suggesting the potential of this complex to detect breast cancer.

Fig. 1 shows the data of tumor tissues after staining with hematoxylin-eosin (HE) and immuno staining specific for cytokeratin (CK). Histopathological analysis revealed that mammary tumors were of diverse histopathologic type. Immunohistochemical staining for cytokeratin confirmed the heterogeneity in histologic phenotypes. All tumors included a population of cells staining immunopositive for cytokeratin, a marker of luminal epithelial cells. In fig. 2, slides A and B are from the mice dissected after injection of ^{99m}Tc-N-(2-hydroxybenzyl)-2-amino-2-deoxy-D-glucose.

CONCLUSION

Carbohydrate based N-(2-hydroxybenzyl)-2-amino-2-deoxy-D-glucose **3** was synthesized and successfully, labeled with ^{99m}Tc. Biodistribution results showed the feasibility of compound **3** as a functional agent for tumor diagnosis.

REFERENCES

Amano S, Inoue T, Tomiyoshi K, Ando T and Endo K (1998). *In vivo* comparison of PET and SPECT radiopharmaceuticals in detecting breast cancer. *J Nucl Med*., **39**: 1424-1427.

Bayly SR, Fisher CL, Storr T, Adam MJ and Orvig C (2004). Carbohydrate conjugates for molecular imaging and radiotherapy: ^{99m}Tc(1) and ^{186}Re(1) Tricarbonyl complexes of N-(2'-Hydroxybenzyl)-2-amino-2-deoxy-D-glucose. *Bioconjugate Chem*., **15**: 923-926.

Beuthien-Baumann B, Hamacher K, Oberdorfer F and Steinbach J (2000). *Carbohydr Res.,* **327**: 1107-1118.

Burger K and Nagy L (1990). Metal complexes of carbohydrate and sugar-type ligands. *In*: Burger K (ed.) Bio-coordination Chemistry, Coordination Equilibrium in Biologically Active Systems. Ellis Horwood, Chichester, pp.236-284.

Czernin J (2002). Clinical applications of FDG-PET in oncology. *Acta Med Aust*., **5**: 162-170.

Dilworth JR and Parrott SJ (1998). The biomedical chemistry of technetium and rhenium. *Chem. Soc. ReV*., **27**: 43-55.

Garvey WT and Birnbaum MJ (1993). Cellular insulin action and insulin resistance. *In*: Ferrannini E (ed.). *In:* Insulin resistance and disease. *Baillière Tindall, London*, pp.785-873.

Gyurcsik B and Nagy L (2000). Carbohydrates as ligands: coordination equilibria and structure of the metal complexes Carbohydrates as ligands: coordination equilibria and structure of the metal complexes. *Coord. Chem. Rev*., **203**: 81-149.

Krohn KA, Mankoff DA, Muzi M, Link JM and Spence AM (2005). True tracers: Comparing FDG with glucose and FLT with thymidine. *Nucl. Med. Biol.,* **32**: 663-671.

Nagy L and Burger K (1992). Novel Results in Chemistry. (A k´emia ´ujabb eredm´enyei), Akad´emiai Kiad´o, *Budapest*, pp.77-145.

Rigo P, Paulus P, Kaschten BJ, Hustinx R, Bury T, Jerusalem G, Benoit T and Foidartwillems J (1996). Oncological applications of positron emission to mography with fluorine-18 fluorode oxyglucose. *Eur. J. Nucl. Med.,* **23**: 1641-1674.

Schelbert HR and Buxton D (1988). Insights into coronary artery disease gained from metabolic imaging. *Circulation*, **78**: 496-505.

Schibli R, La Bella R, Alberto R, Garcia-Garayoa E, Ortner K, Abram U and Schubiger PA (2000). Influence of the denticity of ligand systems on the *in vitro* and *In vivo* behavior of ^{99m}Tc(I)-Tricarbonyl complexes: A hint for the future functionalization of biomolecules. *Bioconjugate Chem.,* **11**: 345-351.

Schibli R, Dumas C, Petrig J, Garcia-Garayoa E, Schubiger PA, Spadola L and Scapozza L (2005). Synthesis and *in vitro* characterization of organometallic rhenium and technetium glucose complexes against glut 1 and hexokinase. *Bioconjugate Chem*., **16**: 105-112.

Segall G (2002). Assessment of myocardial viability by positron emission tomography. *Nucl. Med. Commun.,* **23**: 323-330.

Storr T, Fisher CL, Mikata Y, Yano S, Adam MJ and Orvig C (2005a). A glucosamine-dipicolylamine conjugate of Tc-99m(1) and Re-186(11) for use in imaging and therapy. *Dalton Trans*., **4**: 654-655.

Storr T, Obata M, Fisher CL, Bayly SR, Green DE, Brudzinska I and Mikata Y (2005b). Novel carbohydrate-appended metal complexes for potential use in molecular imaging. *Chem. Eur. J*., **11**: 195-203.

Storr T, Sugai Y, Barta CA, Mikata Y, Adam MJ, Yano S and Orvig C (2005c). Carbohydrate-appended 2,2'-dipicolylamine metal complexes as potential imaging agents. *Inorg Chem*., **44**: 2698-2705.

Weber MJ, Salter DW and McNair TE (1982). Increased glucose transport in malignant cells, analysis of its molecular basis. *In*: Arnott MS, Van Eys J, Wang YM (eds.). Molecular interrelations of nutrition and cancer. Raven Press, New York, pp.183-190.

Yang DJ, Kim CG, Azhdarinia A, Yu DF, Oh CS, Bryant JL, Won JJ and Kim EE (2003). Multifunctional glucose transport system targeted imaging using ^{99m}Tc-EC-deoxyglucose. *Radiology*, **226**: 465-473.