कैंसर देख-भाल में नवाचार

3

प्रोस्टेट कैंसर के निदान हेतु किफ़ायती कार्बनिक लिगेंड के स्वदेशी संश्लेषण में हाल में हुई प्रगति

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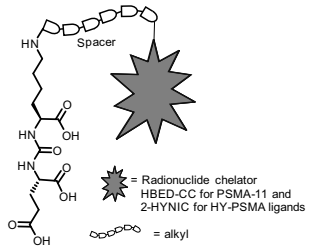

स्व-गृह संश्लेषित 2-हाइड्राज़िनोनिकोटिनिक अम्ल (2-एचवाईएनआईसी) संयुग्मित पीएसएमए लक्षित लिगेंड का चित्रण।

सारांश

किफ़ायती नाभिकीय औषधियों को बनाने के लिए स्वदेशीकरण एक अटल कार्यनीति है जो लक्षित रेडियोलिगैंड उपचार (आरएलटी) विधि को जरूरतमंदों के लिए सुलभ बना सकती है। दो सामान्य रेडियोलिगैंड आधारित नैदानिक विधियों, अर्थात्; एकल फोटॉन उत्सर्जन संगणित टोमोग्राफी (एसपीईसीटी), और पॉज़िट्रॉन उत्सर्जन टोमोग्राफी (पीईटी), में से एसपीईसीटी सबसे व्यापक और सस्ती विधि बनी हुई है। यहाँ, हम उस प्रगति पर चर्चा करेंगे जो हमने एसपीईसीटी और पीईटी आधारित प्रोस्टेट कैंसर निदान के लिए उपयुक्त सिंथेटिक कार्बनिक लिगेंड के अनुसंधान एवं विकास में प्राप्त की है। इसके एक भाग के रूप में, स्थापित पीईटी आधारित लिगैंड, पीएसएमए-11 के अलावा; एसपीईसीटी आधारित प्रोस्टेट कैंसर नैदानिक लिगैंड की मौजूदा आवश्यकता को पूरा करने के लिए, हमने पांच 2-हाइड्राज़िनोनिकोटिनिक अम्ल (2-एचवाईएनआईसी) संयुग्मित पीएसएमए लक्षित लिगैंड का आंतरिक संश्लेषण किया है।

Innovations in Cancer Care

3

Recent Advances in Indigenous Synthesis of Affordable Organic Ligands for Prostate Cancer Diagnosis

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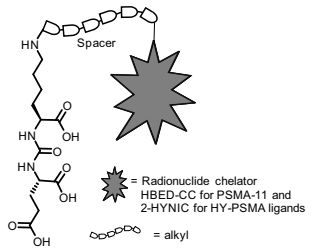

Depiction of in-house synthesis of 2-hydrazinonicotinic acid (2-HYNIC) conjugated PSMA targeting ligands.

ABSTRACT

Indigenization is an unswerving strategy to make affordable nuclear medicines that could make the targeted radioligand treatment (RLT) method accessible to the needy. Among the two common radioligand based diagnostic methods, namely; single-photon emission computed tomography (SPECT), and positron emission tomography (PET), SPECT remains the most widespread and affordable method. Here in, we demonstrate the progress that we have attained in the research and development of synthetic organic ligands appropriate for SPECT and PET based prostate cancer diagnosis. As part of it, apart from the established PET based ligand, PSMA-11; to cater the existing requirement for the SPECT based prostate cancer diagnostic ligands, we have materialized in-house synthesis of five 2-hydrazinonicotinic acid (2-HYNIC) conjugated PSMA targeting ligands.

KEYWORDS: *Diagnosis, Ligands, Cancer, Prostate, PSMA.*

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Introduction

Prostate cancer (PC), a common malignant cancer in men and the second major cause of cancer related deaths is of late showing alarming surge. [1-2] The estimated number of PC cases globally will be ~3 million in a span of next one and half decades [3-5], which would be more than double that was observed in 2020. The surge in the number of cases is also attributed to the advancement in the medical diagnostics, a primary step in the treatment of cancer. Unlike many other human disorders, diagnostic tools are periodically used throughout the course of cancer treatment to estimate the effectiveness of the treatment method adopted. Precise and early stage diagnosis of cancer provides clinicians with the liberty of all available treatment modes. It is perceptible that for such conclusive diagnosis there is still room for the development of diagnostic medicines effective at molecular level. In Indian context, such developments possess great relevance, as India is presently among the cancer hotspots of the world [6,7] due to the significant surge in the number of cancer incidences and associated deaths [8].

Among the existing non-invasive diagnostic techniques; computed tomography (CT), magnetic resonance imaging (MRI) [9], and radio ligand method, the latter is the most sensitive molecular level diagnostic technique, which not only stage the disease but also effectively sketch the treatment progress after cancer therapy. Any effective radio ligand method is based on the manifestation of a biomarker in the targeted cancer cell type. This method is employed in prostate cancer treatment by targeting biomarker, prostate specific membrane antigen (PSMA) or glutamate carboxypeptidase II (GCPII) [10], a type II transmembrane glycoprotein over-expressed on prostate cancer cell surface. To treat prostate cancer through radio ligand method, few effective active pharmaceutical ingredients (APIs), that target PSMA, has been identified and is providing better treatment options for clinicians, globally [11,12]. Unfortunately, most of the APIs used for the radio ligand preparation comes with exorbitant cost, which makes the treatment unaffordable to many patients. PSMA-617 (**1**) and PSMA-11 (**2**), (Fig.1) are few much sought APIs in the therapy and diagnosis, respectively, for prostate cancer management [13-16]. To counter this menace, we initiated the indigenization of APIs along with the development of new APIs for cancer diagnosis and therapy. Over the past 10 years, through our research and development efforts, we successfully undertook the synthesis of some important APIs for radiopharmaceuticals preparations. This includes, three precursors (**3a-c**, Fig.1) for [^{18}F]-FLT (**3**), [17] a positron emission tomography (PET) based brain cancer imaging agent. More recently, few highly important ligands for prostate cancer management, *i.e.* ligand for endotherapeutic application, PSMA-617 (**1**), PET based diagnostic ligand, PSMA-11 (**2**), and five SPECT based 2-hydrazinonicotinic acid (2-HYNIC) conjugated PSMA ligands, HY-1N-I-PSMA (**4**), HY-2N-I-PSMA (**5**), I-PSMA (**6**), I-1N-PSMA (**7**), HY-Hex-I-PSMA (**8**), through indigenously developed synthetic protocols. When it comes to prostate cancer diagnosis, in addition to the established PET based ligand, PSMA-11, it is always advantageous to have SPECT based PSMA ligand as it would give another easily accessible tool at the hands of nuclear medicine physicians.

Practically, the main challenge with the PET based medicine, [^{68}Ga]Ga-PSMA-11, is the adequate accessibility of ^{68}Ge/^{68}Ga generators, [18] which, to an extent can be neutralized with the availability of ^{99}Mo/^{99m}Tc generator driven SPECT based PSMA ligands. It is also to be noted that in India, at present, there are only few tens of functional PET scan centres while the number of functional SPECT scan centres are in hundreds. This obviously depicts the need for the development of SPECT based prostate cancer diagnostic agents so that the diagnosis can reach more number of patients at an affordable cost. Among the bifunctional chelators available for radionuclide, ^{99m}Tc, HYNIC is easily synthesizable from cheap and commercially available resources and is also a proven mono-dentate ligand. Therefore, we opted to synthesize analogues of HYNIC conjugated PSMA ligands employing multi-step organic synthesis and investigate their labelling and diagnostic characteristics.

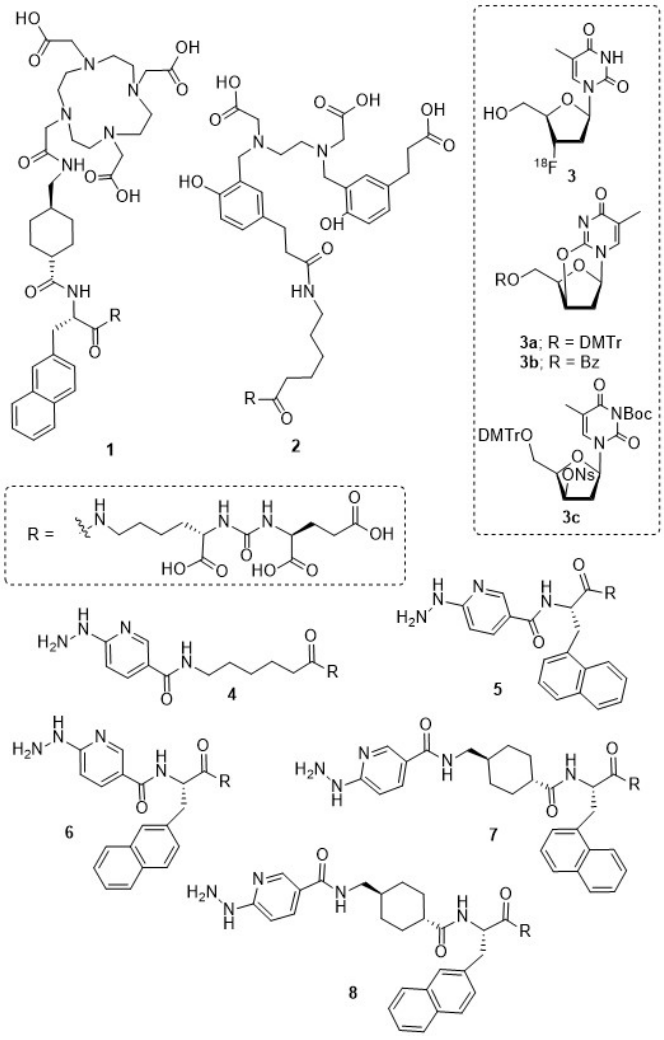


*Fig.1: Indigenous organic ligands/precursors: PSMA-617 (**1**), PSMA-11 (**2**), [^{18}F]-FLT (**3**), precursors for [^{18}F]-FLT (**3a-c**), HY-Hex-I-PSMA (**4**), I-1N-PSMA (**5**), I-PSMA (**6**), HY-1N-I-PSMA (**7**), HY-2N-I-PSMA (**8**).*

The main objective of synthesizing these ligands through indigenous pathway is to make the radionuclide chelated/conjugated ligand based targeted diagnostic modality, nuclear medicine, affordable to the people of our country. In this account, we demonstrate the synthetic pathways that we have adopted for the synthesis of PET and SPECT based organic ligands, PSMA-11 (**2**), HY-1N-I-PSMA (**4**), HY-2N-I-PSMA (**5**), I-PSMA (**6**), I-1N-PSMA (**7**), HY-Hex-I-PSMA (**8**) (Fig.1), useful for prostate cancer diagnosis, in a cost-effective manner.

Results and Discussion

We initiated the synthesis of PSMA targeting diagnostic ligands program with the synthesis of PSMA-11 (**2**), exercising a homogenous solution phase approach. The rationale behind the same is the leeway of espousing various synthetic strategies, like Fmoc-, Boc-, Cbz- and Alloc-, for efficiently achieving the target molecules. Among the three strategies harnessed for the synthesis of PSMA-11; namely, Fmoc-, Boc-, and Cbz-, we fathomed that the Cbz strategy [13,14] is the most economical in achieving the target. Irrespective of the strategies adopted, when it comes to the synthesis of amino acid based urea containing PSMA targeting ligands, there involves two synthetic segments. The first segment comprised of the construction of the urea template, made from glutamic acid and lysine; which serves as the moiety that binds to the membrane bound protein target, PSMA. To achieve the synthesis of this vital part of the target molecule, one can visualize it through the appropriate selection of differently protected hydrochloride salts (Fig.2) of glutamic acid (**9**), and lysine (**10a**) [13-16]. The selection of the side chain protecting group in lysine, and the protecting group at the amino end in the spacer amino acid would decide the strategy towards the target molecule. The second segment involves synthesis of *N*-protected spacer, which in the case of Cbz-strategy for PSMA-11 is *N*-Cbz protected ω-amino-caproic acid (**11a**), can be achieved in three steps from commercially available ω-amino-caproic acid [13]. The final coupling constituent in the synthesis of PSMA-11 is the commercially available *t*-butyl protected chelator, HBED-CC.

With the required amino acid precursors (**9**/**10a**/**11a**) and HBED-CC in hand, the synthesis of PSMA-11 was initiated. Initially, construction of orthogonally protected urea template (**15a**) was achieved from the appropriately protected hydrochloride salts of glutamic acid (**9**) and lysine (**10a**) as shown in scheme 1, using disuccinimidyl carbonate or triphosgene as the conjugating reagent in the presence of a base. Subsequently, the benzyloxycarbamate (cbz) group in the urea template (**15a**) was removed using 10% Pd/C catalysed flow reactor based heterogenous hydrogenation method to furnish amine (**16**) (Scheme 2), which was coupled to amino acid (**11a**) employing dicyclohexylcarbodiimide (DCC) as coupling agent, to yield the adduct (**17a**). Subjecting (**17a**) for hydrogenation reaction yielded amine (**18**), which on coupling with HBED-CC yielded the fully masked organic compound (**19**). Compound (**19**) on acid hydrolysis furnished the required ligand PSMA-11, in purity >99.5%, post HPLC purification. The structural veracity of the synthesized ligand was compared with commercial PSMA-11 ligand and was found to be equivalent. ^{68}Ga labelling studies conducted at BRIT, Vashi further proved the authenticity of the in-house synthesized PSMA-11. The PET based PSMA tracer, [^{68}Ga]Ga-PSMA-11, made from indigenous PSMA-11, is currently an RPC-approved commercial product of BRIT, Vashi [19].

There is long and successful history for the use of SPECT in diagnostic applications. This single-photon emission based technique is still favoured method among clinicians due to the enormous data available from its practice. Also, SPECT unlike PET is relatively less expensive and hence is popular and easily available in our country too. To date, for prostate cancer diagnosis, no FDA approved SPECT based ligands are globally

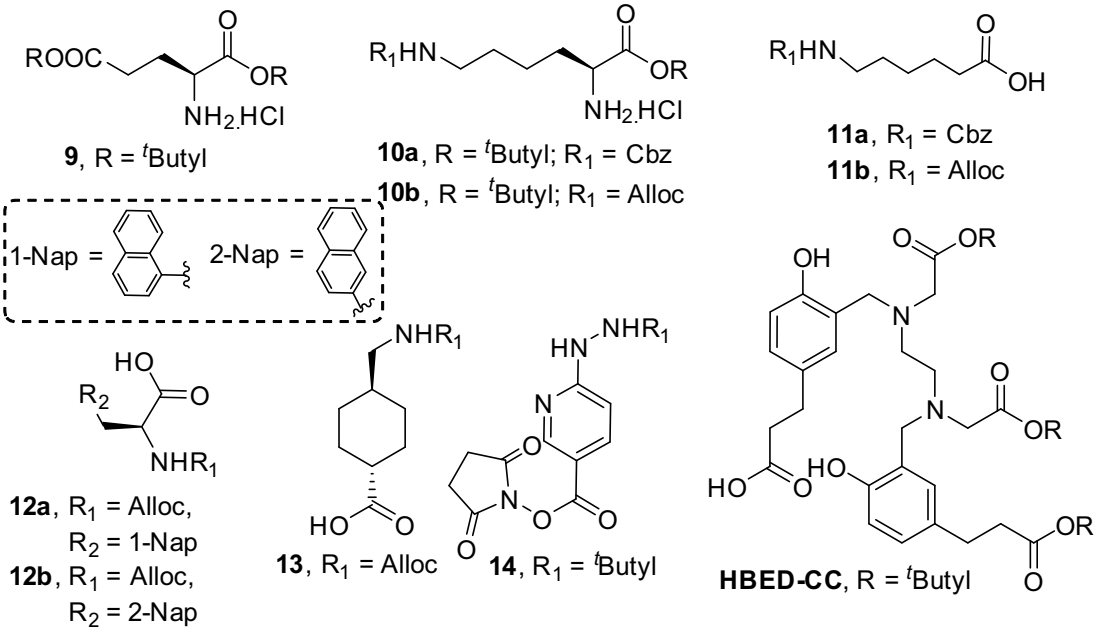
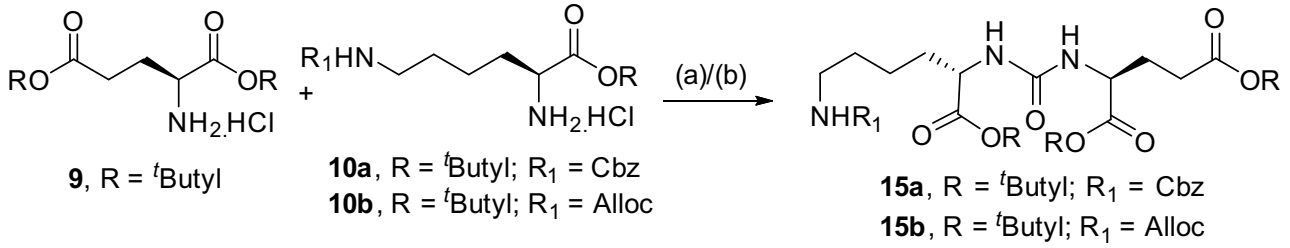
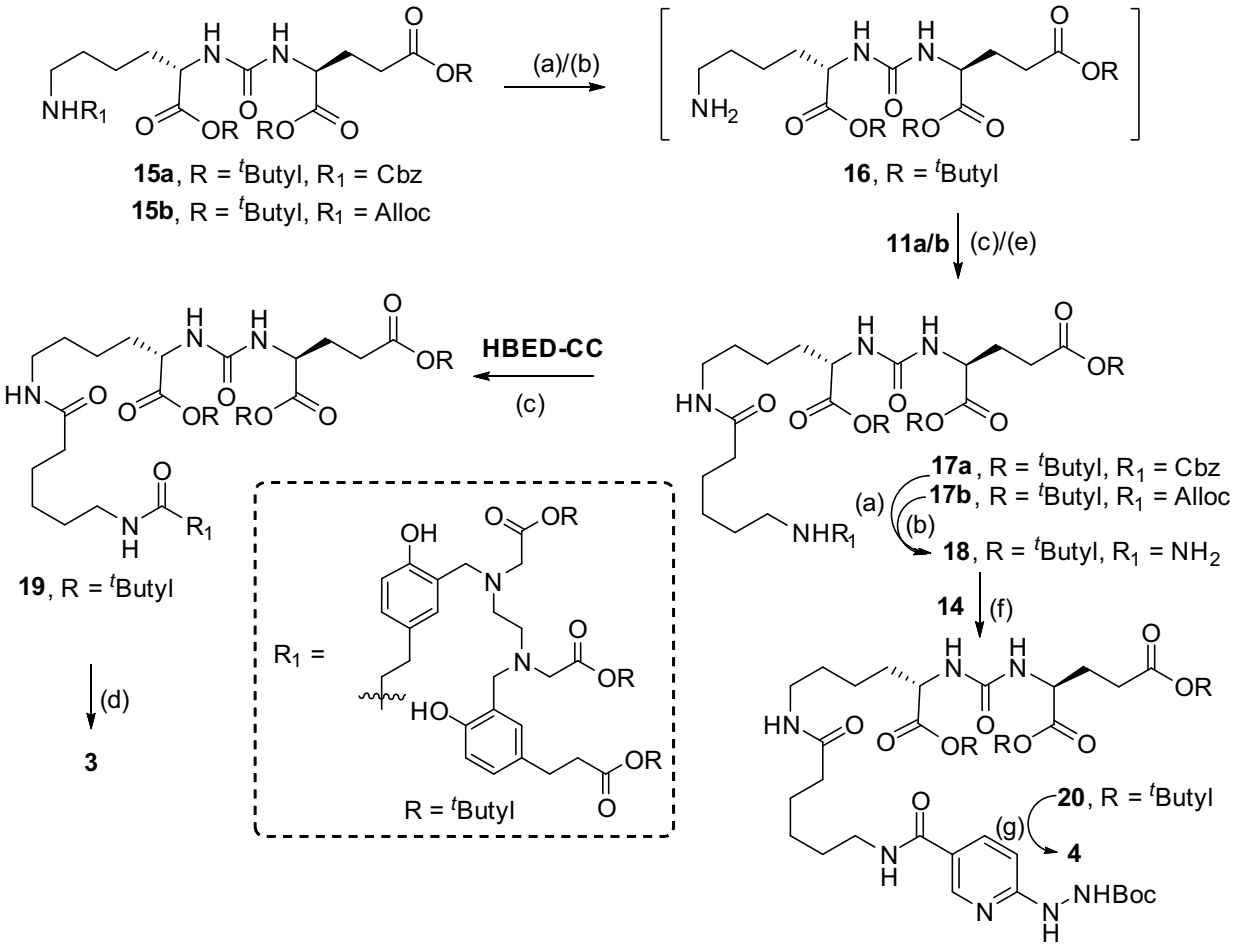


Fig.2: Amino acid constituents/templates for the synthesis of PSMA-11 and HYNIC-PSMA ligands.



*Scheme 1: Synthesis of urea templates (**15a/b**): (a) Disuccinimidyl carbonate, DIEA, CH_2Cl_2; (b) $CO(OCCl_3)_2$, DIEA.*



*Scheme 2: Synthesis of PSMA-11 (**3**) and HY-Hex-I-PSMA (**4**): (a) 10% Pd/C, H_2 (15 Psi), MeOH; (b) $Pd(PPh_3)_4$, Phenyl silane, CH_2Cl_2, 25°C; (c) DCC, DMF; (d) TFA-H_2O-PhSH; (e) HBTU, DIEA, DMF; (f) DIEA, DMF; (g) TFA-H_2O-TIS.*

available, even though extensive research efforts are in place, worldwide, to achieve the same [20-25]. ^{99m}Tc being an important γ-emitting SPECT radionuclide, due to its easy availability through ^{99}Mo/^{99m}Tc generators [26], favourable physical characteristics (γ = 141 keV; 89% abundance), half-life ($T_{1/2}$ = 6 h), and since various chelator based chemistries are available to complex ^{99m}Tc there is major interest in developing ^{99m}Tc labelled organic ligands for imaging applications. Among various chelators, HYNIC being one of the popular and established bifunctional chelator we pursued the synthesis of HYNIC based PSMA ligands in an attempt to find an appropriate API for SPECT based prostate cancer diagnostic agent. With the above perspective we pursued the synthesis of five 2-HYNIC conjugated PSMA targeting ligands, (**4**), (**5**), (**6**), (**7**), and (**8**) as shown in Fig.1. Among them ligand (**4**), is the 2-HYNIC variant of PSMA-11, and can be achieved directly from the amine (**18**) by reacting it with the succinimidyl derivative of *N*Boc-HYNIC (**14**). Alternatively, amine (**18**) can also be accessed through Alloc strategy, as shown in scheme 2, which we have exercised to access all the targeted 2-HYNIC conjugated PSMA ligands. The urea template (**15b**) was achieved by the reaction of glutamic acid derivative (**9**) and lysine hydrochloride derivative (**10b**) (Scheme 1) with triphosgene as the conjugating reagent in the presence of an organic base. Deallylation in (**15b**) was carried out using $Pd(PPh_3)_4$ to achieve amino compound (**16**) which was coupled with alloc protected ω-caproic acid (**11b**) to furnish (**17b**) (Scheme 2). Deallylation of (**17b**) using aforementioned

palladium catalyst yielded amine (**18**). The reaction of (**18**) with HYNIC reagent (**14**) generated the HYNIC conjugated fully masked adduct (**20**). Acid hydrolysis of triester (**20**) followed by purification using HPLC yielded HY-Hex-I-PSMA (**4**) in desired purity (>99.5%) [27].

For the synthesis of ligands I-1N-PSMA (**5**) and I-PSMA (**6**), the amine (**16**) obtained after the deprotection of alloc group in (**15b**) was subjected to coupling with alloc protected amino acids (**12a/b**) to furnish adducts (**21a/b**) (Scheme 3). In the ensuing step, $Pd(PPh_3)_4$ catalyzed alloc deprotection in (**21a/b**) followed by conjugation with HYNIC reagent (**14**) under basic condition furnished fully protected compounds (**22a/b**). Acid hydrolysis of (**22a/b**) in the presence of cation scavenger and subsequent HPLC purification afforded (**5**) and (**6**), respectively [27], in the required purity. Ligands (**5**) and (**6**) were characterized using analytical techniques like spectroscopy ($^{1}H/^{13}C$ NMR, IR), HPLC and MS to ascertain their molecular structures [27].

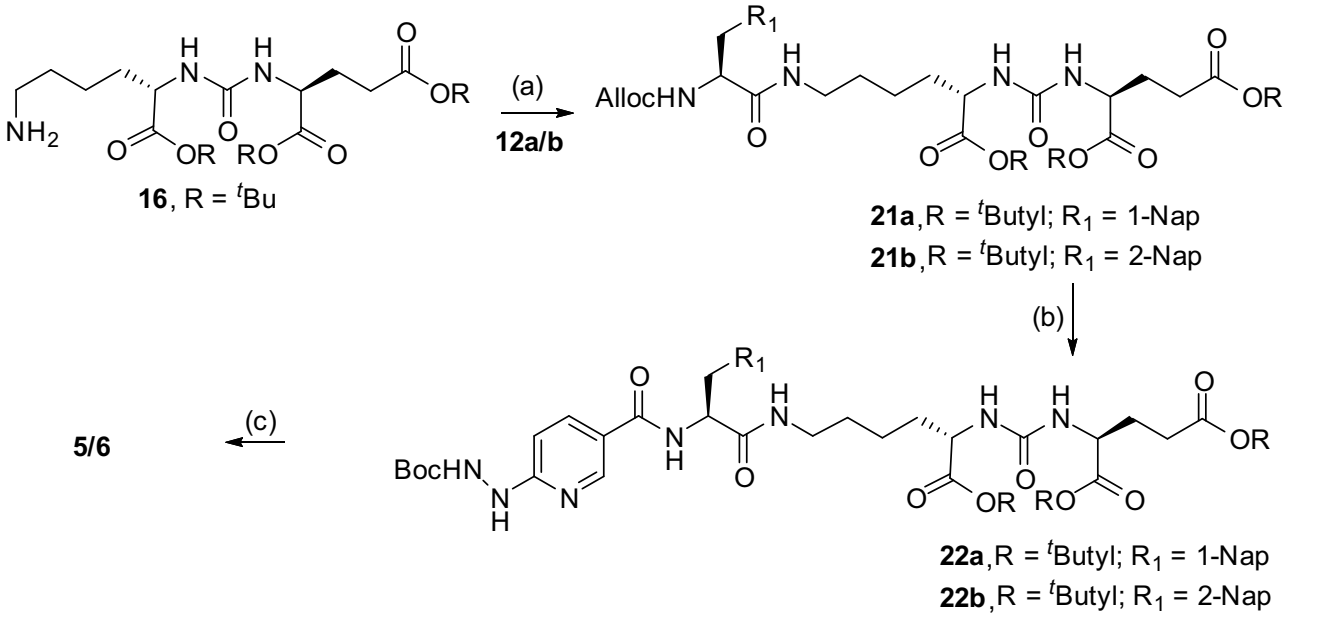
For the synthesis of remaining HYNIC-PSMA analogues, HY-1*N*-I-PSMA (**7**) and HY-2*N*-I-PSMA (**8**), the intermediate compounds (**21a/b**) prepared as per scheme 3 was used as the precursors. Following the demasking of alloc group in (**21a/b**), the amines furnished were coupled with alloc protected tranexamic acid (**13**) to yield corresponding compounds (**23a/b**) (Scheme 4). Deprotection of amino group in (**23a/b**), using the reaction condition mentioned before, followed by ligation with HYNIC reagent (**14**) yielded protected ligands (**24a/b**). Global removal of protecting groups in (**24a/b**) under acidic conditions furnished a crude product that on HPLC purification yielded (**7**) and (**8**), respectively, as foamy white solids. The structural integrity and the purity of the isolated product was confirmed by NMR, HPLC and LCMS analysis [27].

Subsequent to the synthesis of five important 2-HYNIC conjugated PSMA ligands, all the ligands were subjected to labelling studies using SPECT radionuclide ^{99m}Tc, obtained from $^{99}Mo/^{99m}Tc$ generator, at Radiopharmaceuticals Division (RPhD), BARC. The preliminary labelling studies were encouraging, with two among the five ligands exhibited labelling efficiency greater than 90% sufficient for direct human applications and the other three analogues exhibited >70% labelling efficiency, evoking the requirement of further optimization of labelling conditions [27]. Currently, the synthesized HYNIC-PSMA ligands are in different stages of limited clinical evaluation.

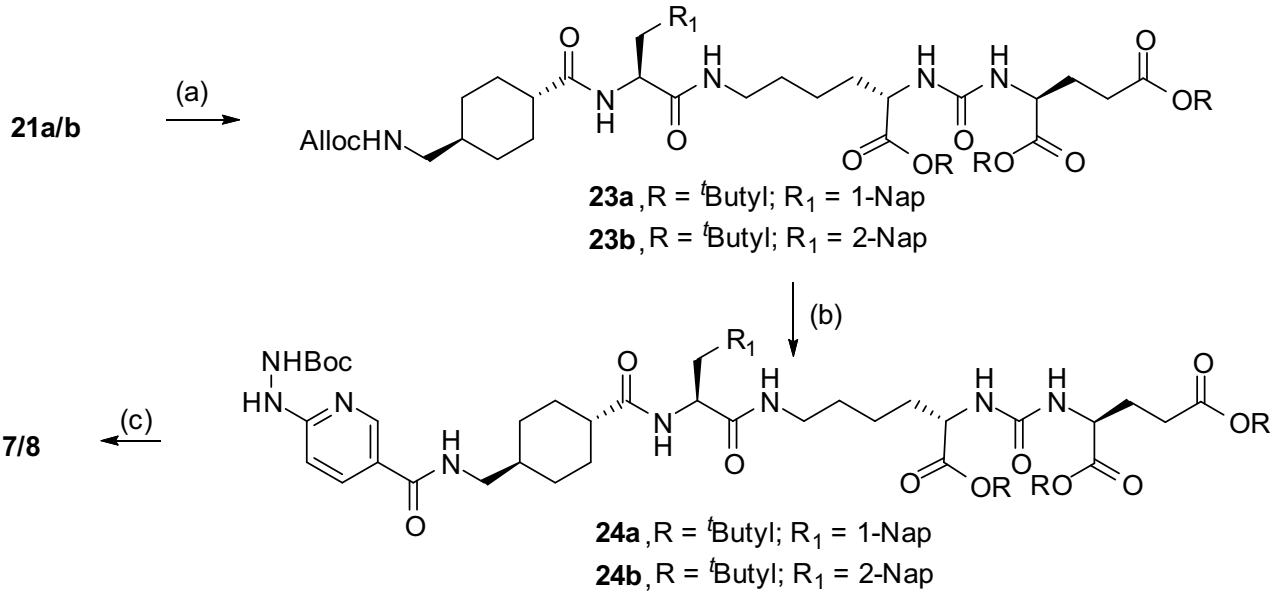
Overall, apart from the pursuit for new ligands for diagnostic applications, through a fruitful collaborative research program with BRIT (Vashi), RMC (Radiation Medicine Centre) and TMH (Tata Memorial Hospital) we were successful in the indigenization of the ligand, PSMA-11, for the preparation of PET based prostate cancer diagnostic agent [^{68}Ga]Ga-PSMA-11. These developments are triggered by the success that we savoured with the fully indigenously developed [^{177}Lu]Lu-PSMA-617, by BOD, BRIT (Vashi), RPhD, RMC, and TMH [28], by virtue of which more than 3000 patients were benefitted through nuclear medicine centres established across India.

Conclusions

Solution phase based Cbz-strategy was found to be an economical method for the synthesis of PET based prostate specific membrane targeting ligand, PSMA-11. Radiolabelling studies of the in-house synthesized PSMA-11 (**2**), carried out at BRIT (Vashi), afforded corresponding labelled products in purity >98% appropriate for direct human applications. Clinical studies conducted at TMH (Parel), using the nuclear medicine, [^{68}Ga]Ga-PSMA-11, made from indigenous PSMA-11 ligand, showed results comparable to the commercial equivalents and is currently an RPC approved product. Through the in-house developed synthetic strategy, we are capable of synthesizing the valuable import substitute, PSMA-11, in sufficient



*Scheme 3: Synthesis of I-1N-PSMA (**5**) and I-PSMA (**6**): (a) HBTU, DIEA, DMF, 0°C to RT; (b) i) $Pd(PPh_3)_4$, Phenyl silane, CH_2Cl_2, 25°C; ii) **14**, DMF, DIEA, 25°C; (c) TFA-H_2O-TIS, 25°C.*



*Scheme 4: Synthesis of HY-1N-I-PSMA (**7**) and HY-2N-I-PSMA (**8**): (a) i) $Pd(PPh_3)_4$, Phenyl silane, CH_2Cl_2, 25°C; ii) HBTU, **13**, DMF, 0°C to RT; (b) i) $Pd(PPh_3)_4$, Phenyl silane, CH_2Cl_2, 25°C; ii) **14**, DIEA, DMF, 25°C; (c) TFA-H_2O-TIS, 25°C.*

quantities with purity >99.5%. In an effort to make SPECT based prostate cancer diagnostic agents we have achieved the synthesis of five 2-HYNIC conjugated-PSMA ligands namely; HY-1N-I-PSMA (**4**), HY-2N-I-PSMA (**5**), I-PSMA (**6**), I-1N-PSMA (**7**), HY-Hex-I-PSMA (**8**), using a solution phase based Alloc-chemistry. Spectroscopic techniques, HPLC, and MS were utilized to establish the structure of the synthesized ligands. Labelling studies using ^{99m}Tc with the synthesized ligands, conducted at RPhD, gave satisfactory results and the fully indigenous [^{99m}Tc]Tc-HYNIC-PSMA tracers are in different phases of clinical evaluation.

Acknowledgments

KSAK is extremely thankful to Prof. B. S Patro, Head, Bio-Organic Division, Prof. P. A. Hassan, Associate Director, Bio-Science Group (BSG) and all the former Group Directors of BSG, particularly, Prof. S. K Nayak, Prof. V. P Venugopalan, Prof. S. K. Ghosh, for their guidance and constant support towards the program. KSAK enthusiastically acknowledge, Prof. Venkatesh Rangarajan (TMH, Parel) for providing clinical data of the studies. KSAK sincerely thank Prof. Sharmila Banerjee (RPhD), Prof. Tapas Das (RPhD), Dr. Usha Pandey (BRIT, Vashi), and Prof. Sandip Basu (RMC, Parel) for their help and support. KSAK is particularly thankful to collaborators, Dr. Madhava B. Mallia (RPhD) and Dr. Anupam Mathur (BRIT, Vashi), for their gusto towards the program. KSAK thankfully acknowledge security personnel of Mod lab for their support.

References

[1] Wang, et. al, (2022). Prostate Cancer Incidence and Mortality: Global Status and Temporal Trends in 89 Countries From 2000 to 2019. *Front. Public Health*, 10, 811044.doi: 10.3389/fpubh.2022.811044.

[2] Lu, et.al, (2023). Molecules, Synthesis and Evaluation of 99mTc-Labeled PSMA-Targeted Tracers Based on the Lys-Urea-Aad Pharmacophore for Detecting Prostate Cancer with Single Photon Emission Computed Tomography. *Molecules*, 28, 5120.doi: 10.3390/molecules28135120.

[3] Ferlay, et. al. (2013). Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur. J. Cancer*. 49,1374-1403. doi.org/10.1016/S0140-6736(24)00651-2.

[4] James, et. al. (2024). The Lancet Commission on prostate cancer: planning for the surge in cases. The Lancet Commissions, 403, 1683-1722. doi:10.1016/j.ejca.2012.12.027.

[5] Siegel, et. al. (2015) Cancer statistics 2015, CA *Cancer J. Clin*. 65, 5-29. doi:10.3322/caac.21254

[6] https://www.apollohospitals.com/apollo-in-the-news/on-world-health-day-apollo-hospitals-has-unveiled-the-4th-edition-of-the-health-of-nation-report, (accessed Sep 17, 2025).

[7] Dhillon, et. al. (2018) India State-Level Disease Burden Initiative Cancer Collaborators. The burden of cancers and their variations across the states of India: the Global Burden of Disease Study 1990-2016. *Lancet Oncol*. 19, 1289-1306. doi:10.1016/S1470-2045(18)30447-9

[8] Sharma, et. al. (2024). Temporal patterns of cancer burden in Asia, 1990–2019: a systematic examination for the Global Burden of Disease 2019 study, *The Lancet Regional Health - Southeast Asia*, 100333. doi:10.1016/j.lansea.2023.100333

[9] Hövels, et. al. (2008). The diagnostic accuracy of CT and MRI in the staging of pelvic lymph nodes in patients with prostate cancer: a meta-analysis. *Clin. Radiol*. 63, 387-395. doi: 10.1016/j.crad.2007.05.022

[10] Clarke, et. al. (2010). Markers for detection of prostate cancer. *Cancers*, 2, 1125. doi: 10.3390/cancers2021125

[11] Maffioli, (2015). Reviews diagnostic And Therapeutic

Management Of Locally Advanced And Advanced Prostate Cancer. Q. *J. Nucl. Med. Mol. Imaging*, 59, 420-438. https://www.minervamedica.it/en/getpdf/WWRvM0htbVI3NGZTeHR1SE1WQlMyNGdycGJrSGp2cEtQaGlITDBUaTk5S3FoRHIrcWsxdkNzK2Z1RHo5M0pUNg%253D%253D/R39Y2015N04A0420.pdf

[12] Santoni, et. al. (2014). Targeting prostate-specific membrane antigen for personalized therapies in prostate cancer: morphologic and molecular backgrounds and future promises. *J. Biol. Regul. Homeost. Agents*, 28, 555-563. PMID: 25620167

[13] Theiss, et. al. (1995). Prognostic significance of capsular invasion and capsular penetration in patients with clinically localized prostate cancer undergoing radical prostatectomy. *Prostate*, 27, 13–17. doi: 10.1002/pros.2990270104

[14] Kumar, & Mathur. (2021). Total chemical synthesis of PSMA-11: API for 68Ga-PSMA-11 used for prostate cancer diagnosis. *Eur. J. Med. Chem. Rep.* 3, 100014. https://doi.org/10.1002/pros.2990270104

[15] Kumar, & Mathur. (2024). Challenges in the solution phase synthesis of PSMA-11 and PSMA-617: Organic ligands for radiopharmaceutical preparations in prostate cancer medication. *Radiochim. Acta*, 112, 651-662. https://doi.org/10.1515/ract-2024-0280

[16] Kumar, & Mathur. (2024). A total chemical synthesis of PSMA-617: A ligand for prostate cancer endotherapeutic applications. *Radiochim. Acta*, 112, 553-563. https://doi.org/10.1515/ract-2023-0205

[17] Kumar, & Mathur. (2024). A convenient total synthesis of PSMA-617: A prostate specific membrane antigen (PSMA) ligand for prostate cancer endotherapeutic applications. *Eur. J. Med. Chem. Rep*. 6, 100084. https://doi.org/10.1016/j.ejmcr.2022.100084

[18] Kumar, et. al. (2020). BARC Newsletter, November-December-2020.

[19] Hennrich & Eder. (2021). [68Ga]Ga-PSMA-11: The First FDA-Approved 68Ga-Radiopharmaceutical for PET Imaging of Prostate Cancer. *Pharmaceuticals*, 14, 713. doi: 10.3390/ph14080713

[20] Kumar, et. al. (2023). *In-house synthesized* PSMA-11 as an *API for manufacture of 68Ga-PSMA-11 radiopharmaceutical for PET imaging of prostate cancer*, May.

[21] Hillier, et.al. (2010). 99mTc-MIP-1340, a small molecule inhibitor of PSMA for molecular imaging of prostate cancer. *J. Nucl. Med*. 51, 481. https://jnm.snmjournals.org/content/51/supplement_2/481

[22] Robu, et. al. (2017) Preclinical Evaluation and First Patient Application of 99mTc-PSMA-I&S for SPECT Imaging and Radioguided Surgery in Prostate Cancer. *J. Nucl. Med*. 58, 235-242. doi: https://doi.org/10.2967/jnumed.116.178939

[23] Rathke, et. al. (2018). Intraindividual Comparison of 99mTc-Methylene Diphosphonate and Prostate-Specific Membrane Antigen Ligand 99mTc-MIP-1427 in Patients with Osseous Metastasized Prostate Cancer. *J. Nucl. Med*. 59, 1373-1379. doi: 10.2967/jnumed.117.200220

[24] Papagiannopoulou, (2020). Handbook of Radiopharmaceuticals: Methodology and Applications, M. R. Kilbourn and P. J. H. Scott, Willey, pp. 375-433.

[25] Urbán, et. al. (2021). Radiation Dosimetry of 99mTc-PSMA I&S: A Single-Center Prospective Study. *J. Nucl. Med*. 62, 1075-1081. doi: 10.2967/jnumed.120.253476

[26] Maurin, et. al. (2022). [99mTc]Tc-PSMA-T4—Novel SPECT Tracer for Metastatic PCa: From Bench to Clinic. Molecules, 27, 7216. https://doi.org/10.3390/molecules27217216

[27] Hasan, & Prelas. (2020). Molybdenum-99 production pathways and the sorbents for 99Mo/99mTc generator systems using (n, γ) 99Mo: a review. SN Appl. Sci. 2, 1782. https://doi.org/10.1007/s42452-020-03524-1

[28] Kumar, & Mallia. (2025). Synthesis and radiolabelling studies of hynic conjugated PSMA targeting ligands. *Radiochim. Acta*, 113, 621-636. https://doi.org/10.1515/ract-2025-0038.

[29] Kumar, et. al. (2019). *^{177}Lu-PSMA-617 'Ready to use' Injectable Formulation*, September.