EFFICIENT METHOD FOR THE SYNTHESIS OF BISAMINO-ETHANETHIOLS AND THEIR PYRROLE CONJUGATES

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Abstract- By following the protection and deprotection approach, an efficient method for the preparation of bisaminoethanethiol ligands (N_2S_2) is discussed. In our attempts to synthesize certain nonpeptide analogs of neurotensin, moldel studies were performed by using these ligands for preparing the corresponding pyrrole based conjugates. This methodology provides an alternate approach for developing various target specific agents that cross the blood brain barrier.

INTRODUCTION

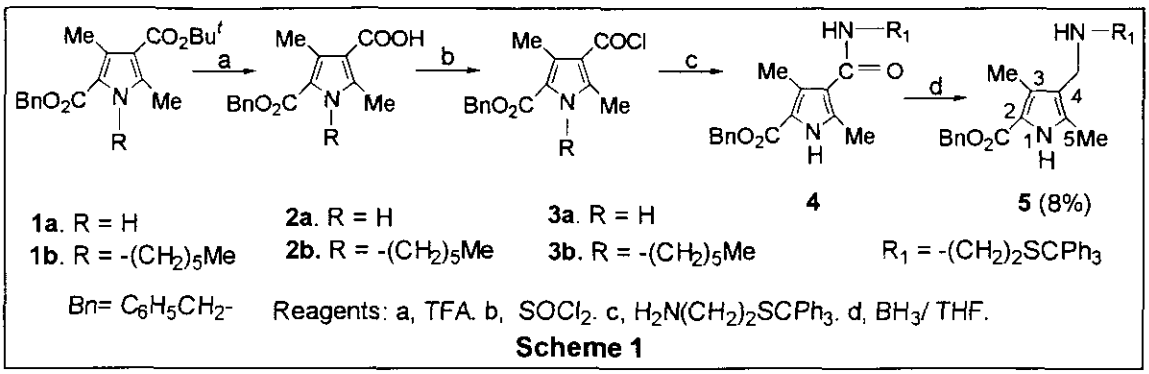
Most of the radiopharmaceuticals used in conventional nuclear medicine are ^{99m}Tc-labeled short half-life and ideal gamma emission. Millicurie quantities can be delivered without excessive radiation to the patient. The monoenergetic 140-KeV photons are readily collimated, producing images of superior spatial resolution. Furthermore, ^{99m}Tc is readily available in a sterile, pyrogen-free, and carrier-free state from ^{99}Mo-^{99m}Tc generators.[1]

A recent report of Hong, Pang and coworkers[2] described certain pyrrole based nonpeptidic analogs of neurotensin are potential candidates for the treatment of neuropsychiatric diseases; *e. g.*, schizophrenia and Parkinson's disease. Our interest was to prepare N_2S_2 conjugates of these compounds as potential brain imaging agents.

RESULTS AND DISCUSSION

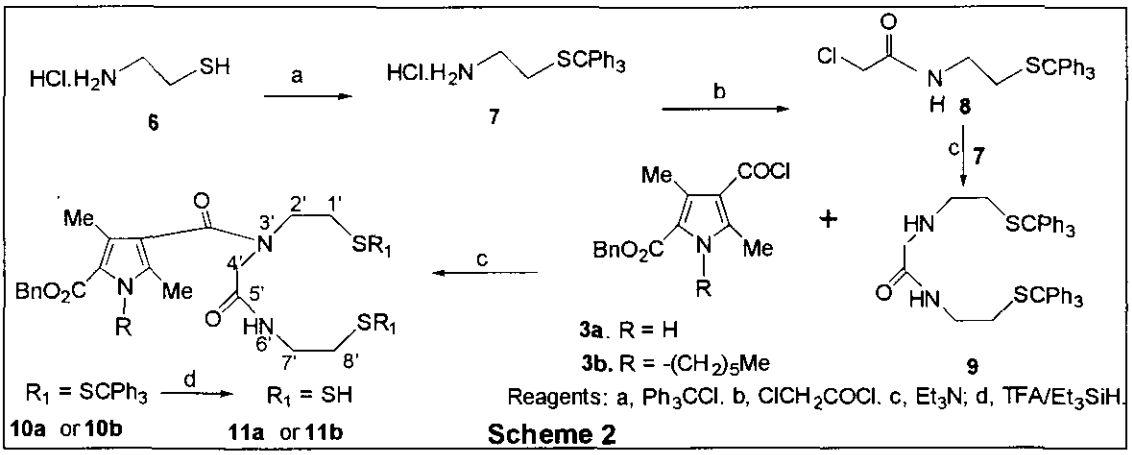
Our initial experiments were aimed to establish the reaction conditions for the preparation of such compounds by performing model studies. In our approach, benzyl 3,5-dimethyl-4-*tert* butoxycarbonylpyrrole-2-carboxylate (**1a**) was prepared by following the standard methodology.[3] Deprotection of **1** with TFA, and subsequent treatment with thionyl chloride gave the corresponding acid

chloride (**3**). The acid chloride was not isolated, but immediately reacted *(in situ)* with protected aminothiol to generate the corresponding amide analog (**4**) in 83 % yield. Our next step was to reduce the amide group, and then to react the intermediate amine with an appropriate aminothiol to generate the



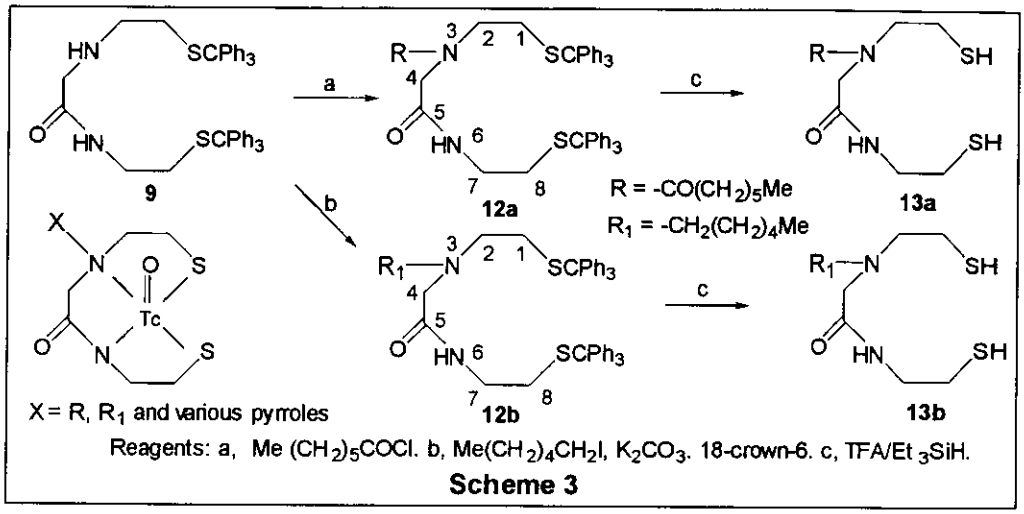
Scheme 1

desired N_2S_2 ligand. Numerous reduction reactions were attempted, but in almost all cases either the starting material was mainly recovered or a complex mixture was obtained. The diborane reaction[4] gave the desired reduced pyrrole **5**, but in low yield (8%). These negative results, therefore, led us to abandon this approach. In our second approach, we decided to prepare the ligand first and then condense it to the desired pyrrole by following the reaction sequence depicted in Scheme 2.



Scheme 2

The protection of the thiol group of 2-aminoethanethiol was achieved by reacting **6** with triphenylmethyl chloride (Ph_3CCl) and the thiol-protected analog (**7**) was obtained in >88% yield. Reaction of **7** with chloroacetyl chloride produced the corresponding chloromethylamide (**8**), which on reaction with **7** produced the thiol-protected N_2S_2 ligand in 71% yield. It was then reacted with pyrrole (**3a**) under inert atmosphere and the related thiol-protected conjugate (**10a**) was isolated in 89% yield. Treatment of **10a** with triethylsilane/TFA efficiently removed the protecting group and the desired compound (**11a**) was obtained in 95% yield. To investigate the effect of lipophilicity on drug uptake in the brain, the *N*-hexylpyrrole (**1b**) was also converted into the related N_2S_2 conjugate **11b** in almost same yield. All



Scheme 3

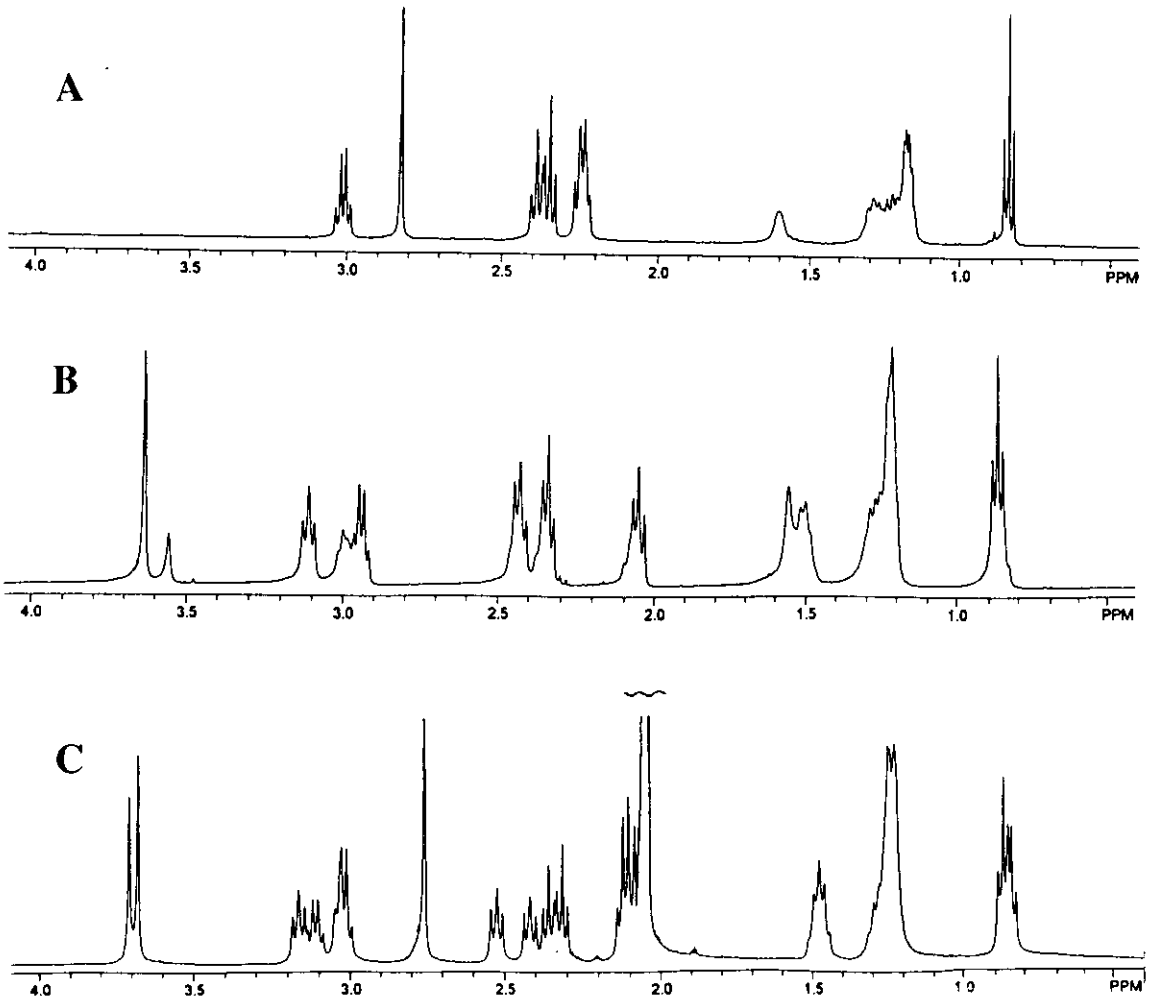


Figure 1. ^{1}H NMR spectra of *N*-heptanoyl N_2S_2 ligand (**12a**) (A), *N*-hexyl N_2S_2 ligand (**12b**) in $CDCl_3$ (B) and *N*-heptanoyl N_2S_2 ligand (**12a**) in acetone-d_6 at 30°C. The aromatic region is not shown.

the final and intermediate products were characterized by NMR and MS spectrometry analyses. The NMR spectra of the N_2S_2conjugates showed splitting in their resonances. These remarkable splitting are most likely due to a slow rotation around N-C bond induced by the acyl substitutions. This slow rotation possibly caused the molecule (and thus all the protons) to become magnetically unequivalent.

To confirm such effects caused by the acyl substituent, two N_2S_2 conjugates (**13a** and **13b**) bearing *N*-heptanoyl and *N*-hexyl group respectively were synthesized. For the preparation of **13a**, heptanoyl chloride was reacted with thiol-protected ligand (**9**) by following the methodology described above. Treatment of **9** with hexyl iodide under similar reaction conditions did not produce the desired product and mainly the starting materials were recovered. However, on reacting these materials in presence of 18-crown-6 and potassium carbonate in acetonitrile solution, the *N*-hexyl derivative was isolated in 81% yield. The thiol groups were then deprotected by following the methodology as discussed for pyrrole conjugate (**10**) and the desired thiols (**13a**) and (**13b**) so obtained were converted into the related corresponding technitium complexes. Similar to the pyrrole conjugates (**10** and **11**), the ^{1}H NMR spectra of bisaminoethanethiol containing a *N*-heptanoyl substituent (**12a**) and (**13a**) also showed similar splitting of most of the resonances due to their magnetically unequivalent nature. The NMR spectrum of the *N*-hexyl N_2S_2 ligand X showed only one set of the resonances (Figure 1 A). However, in the related *N*-heptanoyl analog, splitting was observed for most of the resonances, and the ratio between these individual sets were found to be solvent dependent (Figures 1B and 1C, the aromatic region is not shown).

In conclusion, we have developed a facile method for the preparation of N_2S_2 ligands. This methodology provides an efficient approach to prepare symmetrical and unsymmetrical N_2S_2 ligands in large quantities. To prepare the Tc-99m-labeled organ specific imaging agents, these ligands can be conjugated with a wide variety of receptor specific binding compounds. At present, in our laboratory, efforts are underway to synthesize the conjugates of various receptor specific compounds and certain pyrrole based non-peptide analogs of neurotensin. The details biological studies with these compounds will be reported in our full paper.

EXPERIMENTAL[5]

The melting points are uncorrected. All NMR spectra were recorded on Bruker Am-400 spectrometer and $CDCl_3$ was used as a solvent with TMS as an internal standard. FAB-MS was performed on MAT-90 spectrometer. All organic reagents were obtained from Aldrich Chemical Corporation except that [^{99m}Tc] pertechnetate, which was obtained from department of nuclear medicine of Roswell Park Cancer

Institute. Silica gel 60 F_{254} (Whatman Ltd) plates of 0.25 mm thickness were used for analytical thin-layer chromatography (TLC). Preparative TLC was performed on 20×20 cm TLC plates (Analtech, Inc.).

Compound (10a): A solution containing 248 mg (0.91 mmol) of **2a** and 1081 mg (9.1 mmol) of thionyl chloride in 10 mL of dry methylene chloride was heated to reflux for 3 h. The solvent and excess thionyl chloride were removed with rotavapor and the residue was dried under high vacuum (oil pump) for half hour. The resultant white powder was dissolved in 10 mL of dry methylene chloride, 560 mg (0.83 mmol) of **9** was added and the mixture was stirred at rt for a few minutes, 167 mg (1.65 mmol) of triethylamine was added dropwise, the resultant solution was stirred under dry nitrogen at rt for 16 h, then washed with water, dried over sodium sulfate, and concentrated. The crude product was purified by column chromatography on silica gel with 3% MeOH/CH_2Cl_2 as eluant to give 686 mg of **10a** as a white gum in 89% yield. ^{1}H NMR ($CDCl_3$, δ ppm): 8.69 (1H, s, H-1), 7.60-6.90 (35H, m, aromatic H), 6.54 (1H, s, H-6'), 5.33 (2H, s, CH_2 of benzyl), 3.81 (2H, br, H-4'), 3.15 (2H, br, H-2' or 7'), 2.93 (2H, br, H-2' or 7'), 2.39 (4H, br, H-1' or 8'), 2.13 (3H, s, CH_3-3 or CH_3-5), 2.02 (3H, s, CH_3-3 or CH_3-5). MS (FAB) calculated for $C_{59}H_{55}N_3O_4S_2$: 933. Found: m/z 934 $[M+1]^+$.

Compound (11a): 400 mg (0.43 mmol) of **10a** was dissolved in 3 mL of CH_2Cl_2/TFA (1:1), 218 mg (1.88 mmol) of triethylsilane was added successively. The mixture was stirred under nitrogen at rt for 15 min, then diluted with 30 ml of methylene chloride, washed with water, dried over sodium sulfate, and concentrated. The crude product was purified by column chromatography on silica gel with CH_2Cl_2, 1% MeOH/CH_2Cl_2 and 3% MeOH/CH_2Cl_2 as eluants successively to give 183 mg of **11a** as colorless gum, the yield was 95%. NMR ($CDCl_3$, δ ppm): 9.01(1H, br, H-1), 7.37 (5H, m, -$CH_2C_6\underline{H}_5$), 5.31(2H, s, -$C\underline{H}_2C_6H_5$), 4.12 (2H, br, H-4'), 3.61 (2H, br, H-2' or H-7'), 3.45 (2H, br, H-2' or H- 7'), 2.66 (4H, br, H-1' and H- 8'), 2.30 (3H, s, CH_3-3 or CH_3-5), 2.28 (3H, s, CH_3-3 or CH_3 -5). MS (FAB): calculated for $C_{21}H_{27}N_3O_4S_2$: 449. Found: m/z 450 $[M+1]^+$.

Compound (11b): Compound (**3a**) was converted into the title compound in 91% yield by following the procedure discussed for the preparation of **11a**. NMR ($CDCl_3$, δ ppm): 7.37 (5H, m, -$CH_2C_6\underline{H}_5$) , 5.29 (2H, s, -$C\underline{H}_2C_6H_5$), 4.10 (4H, m, H-4' and H-7), 3.49 (4H, m, H-2' or H- 7'), 2.66 (4H, m, H-1' and H-8'), 2.24 (6H, s, CH_3-3 and CH_3-5), 1.59 (2H, m, H-8), 1.26 (6H, m, H-9, H-10 and H-11), 0.87 (3H, t, J=6.6 Hz, H-12). MS (FAB): calculated for $C_{27}H_{39}N_3O_4S_2$: 533 Found: m/z 534 $[M+1]^+$.

General method for the preparation of Tc-99m analogs:

The appropriate ligand (*e. g.* **11a**) was dissolved in 1 mL of ethanol. 600 μL of HCl (1N), 1 mL of Sn-glucoheptate solution (containing 136 μg of $SnCl_2$ and 200 μg of Na-glucoheptate) and 50 μL of EDTA solution (0.1 N) were successively added. 0.4 mL of [^{99m}Tc]pertechnetate (4.0 mCi) in saline solution was then added. The reaction mixture was heated to reflux for 30 min, cooled to rt and neutralized with a

saturated $NaHCO_3$ solution, then extracted with EtOAc three times (5 mL each). The organic layers were combined, washed with water (5 mL) once and dried over anhydrous Na_2SO_4. Evaporation of the filtrate at reduced pressure (30 °C) gave radiolabeled compound. The ^{99m}Tc complex was formulated in ethanol (0.5 mL), Tween 80 (0.5 mL) and 5 ml of 5% dextrose. The purity was ascertained by HPLC analysis and the resultant clear solution was used for biodistribution experiments.

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5. The experimental details of the key compounds are reported. The synthetic procedure for the preparation of other compounds with detailed biological studies is in progress and will be reported in our full paper.

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