Synthesis of novel bifunctional Schiff-base ligands derived from condensation of 1-(*p*-nitrobenzyl)ethylenediamine and 2-(*p*-nitrobenzyl)-3-monooxo-1,4,7-triazaheptane with salicylaldehyde†

Anil Kumar Mishra,*‡[a] Puja Panwar,[a] Madhu Chopra,[a] Rakesh Kumar Sharma[a] and Jean-Francois Chatal[b]

[a] *Department of Radiopharmaceutical Chemistry, Institute of Nuclear Medicine and Allied Sciences, Brig. S. K. Mazumdar Road, Delhi 110 054, India. E-mail: akmishra@inmas.org*
[b] *INSERM-U463, Institut de Biologie, 9 quai Moncousu, 44093, Nantes cedex, France*

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Two potentially tetradentate (N_2O_2) and pentadentate (N_3O_3) bifunctional Schiff-base ligands, *N*,*N*′-bis(2-hydroxybenzyl)-1-(*p*-aminobenzyl)ethylenediamine (**7**) and *N*,*N*′-bis(2-hydroxybenzyl)-2-(*p*-aminobenzyl)-3-monooxo-1,4,7-triazaheptane (**5**′) have been prepared and characterized by various spectroscopic methods (IR, FAB-MS, NMR). They are derived from the condensation reactions of the C-functionalized diamines 1-(*p*-nitrobenzyl)ethylenediamine and 2-(*p*-nitrobenzyl)-3-monooxo-1,4,7-triazaheptane with 2.1 equiv. of salicylaldehyde. The first complexation trials with ^{99m}Tc are reported.

Radiolabeled (bio)molecules are potentially useful tools for cancer diagnosis and therapy.[1–3] Some radioactive metals, such as ^{99m}Tc(VII), have physical properties that are well-suited for tumor imaging with monoclonal antibodies, while others, such as ^{186}Re(VII) and ^{188}Re(VII), have cytotoxic properties that can be exploited for therapy by antibody-directed tumor targeting.[2–7] To achieve targeted imaging and therapy the radioisotope should be irreversibly bound to the biological molecules. If this condition is met then the tissue specificity and the biological half-life of the antibody *in vivo* and the physical half-life of the decaying radioisotope will determine the total radiation dose delivered to both cancerous and healthy cells.[8] If this condition is not met, most importantly with β-emitting radioisotopes, then the premature release of radionuclide invariably leads to localization of the activity in sensitive organs, such as the kidney, liver, and the bone marrow, which can result in lethal radiotoxic effects. The most common approach used to radiolabel (bio)molecules has involved the use of bifunctional chelating agents.[9]

In the literature several Schiff-base ligands have been reported and most recently, heptadentate (N_3O_4) ones with various ring-substituted salicylaldehydes have been prepared and their coordination chemistry with a number of metal ions has been extensively investigated.[10–26] Another class of N-functionalized aminephenol Schiff-base ligands such as 1,7-bis(2-hydroxybenzyl)-4-(*p*-aminobenzyl)diethylenetriamine (Bhabdt)[27] has been developed and used for the conjugation of proteins to label with radionuclides.

As part of our efforts to develop bifunctional chelating agents,[28] this paper describes a new class of bifunctional Schiff-base ligands and preliminary conjugation and ^{99m}Tc labeling studies. The specific ligands that we have developed are for conjugation of biologically active molecules for clinical applications and are derived from the optically active amino acid L-phenylalanine. The synthesis of potentially tetradentate and pentadentate ligands by the reaction of the C-substituted diamines 1-(*p*-nitrobenzyl)ethylenediamine and 2-(*p*-nitrobenzyl)-3-monooxo-1,4,7-triazaheptane with salicylaldehyde (see Schemes 1 and 2 below) provides a new class of optically active Schiff-base ligands.

Experimental

Materials and methods

The corresponding diamine derivative of the optically active amino acid was synthesized in our laboratory by the standard procedure.[29] Salicylaldehyde, sodium borohydride, stannous chloride dihydrate and other solvents (Aldrich) were used as received. Reduction of amide was performed under argon atmosphere. TLC was run on plastic-backed silica gel plates (0.2 mm thick silica gel 60 F-254, E. Merck, Germany) using a 10% w/v aqueous ammonium acetate–CH_3OH (1:1 v/v) solution as eluent. HPLC was carried out on a Waters 600E System (Millipore Corporation, Milford, MA, USA) equipped with titanium piston washing pump heads. Solvents were mixed using a Dynamax dual-chamber dynamic mixer (Titanium). UV absorbance was measured using an absorbance/fluorescence monitor (Waters 2487) at 254 or 354 nm. A Gilson model 201 fraction collector was used. Reversed-phase HPLC was performed at room temperature with a C_{18} reversed-phase column, generally using a gradient of CH_3OH and 0.1 M ammonium acetate (pH 6) or 0.05% trifluoroacetic acid at a flow rate of 1 mL min^{-1}. All the solvents for HPLC and reaction mixtures were filtered through a nylon 66 Millipore filter (0.45 μm) prior to use. NMR data were recorded on a Bruker 400 MHz apparatus operating near 400 (^{1}H) or

† Electronic supplementary information (ESI) available: mass spectra of compounds **5**–**7** and **3**′–**5**′. See http://www.rsc.org/suppdata/nj/b3/b300621m/.

‡ Max-Planck-Institute for Biological Cybernetics, Spemannstrasse 38, D-72076 Tübingen, Germany. Tel: +49 707 1601 1679; Fax: +49 707 1601 652; E-mail: anil.mishra@tuebingen.mpg.de.

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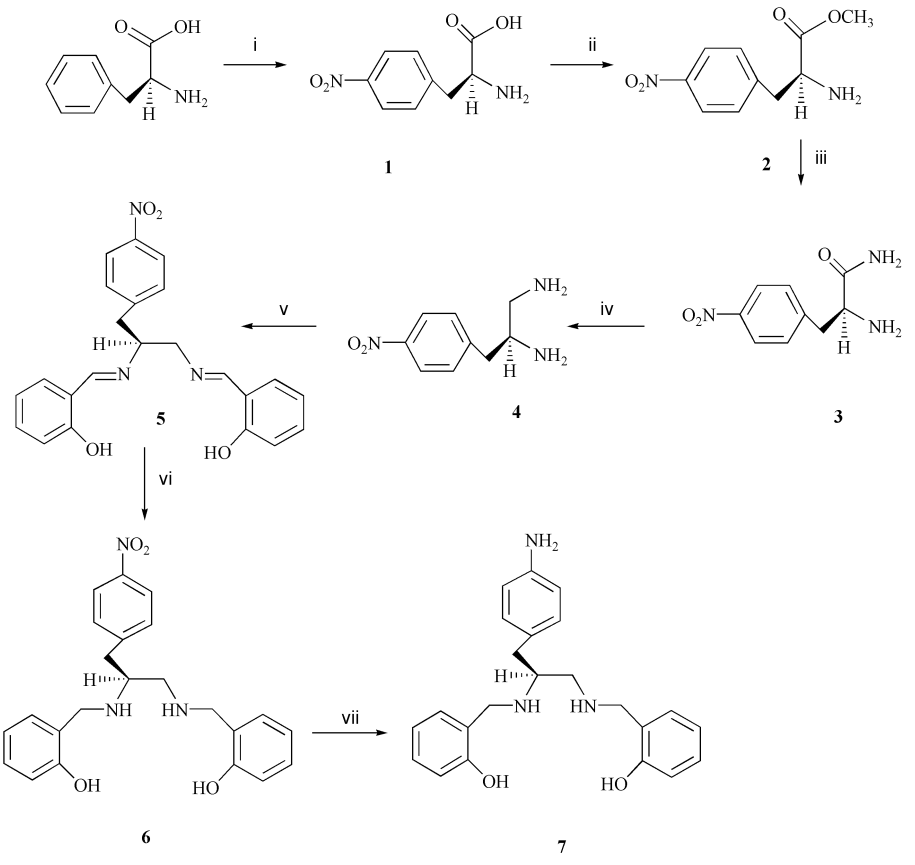
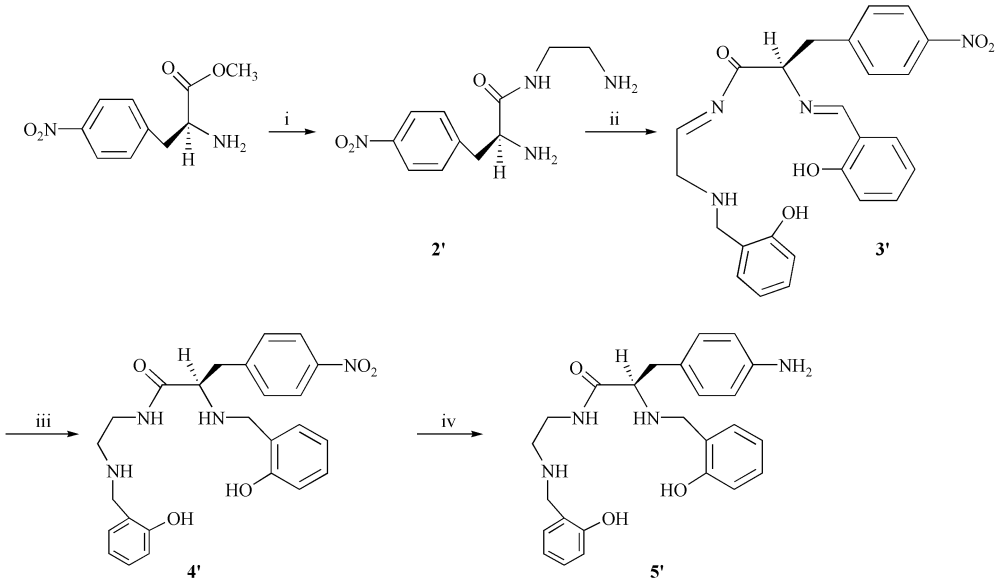


Scheme 1 *Reagents:* (i) H_2SO_4(96%)–HNO_3. (ii) MeOH/$HCl_{(g)}$. (iii) $NH_{3(g)}$. (iv) BH_3·THF. (v) $C_7H_6O_2$. (vi) $NaBH_4$. (vii) $SnCl_2·2H_2O$.

100 (^{13}C) MHz or a Brucker AC 200 operating near 200 (^{1}H) or 50 (^{13}C) MHz. Chemical shifts were relative to either HDO (4.70 ppm) or residual $CHCl_3$ (7.24 ppm).

Syntheses

(L)-*p*-Nitrophenylalanine (1). L-Phenylalanine (99.0 g, 0.6 mol) was dissolved in 96% sulfuric acid (240 mL) with vigorous stirring at 10 °C and concentrated nitric acid (30 mL) was added over a period of 35 min with vigorous stirring at 0–5 °; the reaction mixture was then stirred for an additional 1 h. The reaction mixture was poured on 800 mL of ice cold water and neutralized with 30% ammonium hydroxide. During neutralization precipitation started; the reaction mixture was kept at room temperature for 45 min and then filtered to separate out the precipitate. The precipitate was washed with water until the washings were neutral to litmus. The solid product was recrystallized with hot water to yield (L)-*p*-nitrophenylalanine. IR (KBr) ν/cm^{-1}: 1591 and 1347 (NO_2); ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.87 (d, 2H), 7.23 (d, 2H), 4.20 (t, 1H) 3.12 (m, 2H); anal. calcd. for $C_9H_{10}N_2O_4$: C, 51.43; H, 4.80; N, 13.33; O, 30.45; found: C, 51.60; H, 4.78; N, 13.28; O, 30.60; FAB-MS: Found: m/z 211 $[M+H]^+$; calcd for $C_9H_{10}N_2O_4$: 210.



Scheme 2 *Reagents:* (i) $C_2H_8N_2$. (ii) $C_7H_6O_2$. (iii) $NaBH_4$. (iv) $SnCl_2·2H_2O$.

Synthesis of the methyl ester of *p*-nitrophenylalanine (2). *p*-Nitrophenylalanine (10.0 g, 47.6 mmol) was treated with dry methanol (150 mL) and saturated with HCl gas at 0 °C till dissolution and left to stir at room temperature for 2 h. The clear solution thus obtained was left at 0 °C for 3 h; during this time most of the product precipitated out. The product was filtered, washed with chilled methanol and dried under reduced pressure (1 mm Hg) for 3 h, which yielded 10.9 g (88.3%). TLC of the free amino ester developed in $CHCl_3$–MeOH (4:1) revealed an R_f value of 0.85–0.88. ^{1}H NMR (250 MHz, D_2O): δ 8.19 (d, 2H, $J = 10.0$), 7.48 (d, 2H, $J = 10.0$), 4.48 (t, 1H, $J = 5.0$), 3.78 (s, 3H), 3.36 (m, 2H); FAB-MS: Found: m/z 225 $[M+H]^+$; calcd for $C_{10}H_{12}N_2O_4$: 224; anal. calcd. for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.49; O, 28.54; found: C, 53.61; H, 5.40; N, 12.60; O, 28.65.

***p*-Nitrophenylalanine amide (3).** A slurry of **2** (10.97 g, 42.1 mmol) in dry methanol (5 mL) was treated with triethylamine (6.45 mL). Anhydrous ether (200 mL) was added and the solution was stirred at −10 °C for 2 h. The triethylamine hydrochloride was filtered off and the filtrate concentrated to a viscous orange oil. The oil was added to dry methanol (250 mL) and saturated with NH_3 gas at −5 °C in a fume hood, tightly stoppered and kept at 0–5 °C for 24 h. The precipitated product was collected and dried under reduced pressure. The filtrate was concentrated to dryness and the remaining solid combined with the precipitate gave the amide in 92% yield. ^{1}H NMR (250 MHz, Me_2SO-d_6): δ 8.19 (d, 2H, $J = 8.0$), 7.48 (d, 2H, $J = 8.0$), 7.41 (s, 1H), 7.00 (s, 1H), 3.50 (t, 1H, $J = 3.0$), 3.09 (dd, 1H, $J = 8.0$, 300), 2.81 (dd, 1H, $J = 8.0$, 300); FAB-MS: Found: m/z 210 $[M+H]^+$; calcd for $C_9H_{11}N_3O_3$: 209; anal. calcd. for $C_9H_{11}N_3O_3$: C, 51.67; H, 5.30; N, 20.09; O, 22.94; found: C, 51.02; H, 5.38; N, 21.03; O, 23.01.

1-(*p*-Nitrobenzyl)ethylenediamine (4). To a suspension of **3** (8.12 g, 38.9 mmol in 150 mL dry THF), 200 mL of 1 M diborane in THF at 0 °C was added with a syringe under argon atmosphere and the reaction mixture stirred for 30 min. The temperature of the reaction was raised to 60 °C and the reaction mixture was refluxed for 24 h. After cooling, 20 mL of water was carefully added into the solution to destroy the excess of diborane. The solvent was evaporated under reduced pressure to half the volume and about 100 mL of 18% aqueous hydrochloric acid was added. The reaction mixture was stirred overnight at room temperature and then at 80–100 °C for 15 min. The mixture was cooled, filtered and sufficient ammonium hydroxide was added to the filtrate to give pH > 12. The solution was extracted with chloroform (5 × 100 ml). The combined chloroform extracts were dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure, yielding 88% product as a viscous orange oil. ^{1}H NMR (250 MHz, D_2O): δ 8.23 (d, 2H, $J = 8.0$), 7.64 (d, 2H, $J = 8.0$), 4.12 (m, 1H), 3.53 (dd, 1H, $J = 15.0$, 7.50), 3.46 (dd, 1H, $J = 15.0$, 7.50), 3.39 (dd, 1H, $J = 14.0$, 7.50), 3.26 (dd, 1H, $J = 14.0$, 7.50); FAB-MS: Found: m/z 196 $[M+H]^+$; calcd for $C_9H_{13}N_3O_2$: 195; anal. calcd. for $C_9H_{13}N_3O_2$: C, 55.37; H, 6.71; N, 21.52; O, 16.39; found: C, 55.40; H, 6.73; N, 21.60; O, 16.50.

***N*,*N*′-bis(salicylidene)-1-(*p*-nitrobenzyl)ethylenediamine (5).** 1-(*p*-Nitrobenzyl) ethylenediamine **4** (0.450 g, 2.30 mmol) was taken up in 50 mL acetonitrile. Salicylaldehyde (0.917 g, 4.84 mmol) in 40 mL acetonitrile was added at room temperature and the reaction mixture stirred for 20 h at ambient temperature. The reaction was monitored by TLC. After completion of the reaction, the solvent was removed to dryness and the residue was dissolved in methanol (50 mL), filtered, and the methanol evaporated off to dryness. The yellow solid obtained was dried under vacuum to give the desired product in 85% yield. ^{1}H NMR (250 MHz, $CDCl_3$): δ 3.05 (br s, 1H), 12.92 (br s, 1H), 8.53 (s, 1H), 8.23 (d, 2H, $J = 8.0$), 8.04 (s, 1H), 6.82–7.30 (m, 10H), 4.12 (m, 1H), 3.53 (dd, 1H, $J = 15.0$, 7.50), 3.46 (dd, 1H, $J = 15.0$, 7.50), 3.39 (dd, 1H, $J = 14.0$, 7.50), 3.26 (dd, 1H, $J = 14.0$, 7.50); FAB-MS: Found: m/z 404 $[M+H]^+$; calcd for $C_{23}H_{21}N_3O_4$: 403; anal. calcd. for $C_{23}H_{21}N_3O_4$: C, 68.47; H, 5.25; N, 10.42; O, 15.86; found: C, 68.52; H, 5.32; N, 10.51; O, 15.93.

***N*,*N*′-bis(2-hydroxybenzyl)-1-(*p*-nitrobenzyl)ethylenediamine (6).** To a solution of Schiff base **5** (3.2 g, 7.86 mmol in 100 mL methanol), $NaBH_4$ (5.0 g, 132.17 mmol) was added in several portions and the reaction mixture was allowed to stir at 60 °C for 2 h. Solvent was removed under reduced pressure and the resulting solid was dissolved in $CHCl_3$ (200 mL). The organic phase was separated with water (5 × 200 mL), dried over anhydrous $MgSO_4$, filtered and the solvent evaporated to dryness under vacuum to yield **6** as a yellow brown oil in 90% yield. Reverse-phase C_{18} HPLC: solvent A, 0.01% TFA; solvent B, MeOH; 15–65% B, 0–25 min.; 65–100% B, 30–35 min.; 100–15% B, 35–40 min.; product peak at 17.67 min.^{1}H NMR (250 MHz, $CDCl_3$): 8.23 (d, 2H, $J = 8.0$), 6.82–7.30 (m, 10H), 3.75–3.92 (m, 5H), 3.53 (dd, 1H, $J = 15.0$, 7.50), 3.46 (dd, 1H, $J = 15.0$, 7.50), 3.39 (dd, 1H, $J = 14.0$, 7.50), 3.26 (dd, 1H, $J = 14.0$, 7.50); ^{13}C NMR: a total of 23 peaks have been obtained at 38.57, 48.31, 48.35, 50.16, 50.46, 52.74, 57.76, 116.37, 116.51, 119.49, 119.58, 122.17, 122.28, 123.96, 128.50, 128.70, 129.12, 129.18, 129.94, 145.75, 146.85, 157.51 and 157.62; FAB-MS: Found: m/z 408 $[M+H]^+$; calcd. for $C_{23}H_{25}N_3O_4$: 407; anal. calcd. for $C_{23}H_{25}N_3O_4$: C, 67.80; H, 6.18; N, 10.31; O, 15.71; found: C, 67.85; H, 6.22; N, 10.40; O, 15.79.

***N*,*N*′-bis(2-hydroxybenzyl)-1-(*p*-aminobenzyl)ethylenediamine (7).** To a suspension of product **6** (0.330 g, 0.81 mmol in 200 mL ethanol), $SnCl_2{\cdot}2H_2O$ (0.914 g, 4.05 mmol) was added in small amounts. The reaction mixture was refluxed for 6 h. The reaction was monitored by HPLC using a C_{18} column; the reaction only went to 80% completion to the amino derivative. One additional equivalent of $SnCl_2{\cdot}2H_2O$ was added and the mixture allowed to stir at the ethanol boiling point for an additional 3 h. The reaction mixture was cooled to room temperature and poured onto ice. The pH was adjusted to 8 using NaOH (2 M) and the reaction mixture extracted with ethyl acetate (4 × 100 mL). The combined ethyl acetate layers was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The brownish solid was purified on HPLC and lyophilized on Lyovac to give **7** (yield 60%).^{1}H NMR (250 MHz, $CDCl_3$): δ 8.23 (d, 2H, $J = 8.0$), 6.82–7.30 (m, 10H), 3.75–3.92 (m, 5H), 3.53 (dd, 1H, $J = 15.0$, 7.50), 3.46 (dd, 1H, $J = 15.0$, 7.50), 3.39 (dd, 1H, $J = 14.0$, 7.50), 3.26 (dd, 1H, $J = 14.0$, 7.50); ^{13}C NMR: a total of 23 peaks have been obtained at 38.57, 42.31, 48.81, 50.16, 50.46, 52.74, 57.76, 116.37, 116.51, 119.49, 119.58, 122.17, 122.28, 123.96, 128.50, 128.70, 129.12, 129.18, 129.94, 145.75, 146.85, 157.51, and 157.62; FAB-MS: Found: m/z 378 $[M+H]^+$; calcd. for $C_{23}H_{27}N_3O_2$: 377; anal. calcd. for $C_{23}H_{27}N_3O_2$: C, 73.18; H, 7.21; N, 11.13; O, 8.48; found: C, 73.22; H, 7.24; N, 10.93; O, 8.77.

2-(*p*-Nitrobenzyl)-3-monooxo-1,4,7-triazaheptane (2′). The methyl ester of *p*-nitrophenylalanine (**2**; 4.4 g, 17.9 mmol) was dissolved in neat ethylenediamine at 0–5 °C and the reaction mixture was stirred for 24 h at ambient temperature. By the end of this time, the reaction was completed (as monitored by TLC) and remaining ethylenediamine was removed under reduced pressure. The obtained oily viscous product was dissolved in chloroform (100 mL) and allowed to evaporate slowly at room temperature; the brown solid thus precipitated was filtered and washed with chilled chloroform to give **2′** in 70% yield. ^{1}H NMR (250 MHz, D_2O): δ 8.23 (d, 2H, $J = 8.0$), 7.54 (d, 2H, $J = 8.0$), 4.01 (t, 1H, $J = 7.00$), 3.27

(m, 1H), 3.04 (m, 3H), 2.77 (m, 2H); FAB-MS: Found: m/z 253 $[M+H]^+$; calcd for $C_{11}H_{16}N_4O_3$: 252; anal. calcd. for $C_{11}H_{16}N_4O_3$: C, 52.37; H, 6.39; N, 22.21; O, 19.03; found: C, 52.42; H, 6.43; N, 22.17; O, 19.11.

***N,N′*-Bis(salicylidene)-2-(*p*-nitrobenzyl)-3-monooxo-1,4,7-triazaheptane (3′).** To a solution of **2′** (0.232 g, 0.92 mmol in 50 mL methanol), salicylaldehyde (0.254 g, 2.4 mmol) in 60 mL of methanol was added dropwise over 30 min. The reaction mixture was allowed to stir at 40 °C for 18 h and monitored by TLC. After completion of the reaction solvent was removed to dryness and the residue dissolved in methanol (50 mL), filtered, and methanol evaporated to reduce the volume to half. The obtained yellow solid was filtered to give the desired product in 88% yield. ^{1}H NMR (250 MHz, $CDCl_3$): δ12.85 (br s, 1H), 12.03 (br s, 1H), 8.28 (s, 1H), 8.12 (d, 2H,), 8.02 (s, 1H), 6.81–7.39 (m, 10H), 6.13(t, 1H), 4.12 (t, 1H), 3.23–3.72 (m, 6H); FAB-MS: Found: m/z 461 $[M+H]^+$; calcd for $C_{25}H_{24}N_4O_5$: 460; anal. calcd. for $C_{25}H_{24}N_4O_5$: C, 65.21; H, 5.25; N, 12.17; O, 17.37; found: C, 65.25; H, 5.30; N, 12.26; O, 17.43.

***N,N′*-Bis(2-hydroxybenzyl)-2-(*p*-nitrobenzyl)-3-monooxo-1,4,7-triazaheptane (4′).** To a solution of Schiff base **3′** (1.5 g, 3.2 mmol in 150 mL methanol), $NaBH_4$ (2.5 g, 66 mmol) was added in several portions and the reaction mixture was allowed to stir at 60 °C for 2 h. Solvent was removed under reduced pressure and the resulting solid was dissolved in $CHCl_3$ (200 mL) and washed with water (5 × 200 mL). The organic layers were separated, dried over anhydrous $MgSO_4$, filtered and the solvent was removed to dryness under reduced pressure to give an oily yellow brown product (yield 90%). Reverse-phase C_{18} HPLC: solvent A, 0.01% TFA; solvent B, MeOH; 15–65% B, 0–25 min.; 65–100% B, 30–35 min.; 100–15% B, 35–40 min.; product peak at 17.9 min. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.12 (d, 2H), 6.73–7.31 (m, 10H), 6.34 (t, 1H), 4.00 (t, 1H), 3.23–3.72 (m, 10H); FAB-MS: Found: m/z 465 $[M+H]^+$; calcd for $C_{25}H_{28}N_4O_5$: 464; anal. calcd. for $C_{25}H_{28}N_4O_5$: C, 64.64; H, 6.08; N, 12.06; O, 17.22; found: C, 64.24; H, 6.11; N, 12.03; O, 17.32.

***N,N′*-Bis(2-hydroxybenzyl)-2-(*p*-aminobenzyl)-3-monooxo-1,4,7-triazaheptane (5′).** To a suspension of **4′** (0.390 g, 0.84 mmol in ethanol 200 mL), $SnCl_2{\cdot}2H_2O$ (1.59 mg, 7.05 mmol) was added at room temperature. The reaction mixture was refluxed for 6 h with the reaction being monitored by HPLC using a C_{18} column. One additional equivalent of $SnCl_2{\cdot}2H_2O$ was added and the mixture allowed to stir at the boiling point of ethanol for an additional 3 h. The reaction mixture was cooled to room temperature and poured onto ice. The pH was adjusted to 8 using NaOH (2 M) and the mixture was extracted with ethyl acetate (400 mL). The ethyl acetate layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The brownish solid was purified on HPLC and lyophilized on Lyovac (yield 70%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.12 (d, 2H), 6.73–7.31 (m, 10H), 6.34 (t, 1H), 4.00 (t, 1H), 3.23–3.72 (m, 10H); FAB-MS: Found: m/z 435 $[M+H]^+$; calcd for $C_{25}H_{30}N_4O_3$: 434; anal. calcd. for $C_{25}H_{30}N_4O_3$: C, 69.10; H, 6.96; N, 12.89; O, 11.05; found: C, 69.16; H, 7.01; N, 12.93; O, 11.13.

Complexation studies

Preliminary complexation studies with technetium-99m metal ion with compounds **7** and **5′** were carried out by the reduction of TcO_4^- (500–740 MBq) with stannous chloride dihydrate (3.4 μM) in the presence of ligands (10 μM) in the pH range 5.5 to 6.0 at 25 °C. Strip chromatography with ITLC-SG (Gelman Sciences, Ann Arbor, MI, USA) using acetone eluent, in which pertechnetate migrates, and using saline eluent, in which both labeled chelate and pertechnetate migrate, were used to determine the radiochemical purity of the labeled complexes. On the basis of chromatographic analysis the radiolabeling efficiency was found to be > 98%. Labeled complex was incubated at 37 °C in fresh human serum at a concentration of 100 nM mL^{-1}. Stability as assessed by TSK 3000 gel filtration HPLC showed that the metal ion was intact under physiological conditions and not taken up by the protein. Unbound radioactivity was less than 0.5% in 7 h.

Conjugation

The amino group of the Schiff-base ligand was converted to the isothiocyanato group by reacting with thiophosgene at pH 2. Compounds **7** and **5′** (1.5 mmol) in 5 mL of 3 M HCl were added to 3 mL of $CSCl_2$ (85% in CCl_4) at room temperature. The reaction mixture was stirred vigorously for 4 h. The aqueous phase was washed with $CHCl_3$ (4 × 5 mL) in a fume hood to remove excess $CSCl_2$ and then purified by reverse phase HPLC. Conjugation of EGFr monoclonal antibody was performed by adding 25 μL of the 20 mM chelate solution to 300 μL of a solution containing 3 mg of antibody in 0.1 M sodium phosphate, pH 7. Saturated trisodium phosphate solution (40 μL) was added to make the pH 8.5. The reaction mixture was incubated at 37 °C for 60 min and then subjected to centrifuged column gel chromatography, which removed the unreacted chelate, and the buffer changed to 0.1 M sodium acetate, pH 5.5. UV absorbance at 280 nm for the centrifuged column effluent was used to determine the antibody concentration and ^{57}Co assay used to obtain the bound chelate concentration. In short, 1 nmol of conjugated antibody, 2 nmol of ^{59}Co and a tracer of ^{57}Co were mixed and incubated at pH 5.5 at room temperature for 30 min. An aliquot was applied to thin layer chromatography plates with ITLC-SG (instant thin layer chromatography-silica gel; Gelman Sciences, Ann Arbor, MI, USA) with ammonium acetate–CH_3OH (1:1 v/v) solution as eluent. Free cobalt migrates to $R_f = 1.0$ while labeled cobalt with immuniconjugate stays at $R_f = 0$. The ^{57}Co binding assay indicates 2.1 ± 0.1 chelate molecules were conjugated per antibody molecule.

Results and discussion

The preparation of diamines has been achieved in high yields starting from optically active L-(−)-phenylalanine followed by nitration, esterification, aminolysis and reduction with diborane and $SnCl_2{\cdot}2H_2O$. The reported method[29] for the reduction of amide with diborane to the corresponding amine followed by HCl gas often gives lower yields, which can be increased by passing HCl gas in several installments, while in the present studies we have used 6 M HCl, which provides better yield and easy workup. The previously described method[29] for the reduction of the nitro group uses 10% Pd/C under hydrogen atmosphere at 4 °C in basic media while we have followed the method reported in the literature[30] using $SnCl_2{\cdot}2H_2O$ in absolute ethanol, which gave quantitative yields. All the products were fully characterized on the basis of spectral studies.

The Schiff-base complexing agents (**7** and **5′**) obtained by the condensation of a salicylaldehyde with the corresponding functionalized amines are bifunctional in nature and can be used for conjugation and radiolabeling as reported in the literature,[27] with better stability under physiological conditions by conversion of primary amines to the isothiocyanato group. It has been noted that carbon-backbone-substituted chelating agents form more stable radiolabeled complexes or immunoconjugates, which have higher serum stability than the N-attached (including amide-attached) derivatives. The ligand in the –NCS derivative was found to be stable when stored at −20 °C in 0.3 M HCl.

Monoclonal antibodies against abundantly expressed antigens could prove to be effective tracer molecules for

radioimmunodiagnosis. The mAb ior egf/r3 recognizes EGFr and inhibits the binding to its ligand. This antigen has been used successfully as a target for imaging and therapy because of its over-expression in tumors of epithelial origin.[31] Although normal cells also express EGFr, the elevated number of receptors on tumor cells confers a degree of targeting specificity. Therefore, the tumor cells can proportionally bind more radiolabeled mAbs.

Conjugation was performed in the ratio of 1:20. UV absorbance and the ^{57}Co binding assay indicated 2.1 ± 0.1 chelate molecules per monoclonal antibody, which was labeled with a specific activity of 20–30 mCi mg^{-1} of protein. Labeling efficiency was measured by ascending paper chromatography on ITLC-SG strips. Preliminary complexation with ^{99m}Tc was found to give sufficiently stable complexes under physiological conditions. In conclusion, the preliminary studies with these novel Schiff-base ligands are encouraging to carry out further *in vivo* experiments for targeted imaging of human tumors in animal models.

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