Solid-Phase Organic Synthesis (SPOS) of Modulators of Estrogenic and Androgenic Action

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Abstract: Estrogens and androgens are key growing factors involved in a large series of disorders. Two main strategies are possible for controlling their undesirable agonist effects: (1) blocking their biosynthesis by using selective enzyme inhibitors, and (2) antagonizing their hormonal action on a receptor with an antiestrogen or an antiandrogen. In this review, we will briefly discuss the identification of a series of important therapeutic targets, through the study of steroidogenesis of potent estrogens, estrone and estradiol, and potent androgens, testosterone and dihydrotestosterone, as well as of their nuclear receptors. We will next review the solid-phase synthesis of steroidogenic enzyme (steroid sulfatase and 17β-hydroxysteroid dehydrogenases) inhibitors and steroid (estrogen and androgen) receptor modulators, all being potential therapeutic agents for the treatment of hormone-sensitive diseases.

INTRODUCTION

Steroidal compounds are essential modulators of a large series of important biological functions [1]. The different families of steroidal hormones, such as progestins, mineralocorticoids, glucocorticoids, androgens, and estrogens, all originate from the same precursor that is cholesterol (Fig. (**1**)). The biosynthesis of steroidal hormones is a multi-step process named *steroidogenesis*, which consists in successive transformations involving different enzymes [2]. These steroidogenic enzymes are thus responsible for the synthesis of each steroidal hormone in specific secretory organs (endocrine production), but it is now accepted that peripheral tissues contain all necessary enzymes for the intracellular local synthesis of active steroids (intracrine production) [3-5]. Since these hormones will activate the steroid receptors or will decrease by competition the antagonistic effect of an antihormone, it is very important to take into consideration both the formation and the action of steroid hormones.

Among the families of steroidal hormones, estrogenic and androgenic sexual steroids are both very important being involved in several diseases [5-10]. The most potent estrogen, E_2, controls differentiation, growth and function of female reproductive tissues and is involved in the growth of estrogen-sensitive breast and endometrial cancers. Similarly, DHT and T, the most potent androgens, are responsible for male characters and are involved in the growth of androgen-sensitive prostate cancer, as well as in acne, alopecia, and hirsutism. Sexual hormones act *via* specific nuclear receptors, such as estrogen and androgen receptors, and they are known to respectively play key roles in the regulation of the growth of human breast and prostate cancers. Consequently, a therapy based on an antiestrogen [11-18] or an antiandrogen [19-22], a receptor antagonist able to block the action of estrogens or androgens by competing with the natural receptor ligand, is a logical approach for the treatment of such cancers. Furthermore, the inhibition of targeted steroidogenic enzymes is a strategy for reducing the level of active steroids (E_1, E_2, T, and DHT), thus decreasing their mitogenic effects. Enzymes involved in the steroidogenesis of estrogens and androgens (especially *C*, *E1*, *E3*, *E5*, *E7*, *G*, *H1*, and *H2*) as well as hormone receptors (ER and AR) constitute very suitable therapeutic targets (Fig. (**2**)). Although some of these targets have been known for a long time and medicinal chemists have synthesized potent receptor antagonists and enzyme inhibitors by classical chemistry, the contribution of solid-phase organic synthesis (SPOS) appeared only recently in the literature. The present article will review the SPOS of steroidogenic enzyme inhibitors, receptor agonists and receptor antagonists, all modulators of estrogen and androgen actions.

STEROID SULFATASE

Sulfotransferase and steroid sulfatase are respectively responsible for the sulfatation and desulfatation of steroids. The former enzyme catalyzes the transfer of a sulfuryl group from 3'-phosphoadenosine to a hydroxyl group on a steroid. Since sulfated steroids are neither ligands of steroid receptors nor active hormones, there is no point in inhibiting sulfotransferase during treatment of estrogen or androgen-sensitive cancers. On the other hand, the inhibition of steroid sulfatase is desirable as it hydrolyses sulfated steroids such as DHEAS and E_1S into unconjugated DHEA and E_1, respectively; it thus controls the first step in hormone synthesis at the cellular level (Fig. (**2**)). Furthermore DHEAS is the major sulfated steroid found in human blood circulation [3], and plasmatic concentration of E_1S is relatively high, being a major source of active estrogens in mammary tumors of menopaused women [23]. Since DHEAS and E_1S are two important sources of estrogens and androgens, the inhibition of steroid sulfatase will reduce the amounts of substrates for the enzymes involved in the final steps of sex steroid production.

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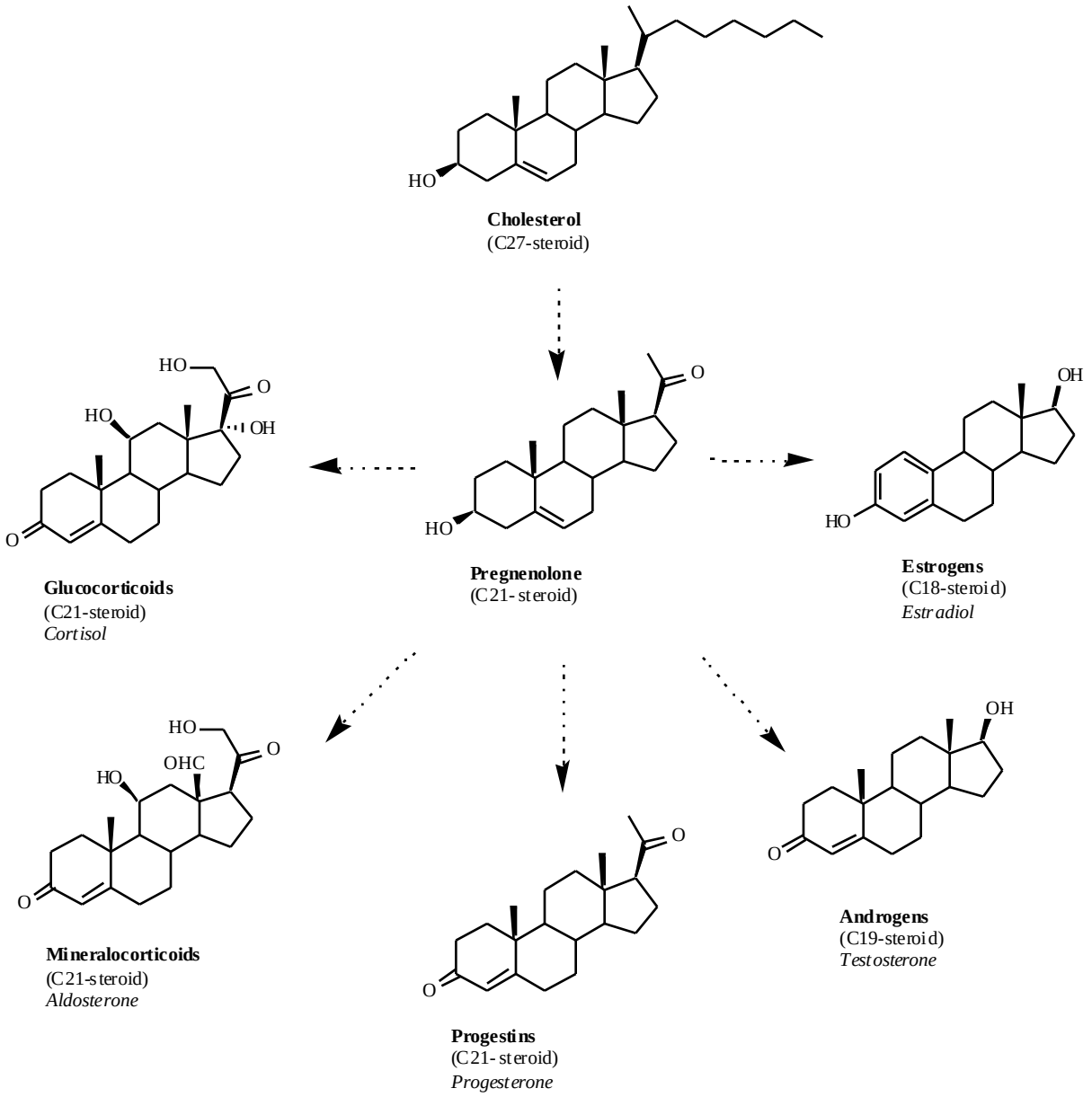

Fig. (1). Representative members (italic) of each family (bold) of steroidal hormones synthesized from cholesterol.

Inhibitors of steroid sulfatase are thus valuable therapeutic agents for estrogen and androgen-sensitive diseases.

In the last fifteen years, many research groups became interested in developing steroid sulfatase inhibitors [24-30] and successfully synthesized compounds containing two crucial elements, an arylsulfamate moiety inactivating the enzyme [31] and a hydrophobic substituent in the proximity of the steroidal D-ring for the reversible nature of the interaction [32]. Considering the great importance of the arylsulfamate group, a new multidetachable linker was developed allowing the SPOS of both sulfamate and phenol derivatives, depending on cleavage conditions [33,34].

This sulfamate linker was used for the parallel synthesis of two model libraries of *N*-derivatives 17α-piperazinomethyl-E_2 as sulfamates and phenols (Fig. (**3**)) [35]. The key precursor **1** was first synthesized in solution, loaded on trityl chloride resin to afford **3**, and sequential acylation reactions with five Fmoc protected amino acids and five carboxylic acids were performed in order to introduce two levels of molecular diversity. Finally, the resins **5** were split in two parts, one undergoing acidic cleavage (5% trifluoroacetic acid in dichloromethane) and the other, nucleophilic cleavage (piperazine in tetrahydrofuran). These two libraries of compounds **6** (5 x 5 sulfamates) and **7** (5 x 5 phenols) were generated in overall yields of 18-66% and high purities (87-96%), without purification steps. A preliminary screening test for inhibition of steroid sulfatase showed that the phenols were clearly weaker inhibitors, compared to their sulfamate analogs. The sulfamate inhibitors bearing a suitable hydrophobic amino acid and a carboxylic acid as elements of molecular diversity were as potent as the leading inhibitor 17α-*tert*-butylbenzyl-E_2 3-*O*-sulfamate previously reported in the literature.

The sulfamate linker and the same sequence of reactions were also used for generating two libraries of 48 (8 x 6) 16β-

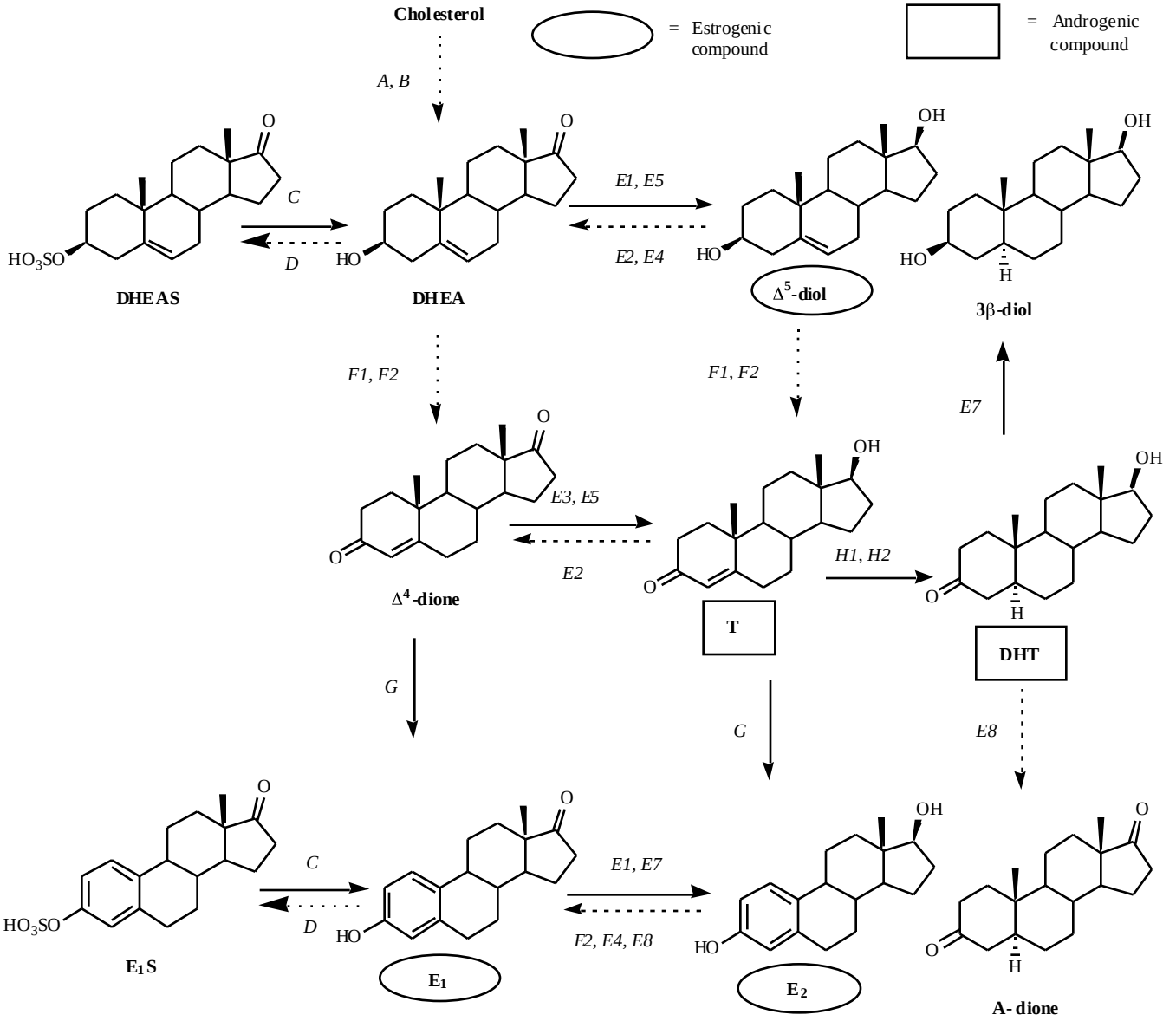

Fig. (2). The formation of estrogens and androgens from cholesterol (steroidogenesis). Good therapeutic targets for potential treatment of estrogen- and androgen-sensitive diseases are represented by a solid arrow (—›). Enzymes are *A*: P450 side-chain cleavage; *B*: P450 17α-hydroxylase/P450 17,20-lyase; *C*: steroid sulfatase; *D*: sulfotransferase; *E*: 17β-hydroxysteroid dehydrogenases (types 1-8); *F*: 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerases (types 1, 2); *G*: aromatase; *H*: 5α-reductases (types 1, 2). Steroids are *DHEA*: dehydroepiandrosterone; *DHEAS*: dehydroepiandrosterone sulfate; *DHT*: dihydrotestosterone; *3β-diol*: 5α-androstane-3β,17β-diol; *Δ^5-diol*: 5-androstene-3β,17β-diol; *Δ^4-dione*: 4-androstene-3,17-dione; *A-dione*: 5α-androstane-3,17-dione: E_1: estrone; E_1S: estrone sulfate; E_2: estradiol; *T*: testosterone.

derivatives of E_2, sulfamoylated or not (Fig. (**4**)) [36,37]. The building blocks (amino acids and carboxylic acids) were selected according to their potential to contribute to interactions with an important hydrophobic area. Libraries of compounds **12** and **13** were obtained in average crude overall yields of 40% and 32% for the solid-phase reactions while the average purities were 91% for sulfamates and 93% for phenols. These results were found satisfactory since no chromatographic purification was required after the final cleavage with hexafluoro-2-propanol or piperazine. A preliminary screening identified 17 compounds with better steroid sulfatase inhibitory potency than the reference inhibitor estrone-3-*O*-sulfamate.

Using a tyramine nucleus and the sulfamate linker, two libraries of 25 sulfamates and 25 phenols were generated by SPOS as potential non-steroidal inhibitors of steroid sulfatase (Fig. (**5**)) [34]. The *N*-Fmoc-tyramine-*O*-sulfamate (**14**) was first added to a trityl chloride resin and two levels of molecular diversity (amino acids and carboxylic acids) introduced onto **15** before the final dual (acid and nucleophile) cleavages. Yields (49-97%) and purities (38-68% and 39-82% for sulfamates **18** and phenols **19**, respectively) were found acceptable for screening assays, but biological results were not yet published. Since the multidetachable sulfamate linker proved to be very useful for the synthesis of sulfamoylated and phenolic inhibitors of the

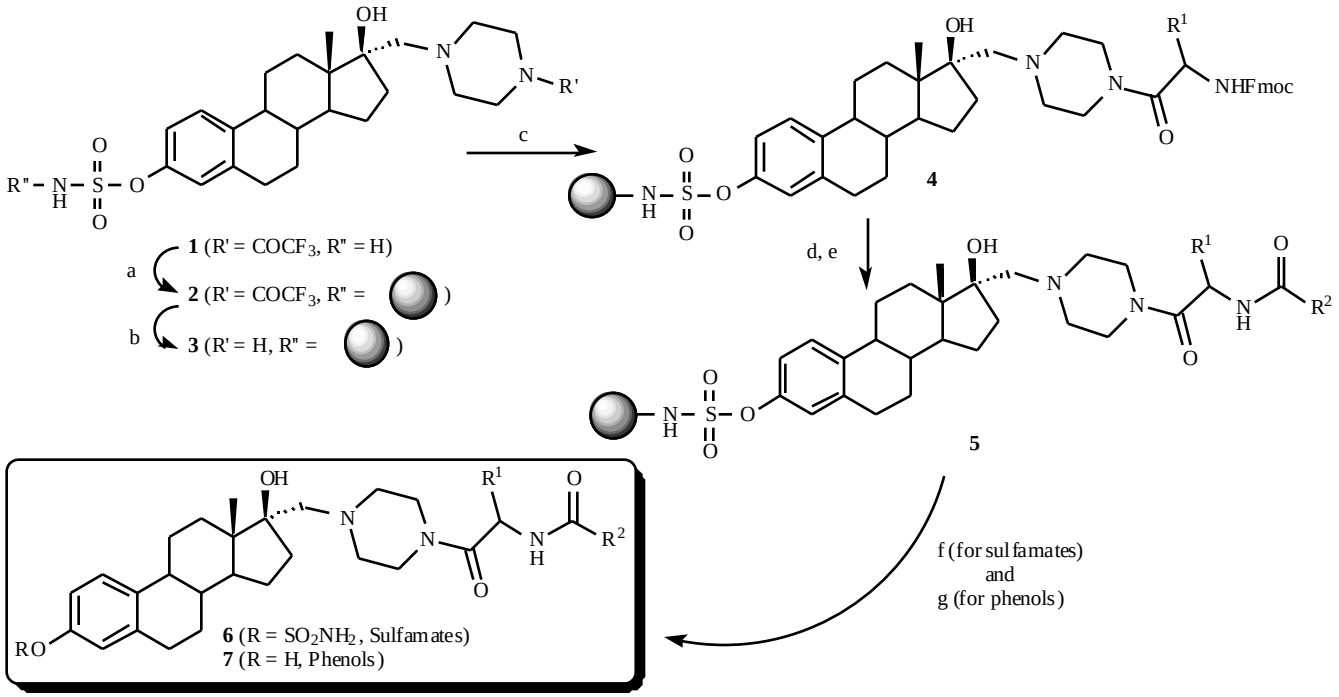

Fig. (3). SPOS of *N*-derivatives of 17α-piperazinomethyl-E_2 as sulfamates **6** and phenols **7**. (**a**) Polystyrene trityl chloride resin, DIPEA, CH_2Cl_2, rt; (**b**) 3N NaOH, THF, rt; (**c**) PyBroP, HOBt, $R^1CH(NHFmoc)COOH$, DIPEA, DMF, rt; (**d**) 20% piperidine in CH_2Cl_2, rt; (**e**) PyBOP, R^2COOH, DIPEA, DMF, rt; (**f**) 5% TFA in CH_2Cl_2, rt; (**g**) piperazine, THF, 45-50 ºC.

steroid sulfatase, the same strategy could easily be extended to other therapeutic targets.

17β-HYDROXYSTEROID DEHYDROGENASES

17β-Hydroxysteroid dehydrogenases (17β-HSDs) catalyze the reduction of 17-ketosteroids or the oxidation of 17β-hydroxysteroids, with nicotinamide adenine dinucleotide (phosphate), reduced or not, as cofactor [38-40]. As it is thus involved in the last step of the biosynthesis of sex steroids from cholesterol, the 17β-HSD family constitutes a very suitable target for controlling the concentration of E_2, Δ^5-diol, T, or DHT (Fig. (**2**)). The enzymatic activities associated with the different isoforms are widespread in

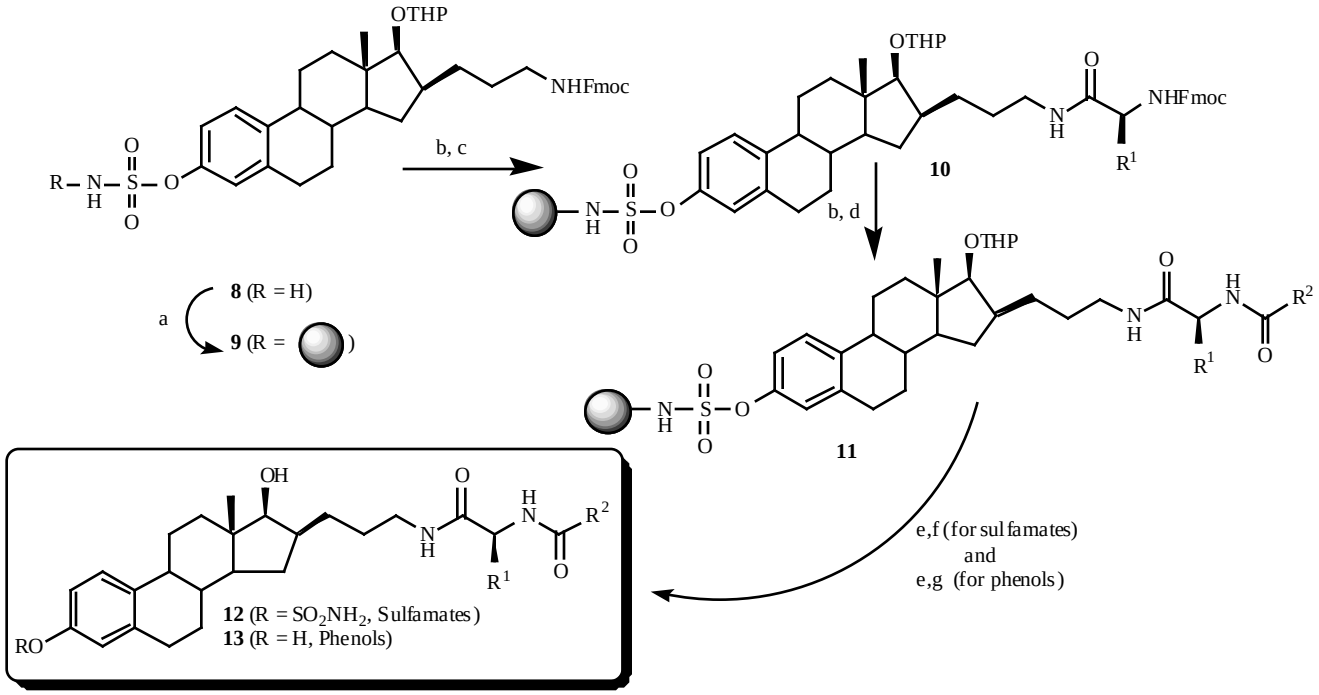

Fig. (4). SPOS of *N*-derivatives of 16β-aminopropyl-E_2 as sulfamates **12** and phenols **13**. (**a**) Polystyrene trityl chloride resin, DIPEA, CH_2Cl_2, rt; (**b**) 20% piperidine in CH_2Cl_2, rt; (**c**) PyBOP, HOBt, $R^1CH(NHFmoc)COOH$, DIPEA, DMF, rt; (**d**) PyBroP, HOBt, R^2COOH, DIPEA, DMF, rt; (**e**) 0.07M *p*-TSA in 1-butanol:$ClCH_2CH_2Cl$ (1:1), rt; (**f**) 30% HFIP in CH_2Cl_2, rt; (**g**) piperazine, THF, 45–50 ºC.

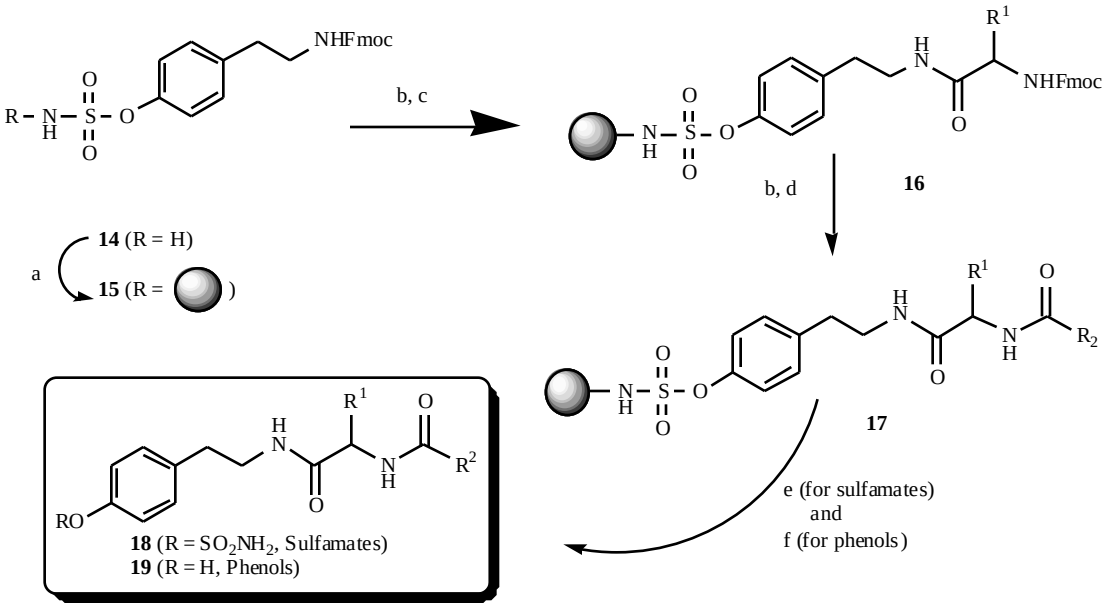

Fig. (5). SPOS synthesis *N*-derivatives of tyramine as sulfamates **18** and phenols **19**. (**a**) Polystyrene trityl chloride resin, DIPEA, CH_2Cl_2, rt; (**b**) 20% piperidine in CH_2Cl_2, rt; (**c**) PyBOP, HOBt, $R^1CH(NHFmoc)COOH$, DIPEA, DMF, rt; (**d**) PyBroP, HOBt, R^2COOH, DIPEA, DMF, rt; (**e**) 30% HFIP in CH_2Cl_2, rt; (**f**) 30% DEA in THF, rt.

human tissues, not only in classic steroidogenic tissues, such as the testis, ovary, and placenta, but also in a large series of peripheral intracrine tissues. In the nineties, several new types of 17β-HSD were reported, indicating that a fine regulation is carried out [41-43]. More importantly, each 17β-HSD isoform has a specific tissue distribution and displays a selective substrate affinity, and moreover, in intact cells, its activity is unidirectional (reductive or oxidative) [44,45]. These findings indicate that selectivity of drug action could be achieved by targeting a particular 17β-HSD isoform. Selective inhibitors are useful tools for elucidating the role of these enzymes in specific biological systems or for therapeutic purposes [25,46-49]. In order to block the formation of hydroxysteroids that stimulate sex steroid-sensitive pathologies, the reductive 17β-HSDs (types 1, 3, 5, and 7) must be targeted. To date however, SPOS of 17β-HSD inhibitors has only been reported for types 1 and 3.

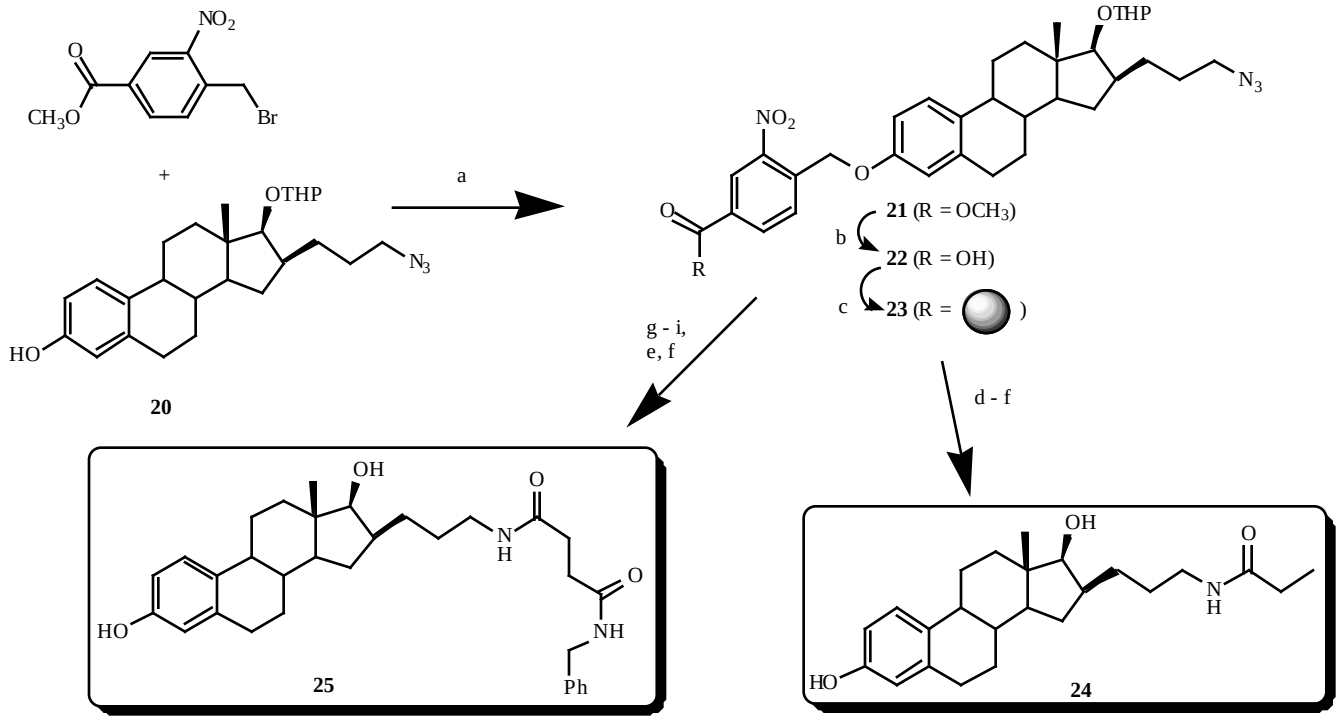

Fig. (6). SPOS of *N*-derivatives of 16β-aminopropyl-E_2 (compounds **24** and **25**). (**a**) Cs_2CO_3, CH_3CN:DMF (4:1), rt; (**b**) LiOH aq, THF, rt; (**c**) Polystyrene aminomethyl resin, DIPC, HOBt, DMF, rt; (**d**) propionic acid, EDC, HOBt, dioxane, Bu_3P in toluene, rt; (**e**) hυ (350 nm), MeOH, rt; (**f**) 2% HCl, MeOH, rt; (**g**) *i*. PPh_3, THF, rt; *ii*. H_2O, 70°C; (**h**) succinic anhydride, pyridine, rt; (**i**) $PhCH_2NH_2$, PyBOP, DIPEA, DMF, rt.

Type 1 17β-HSD

A SPOS approach was developed by Tremblay and Poirier [50,51] for optimizing the inhibitory activity of E_2 derivatives containing a functionalized side-chain at position 16. They first investigated the effectiveness of five different linkers to attach the phenolic function of a 16β-(azidopropyl)-E_2 (**20**) to a polystyrene resin. A short sequence of reactions was performed, including reduction of the azide and acylation of the corresponding amine on the polymer-bound E_2 derivative. Although all the tested linkers (benzylic ether from Merrifield resin, 4-alkoxy-benzylic ethers from Wang or Sheppard resin, tetrahydropyranyl ether, benzoic ester, and *o*-nitrobenzyl ether) proved to be effective in attaching the phenol functionality of the precursor, only the *o*-nitrobenzyl ether photolabile linker enabled the release of the final products in acceptable purities without purification. Consequently, this linker was selected for the preparation of two E_2 derivatives, **24** and **25,** from resin **23** giving acceptable yields (20-31%) and excellent purities (Fig. (**6**)). These two model compounds were however not tested as inhibitors of type 1 17β-HSD, but the combinatorial synthesis of larger libraries is now possible using commercially available building blocks, such as carboxylic acids, anhydrides and amines.

The multidetachable sulfamate linker was also used for the synthesis of another series of E_2 derivatives as inhibitors of type 1 17β-HSD [36]. The strategy was the same as exemplified in Fig. (**4**) for the synthesis of steroid sulfatase inhibitors **12** and **13**, except that a 17β-acetate protecting group (hydrolysed with 0.1M MeONa/MeOH:THF (25:75)) was used instead of a tetrahydropyranyl group. Furthermore, the building blocks used to introduce the second level of diversity, a series of polar carboxylic acids, were selected for potential interaction with the cofactor-binding domain of the enzyme. A model library of 30 phenols (16β-derivatives of E_2) was generated in moderate yields (16-62%) and good purities (85-95%), but preliminary testing showed a weak inhibitory activity on type 1 17β-HSD, suggesting that the selected building block did not efficiently mimic the adenosine component of the lead inhibitor. These compounds were synthesized to mimic a new family of potent E_2-adenosine hybrid inhibitors of 17β-HSDs [52-53], which interacts with both substrate- and cofactor-binding domains of the enzyme. As part of a combinatorial approach, the sulfamate linker was recently used by Potter's group to allow rapid access to a series of 16β-derivatives of 2-ethyl-E_1 as potent inhibitors of type 1 17β-HSD [54].

Type 3 17β-HSD

The synthesis of androsterone (ADT) derivatives **30**-**32**, bearing a 3β-side chain with 0 to 2 amino acid units (1, 2, or 3 levels of diversity) and a carboxylic acid at the end of the sequence, was performed in good yields (23-58%) and high purities (80-96%) using the Leznoff's acetal linker (Fig. (**7**)) [55]. The steroidal ketone **26** was previously linked onto polymer-bound glycerol by an efficient transacetalization

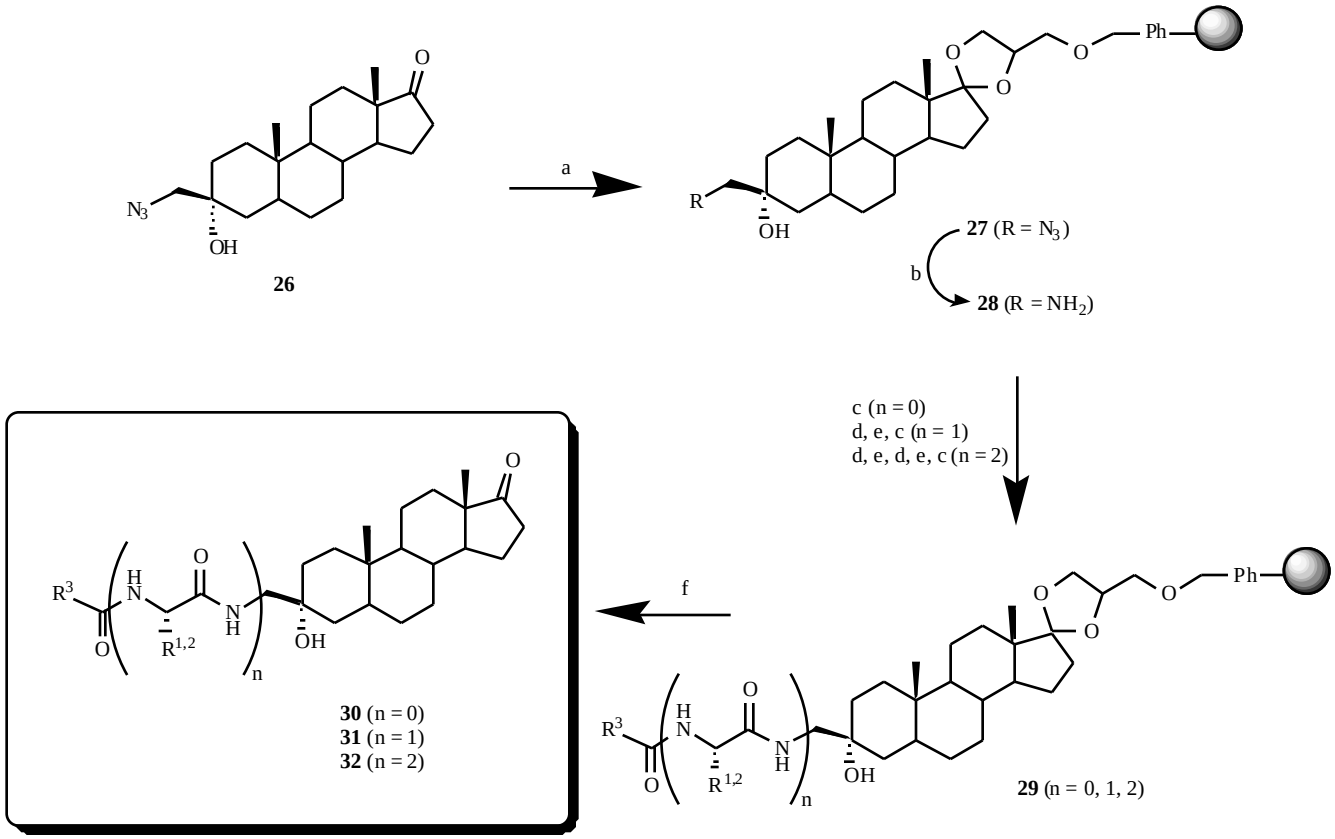

Fig. (7). SPOS of *N*-derivatives of 3β-aminomethyl-ADT (compounds **30**-**32**). (**a**) Polymer-bound glycerol, TMOF, $Sc(OTf)_3$, toluene, rt; (**b**) $SnCl_2$ (0.2M), PhSH (0.8M), TEA (1M); (**c**) R^3COOH, PyBOP, HOBt, DIPEA, DMF, rt; (**d**) $R^{1,2}CH(NHFmoc)COOH$, PyBOP, HOBt, DIPEA, DMF, rt; (**e**) 20% piperidine in CH_2Cl_2, rt; (**f**) 2N HCl in dioxane (containing 1% of H_2O), rt.

reaction [56], and the sequence of reactions was followed by the addition of a selection of hydrophobic amino acids on the aminomethyl group, capping with a carboxylic acid, and the final release of the 3β-ADT derivatives **30**-**32**. Three model libraries (n = 0, 1, and 2) were obtained in a parallel format giving individual compounds bearing hydrophobic diversity groups, an important requirement for a good inhibitor of type 3 17β-HSD. Potent inhibitors were identified, especially six members of the 3-level library having at least one benzyl group. One of them (compound **32**; R^1 = *i*-butyl, R^2 = benzyl, and R^3 = hexyl) inhibited the enzyme with an IC_{50} value of 227 nM.

In another study by Maltais *et al.* [57], the diethylsilyloxy linker was found suitable for efficiently attaching both secondary and primary hydroxysteroids. Hence, the authors successfully generated a model library of 20 (5 x 4) 3β-derivatives of androstane-3α,17β-diol in good yields (49-63%) and purities (95-100%) (Fig. (**8**)). From the steroidal 3α-oxirane **33** coupled onto polystyrene butyldiethylsilane resin, a 2-level library was elaborated by (1) aminolysis of the oxirane **34** using an excess of five alkylamines, (2) acylation of **35** with four acyl chlorides, and (3) cleavage of C19-steroids diversified at position 3β as compounds **36**. The acyl chloride was also successfully replaced by a carboxylic acid, an amino acid or an α-bromo-acetic acid (as peptoid precursor) allowing additional possibilities for diversification (compounds **36I-IV**). These four compounds and the model library were not tested as inhibitors, but future libraries generated by this methodology will assess the relative importance of 17β-OH versus the carbonyl group of previously reported type 3 17β-HSD inhibitors.

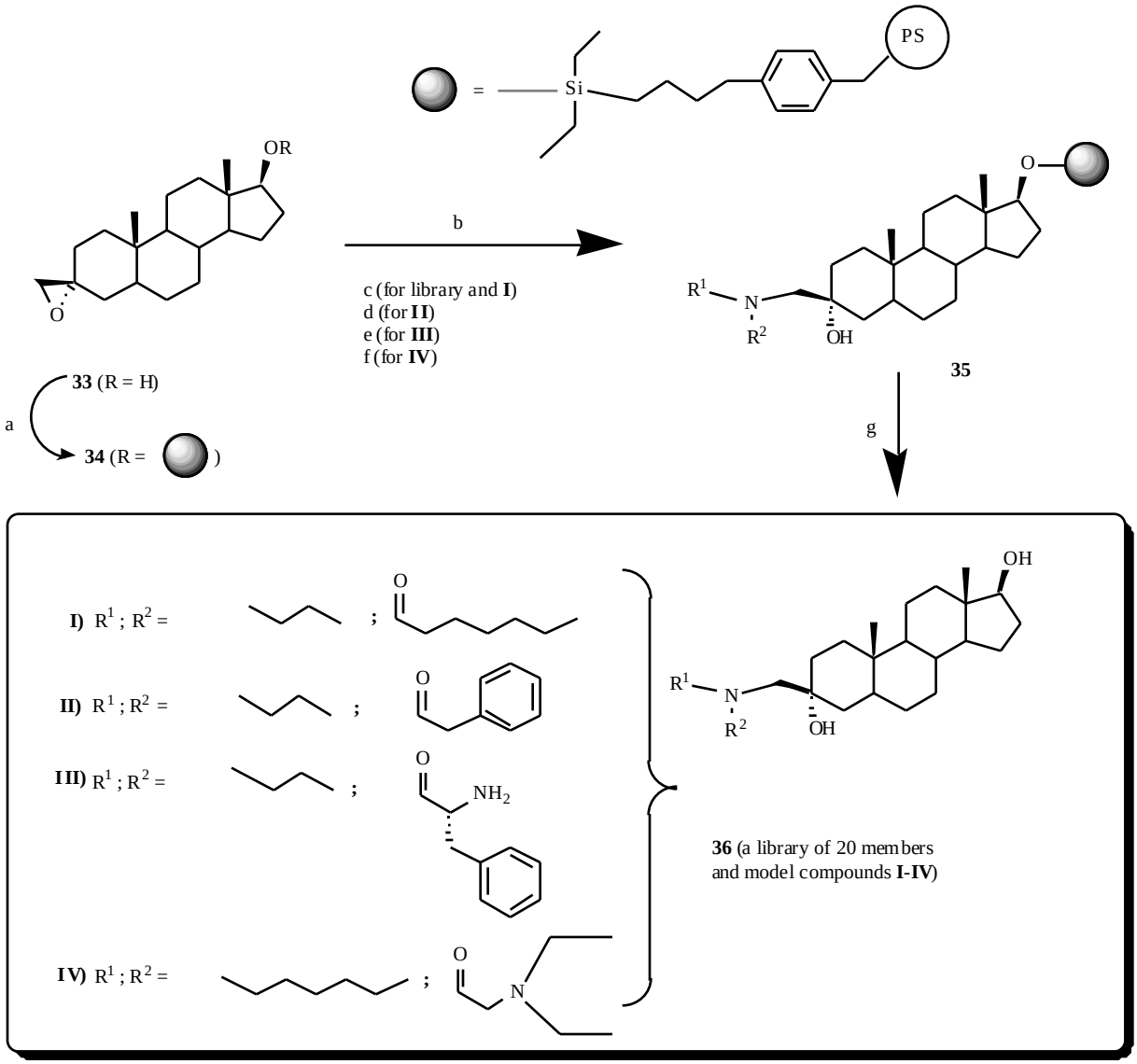

Fig. (8). SPOS of *N*-derivatives of 3β-aminomethyl-5α-androstane-3α,17β-diol (compounds **36**). (**a**) *i.* Polystyrene butyldiethylsilane resin, 1,3-dichloro-5,5-dimethylhydantoin, CH_2Cl_2, rt; *ii.* imidazole, CH_2Cl_2, rt; (**b**) alkylamine:EtOH (1:1), 60°C; (**c**) acyl chloride (for library) or hexanoyl chloride (for compound **I**), pyridine, CH_2Cl_2, rt; (**d**) phenylacetic acid, PyBroP, HOBt, DIPEA, DMF, rt (for compound **II**); (**e**) *i*. $PhCH_2CH(NHFmoc)COOH$, PyBroP, HOBt, DIPEA, DMF, rt; *ii*. 20% piperidine in CH_2Cl_2, rt (for compound **III**); (**f**) *i.* bromoacetic acid, DIPC, DMF, rt; *ii.* 2.5M diethylamine in DMSO, rt (for compound **IV**); (**g**) HF-pyridine in THF, rt.

OTHER STEROIDOGENIC ENZYMES

In addition to steroid sulfatase and reductive 17β-HSDs (types 1, 3, 5, and 7), other enzymes are involved in the biosynthesis of estrogens and androgens from cholesterol, namely P450 side-chain cleavage, P450 17α-hydroxylase/P450 17,20-lyases, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerases, 5α-reductases, and aromatase (Fig. (**2**)). Since the first three also participate in the synthesis of mineralocorticoids and glucocorticoids, it is better not to inhibit them in order to avoid steroid depletion and severe side effects. On the opposite, aromatase and 5α-reductases are involved exclusively at the end of sex steroid formation. Their inhibition thus constitutes a much more appropriate strategy, which has been adopted by numerous research groups. The 5α-reductases catalyze the double bond reduction of T into DHT, the most potent androgen. There are two types; the first one predominates in the skin, while type 2 predominates in the prostate. Inhibitors of both types have been developed in the past and been used for the treatment of benign prostatic hyperplasia, prostate cancer and alopecia [23,25,58-60]. Aromatase catalyses the crucial transformation (aromatization) of the C19-steroid 4-androstene-3,17-dione into the estrogenic C18-steroid E_1. Aromatase inhibitors have been known for a long time and encouraging results were recently reported for breast cancer treatment with the now available selective third-generation inhibitors [25,61-68]. Although the synthesis of inhibitors of P450 lyases, 5α-reductases and aromatase is still going on, most of the synthetic efforts were done in past years, and consequently, no strategy using SPOS has been published until now.

ESTROGEN RECEPTOR

Estrogens exert their action *via* nuclear estrogen receptors (ER) giving transcription-activator complexes that bind to specific regulating sequences of estrogen-sensitive genes [6]. Based on this principle, antiestrogens have been designed to interfere with the binding of estrogens to their receptors and/or with the binding of the complex to deoxyribonucleic acid [11-17]. Recent advances in the comprehension at the molecular level of regulatory mechanisms of estrogen-

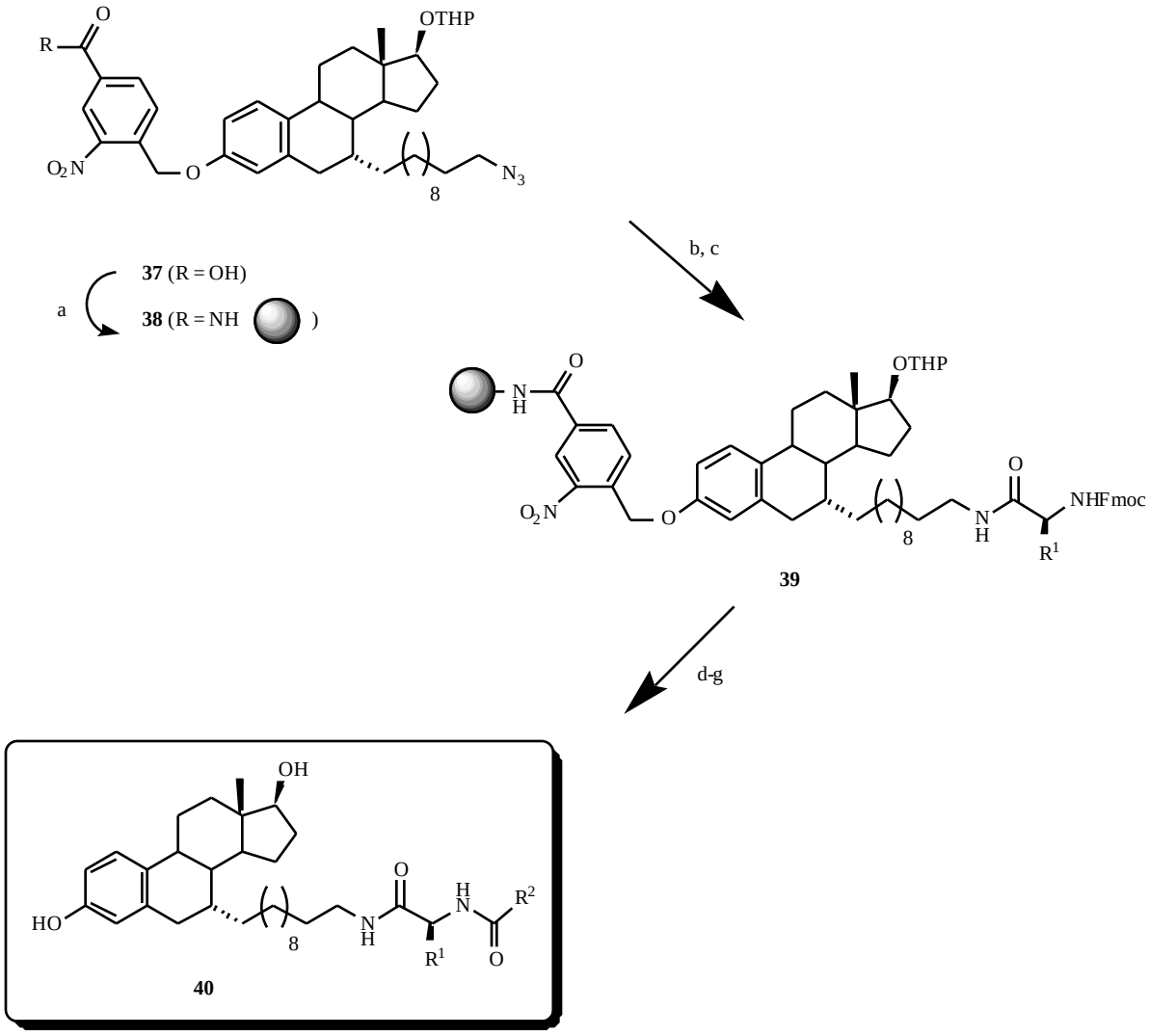

Fig. (9). SPOS of 7α-(alkylamide)-E_2 derivatives **40**. (**a**) Polystyrene aminomethyl resin, EDC, HOBt, DMF, rt; (**b**) *i*. PPh_3, THF, rt; *ii*. H_2O, 70ºC; (**c**) $R^1CH(NHFmoc)COOH$, HBTU, HOBt, DIPEA, DMF, rt; (**d**) 20% piperidine in DMF, rt; (**e**) R^2COOH, HBTU, HOBt, DIPEA, DMF, rt; (**f**) *p*-TSA, 1-butanol:$ClCH_2CH_2Cl$ (1:1), rt; (**g**) hυ (350 nm), MeOH, rt.

sensitive genes have led to the concept of selective estrogen receptor modulators [18]. These compounds produce antiestrogenic effects in certain tissues, while displaying estrogen-like activity in others. It is noteworthy that differences between a complete agonist, an antagonist, and a modulator are minimal from a molecular standpoint. The emergence of SPOS as a tool for parallel and combinatorial chemistry has provided the opportunity of creating large libraries of compounds. SPOS has thus increased the capacity to investigate, in an exhaustive fashion, the molecular determinants of the complex interactions between the receptor (ER-α or ER-β), the ligand, and the other proteins involved in biological responses. During the last decade, various libraries were designed by academic and pharmaceutical researchers with different strategies of diversification around the steroidal or non-steroidal templates with the goal of exploring receptor binding and/or estrogenic/antiestrogenic effects.

Steroidal Templates

The natural ligand of ER, E_2, constitutes a valuable template in the investigation of ER modulators. A pure steroidal antiestrogen, ICI 164,384 [69], was chosen as a lead structure by Poirier's group to generate a novel library of 7α-alkylamide-E_2 derivatives (Fig. (**9**)) [70]. The steroid scaffold **37** was first prepared following a 14-step solution-phase synthetic sequence and then coupled to an aminomethyl resin *via* the *o*-nitrobenzyl linker. Following reduction of azide **38**, the first level of diversity was introduced by acylation with four activated *N*-Fmoc amino acids to give **39**. The second level of diversity was then obtained by a second reaction of acylation with five activated carboxylic acids and final E_2 derivatives **40** (a 20-member library of ICI 164,384 analogs) released by photocleavage. This approach allowed synthesis of a library of antiestrogen-related compounds in moderate yields (12-38%) and good purities (44-95%). Each compound was tested to assess its ability to inhibit E_2-induced proliferation of T-47D human breast cancer cells in culture. Most of the compounds displayed antiproliferative activities at 0.1 μM, thus showing potencies close to that of the antiestrogen ICI 164,384.

Hanson's group investigated the tolerance of ER for a series of 17α-E/Z-(X-phenyl)-vinyl E_2 derivatives (Fig. (**10**)) [71]. The synthesis was accomplished by coupling the 17α-ethynyl-E_2 (**41**) to polystyrene carboxy resin, hydrostannylating the triple bond of **42** into **43**, and then performing the Stille coupling reaction to introduce a variety of terminal phenyl groups before the final cleavage giving **45** (8 compounds) in 17-75% yields after purification. These compounds, and others from the same group, were evaluated for binding affinity and results reported in a study aimed at developing probes for ER-α [72,73].

In the field of radiopharmaceutical ligands, Arterburn's group was interested in the radiolabelling of E_2-based compounds for targeting ER (Fig. (**11**)) [74]. The amine **46** was linked to Tentagel carboxy resin. The reaction of solid-supported acetyl hydrazine **47** with $ReOCl_3(PPh_3)_2$ and PPh_3 provided the air- and moisture-stable rhenium complex **48**. This short and efficient synthesis of **48** illustrates a new

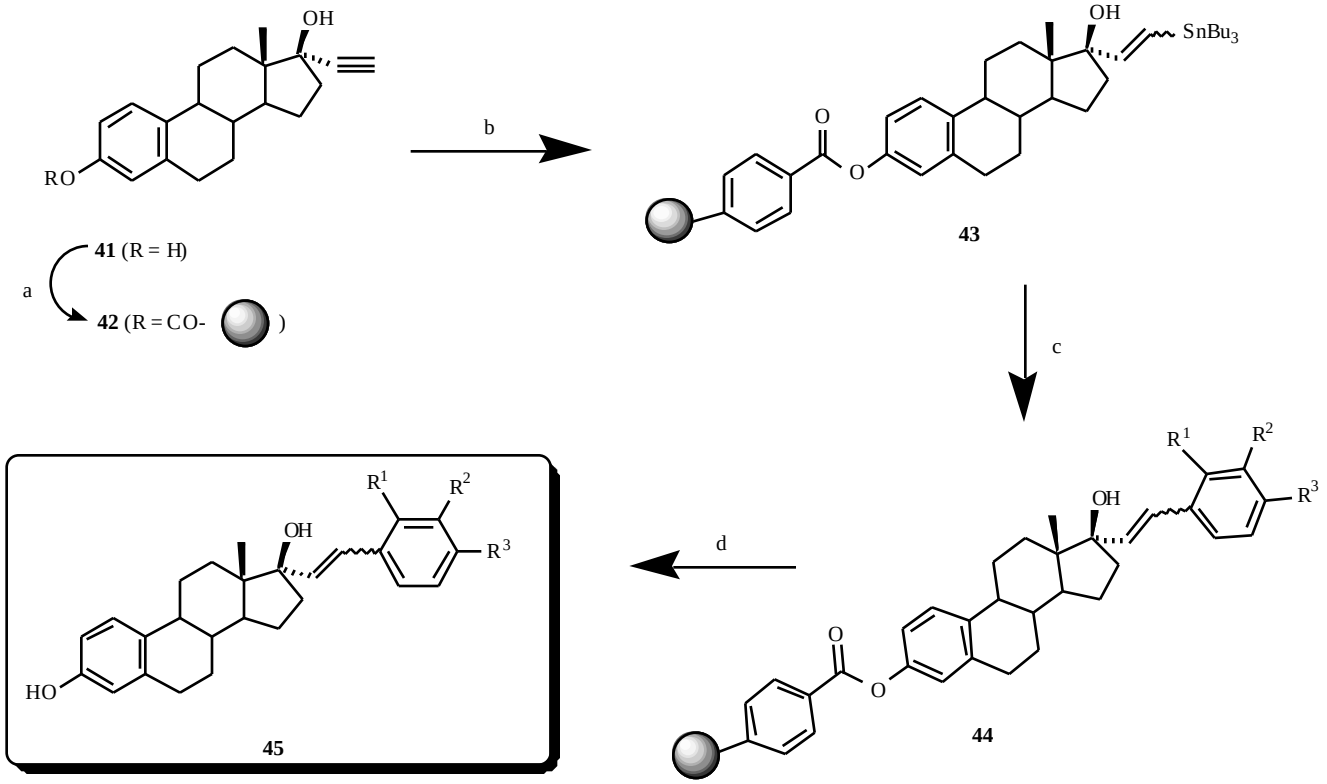

Fig. (10). SPOS of 17α-E/Z-(X-phenyl)-vinyl E_2 derivatives **45**. (**a**) Polystyrene carboxy resin, DCC, DMAP, CH_2Cl_2, rt; (**b**) $HSnBu_3$, Et_3B, THF, 50-60ºC; (**c**) R^{1-3}-Aryl-X, Pd, $(PPh_3)_4$, BHT, toluene, N_2, reflux; (**d**) *i.* 5N NaOH in MeOH:dioxane (1:3), CH_2Cl_2, rt; *ii.* 5% AcOH, rt; *iii.* 10% $NaHCO_3$, rt.

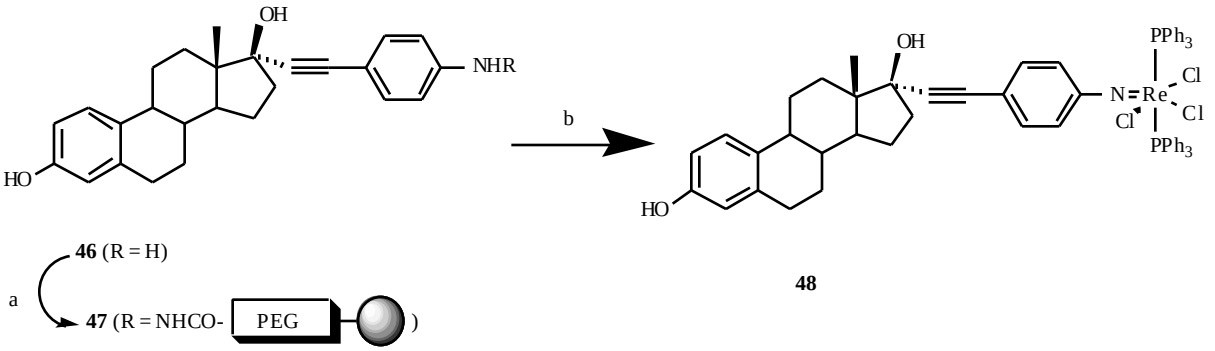

Fig. (11). A solid-supported hydrazine E_2 ligand for radiolabelling ER (compound **48**). (**a**) Tentagel carboxy resin, PyBOP, DIPEA, CH_2Cl_2, rt; (**b**) $ReOCl_3(PPh_3)_2$, PPh_3, CH_2Cl_2, 40ºC.

strategy for labelling E_2 ligands, which will be extended to technetium analogs.

Non Steroidal Templates

A library of hydroxystilbenes (23 members) was prepared by Williard *et al*. by adapting the classic Horner-Emmons olefination chemistry to solid phase (Fig. (**12**)) [75]. The hydroxystilbene structure was found suitable as a molecular scaffold since similar hydroxylated aromatic molecules are estrogenic compounds. Four hydroxybenzaldehydes were attached to polystyrene aminomethyl resin with the 4-(hydroxymethyl)phenoxy linker, and the resins **49** were treated with a solution of six benzyl phosphonate anions **50**. After cleavage in acid conditions, the resulting hydroxystilbenes **51** were recovered in variable yields (7-85%), but no purification was necessary. The library was screened for estrogenic and antiestrogenic activities and three compounds proved to have estrogenic activity in the 5-15 μM range.

The Katzenellenbogen's group turned their attention to a tetrasubstituted pyrazole template, which allowed a multiparameter study on structural requirements for the

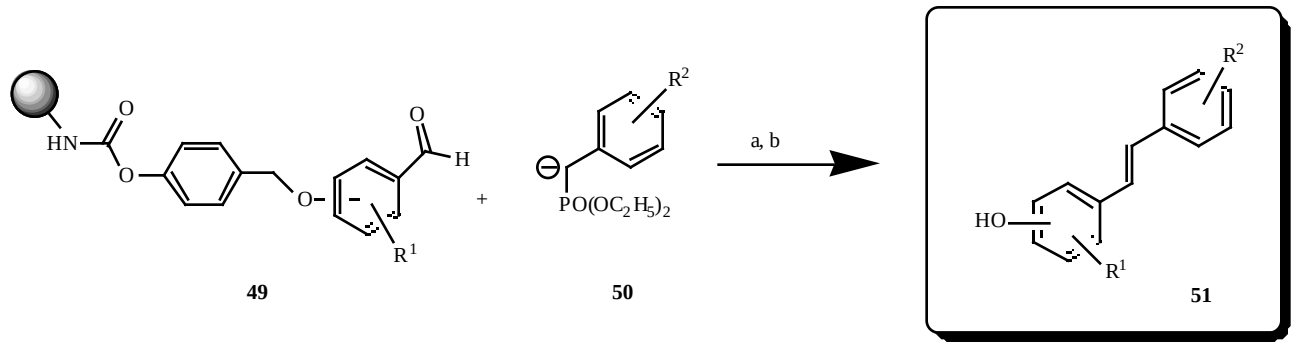

Fig. (12). SPOS of hydroxystilbenes **51**. (**a**) DMF, rt; (**b**) TFA:H_2O (95:5), rt.

design of selective estrogen receptor modulators (Fig. (**13**)) [76-78]. The synthesis was accomplished by first immobilizing different alkylphenones **52** on Merrifield resin and then submitting the resins **53** to a crossed-Claisen condensation reaction to obtain the β-diketones **54**. These were next treated with alkylhydrazines to form, after cleavage, the desired tetrasubstituted pyrazoles **55**. Using this approach, a 12-member model library (>80% of purity

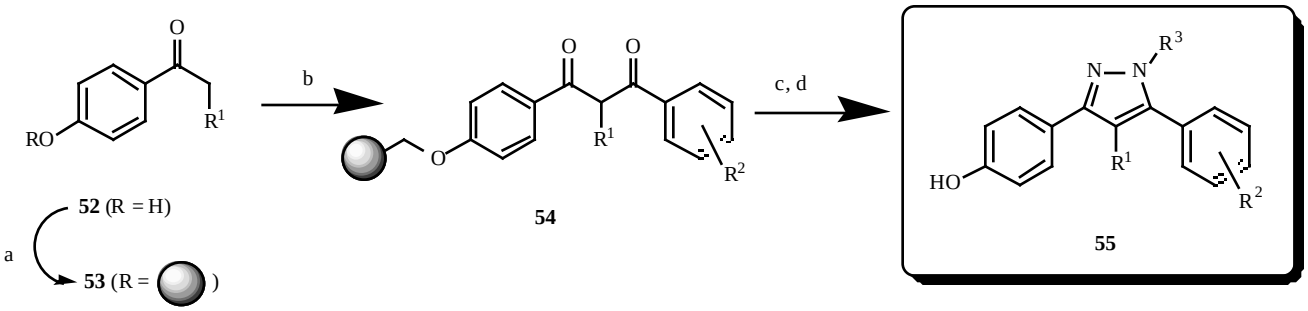

Fig. (13). SPOS of pyrazole derivatives **55**. (**a**) Merrifield resin, NaH, DMF, 50ºC; (**b**) $R^2PhCOOPhNO_2$, LHMDS, THF, 40ºC; (**c**) $R^3NHNH_2 \cdot HCl$, TEA, EtOH, 65ºC or toluene 80ºC; (**d**) *i*. BBr_3, CH_2Cl_2, 0ºC-rt; *ii*. MeOH, 0ºC or/and trimethylammonium carbonate resin.

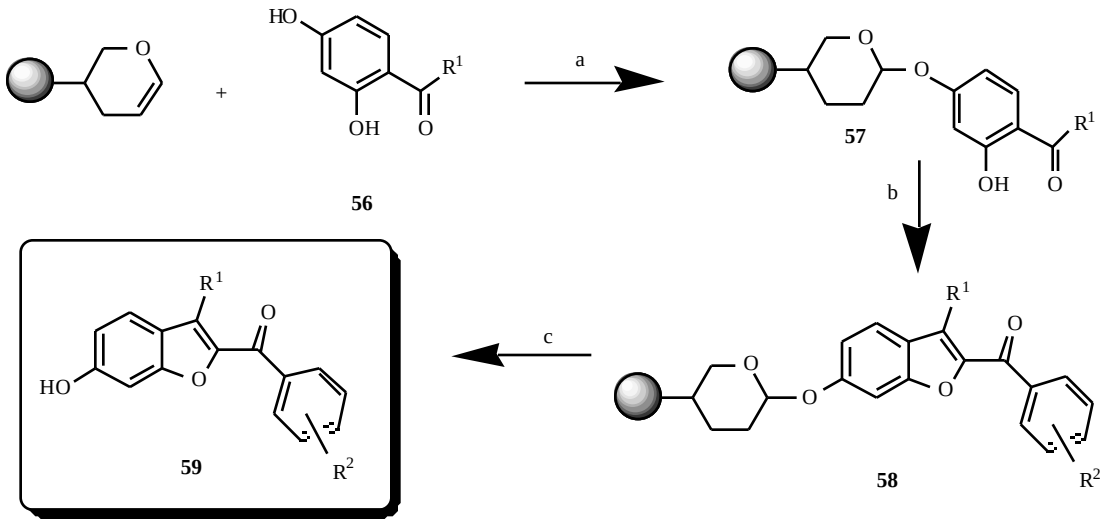

Fig. (14). SPOS of benzofuran derivatives **59**. (**a**) $ClCH_2CH_2Cl$, PPTS, 80ºC or *p*-TSA, 0ºC; (**b**) α-bromoketones, DBU, NMP, 80ºC; (**c**) TFA:CH_2Cl_2:MeOH (1:5:1).

after radial chromatography) and a 96-member library (50% of average purity without purification) were obtained in a parallel format and acceptable yields. All library members were tested for ER-α relative binding affinity (RBA = 0.1-14%) and structure-activity relationships have provided new directions for further development.

Smith *et al*. [79] designed a solid-phase approach to the synthesis of benzofuran derivatives, based on mimicking the antiestrogen raloxifene (Fig. (**14**)). A library of 320 members was generated by immobilizing a variety of dihydroxyphenyl ketones **56** on dihydropyran resin and reacting **57** with various α-bromoacetophenone building blocks. Benzofurans **58** were then formed by a modification of Boehm and Showalter's method using DBU in NMP and released from solid support by acid cleavage to give the desired compounds **59** as the major component (LC/MS analysis) in an overall yield of 35%. Several analogs were identified as potent ligands for ER-α and ER-β with some compounds exhibiting selectivity for ER-α.

In a patent from American Home Products Corporation, Chiu *et al*. [80] used a series of 2,4,6-trisubstituted pyridines as templates for the synthesis of estrogenic

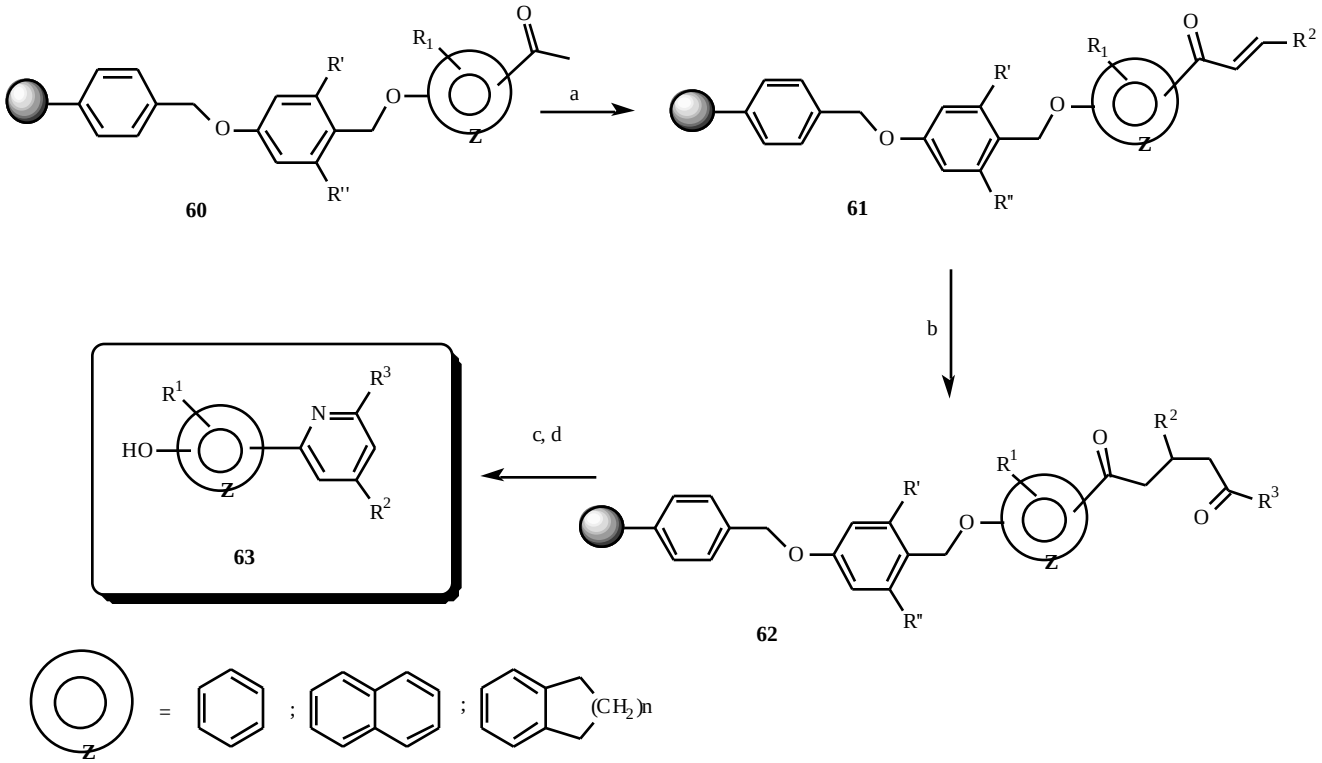

Fig. (15). SPOS of 2,4,6-trisubstituted pyridines **63**. (**a**) R^2CHO, 25% NaOMe in MeOH, TMOF, 0-50ºC; (**b**) 1-trimethylsilyloxy-1-R^3-ethylene, CsF, DMSO, 70ºC; (**c**) NH_4OAc, AcOH, DMF, 100ºC; (**d**) 50% TFA in CH_2Cl_2, rt.

compounds **63** (Fig. (**15**)). Different hydroxyacetophenones (R^1) were coupled onto chloro-activated Wang resin and the acetophenone functionality of **60** reacted with different aldehydes (R^2) in the presence of NaOMe to give the corresponding olefins **61**. These were treated with silylenol ethers (R^3) in the presence of cesium fluoride to produce the 1,5-diketones **62** as key intermediates. Finally, compounds **62** were treated with ammonium acetate to yield the pyridine derivatives, which were cleaved from solid support by an acid treatment and purified by chromatography giving **63** (10 compounds). The estrogenicity of some final compounds was reported but appears to be very weak at a concentration of 1 μM.

The parallel solid-phase synthesis of 25 triphenylethylene derivatives based on a tamoxifen template was achieved by Brown and Armstrong [81] (Fig. (**16**)). The library was initiated in solution phase by using 5 alkynes **64** (R^1 and R^2) that were converted into the corresponding bis(boryl)alkenes **65** by a platinum catalyzed reaction. The diboronate intermediates were then submitted to a Suzuki reaction using five aryl halides (R^3) to give mixtures of regioisomers **66**. Boronates **66** were then sequestered by the resin capture technique using **67** to afford the solid-supported triarylethylene compounds **68**. The cleavage of the traceless silyl linker yielded the desired library members **69** in moderate to good yields (13-68%) and purity >90% (as mixtures of regioisomers). Herein, the resin capture strategy was used to combine the flexibility of traditional solution synthesis with the purity of compounds produced on solid support. Although not reported, the resin **67** could also be varied allowing the introduction of an additional level of diversity. No biological evaluation of these tamoxifen analogs as antiestrogens was reported.

Defreest *et al.* [82] investigated the use of short synthetic peptides, derived from an α-fetoprotein (AFP), for inhibiting the growth of human ER^+ breast cancers. Although the anti-breast cancer activity of the linear and cyclic AFP-derived peptides [83,84] is well documented, the pharmacophore has not been elucidated. The authors synthesized a series of linear and cyclized peptides as analogs of the *EMTPVNPG* peptide to determine which amino acids were crucial for activity. Linear octapeptides were prepared by SPOS with a peptide-synthesis system. Briefly, a first *N*-Fmoc amino acid was added on Fmoc-PAL-PEG polystyrene resin (HATU as coupling reagent and DIPEA), other amino acids were sequentially coupled by Fmoc chemistry, and the final peptide released by incubation in a solution of TFA, anisole and ethane dithiol. The antiestrogenic activity of synthetic peptides was determined with an immature mouse uterine growth assay and results showed the relative importance of each amino acid.

Short linear peptides that display estrogen-like activity were also reported by Kasher *et al.* [85]. After identification of an *LPALDPTKRWFFETK* peptide (peptide H5), which recognizes ER-α and interacts specifically with a monoclonal antibody against estradiol (mAb-E_2) with an IC_{50} value of 1 μM, the authors undertook a study with the aim of generating peptides with tissue-selective estrogenic activity and increased affinity for ER. Peptides were prepared with an automated solid-phase multiple peptide synthesizer using a Wang resin or a rink-amide 4-methyl-benzhydrylamine resin and Fmoc chemistry following

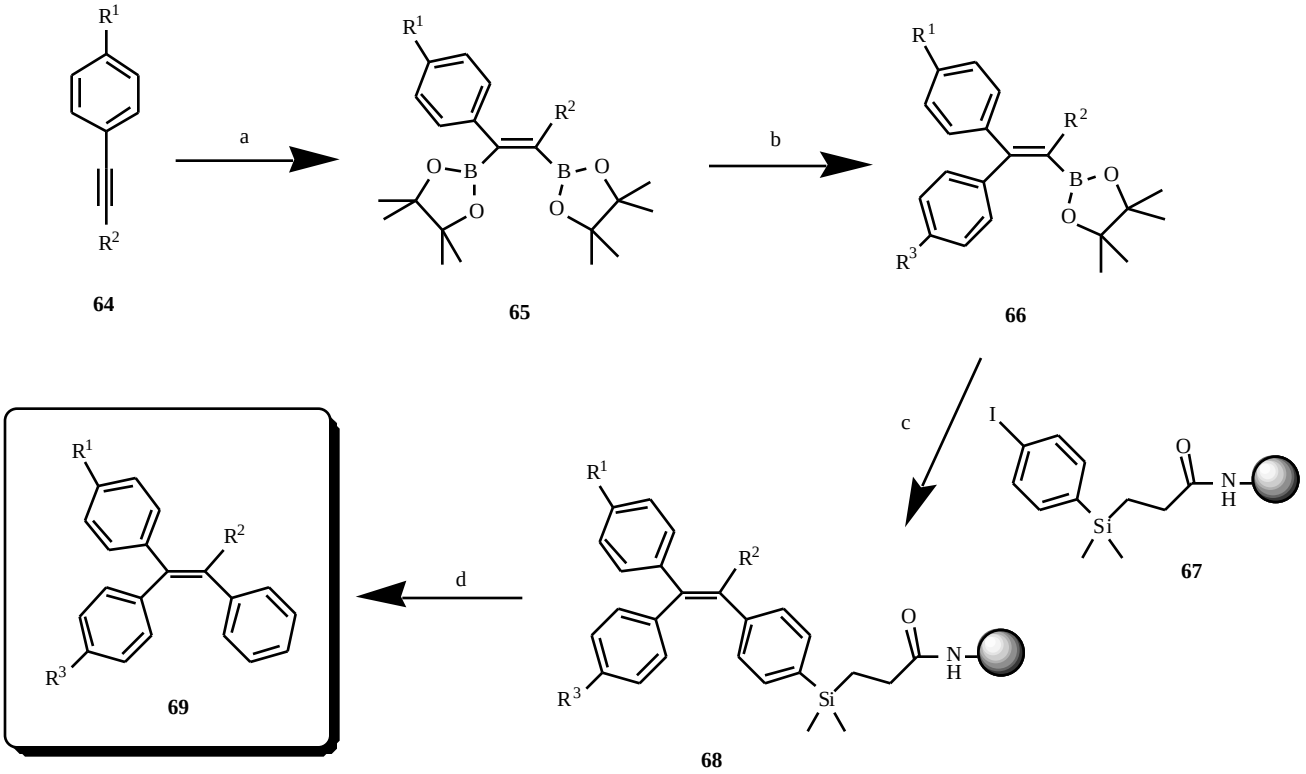

Fig. (16). SPOS of triethylene derivatives **69** (only one of the possible two regioisomers is represented). (**a**) Bis-(pinacolato)diboron, $Pt(PPh_3)_4$, DMF, 80ºC; (**b**) R^3PhX, $Pd(dppf)Cl_2$, 3,5-dimethoxyphenol, 6M KOH, DME, rt; (**c**) resin **67**, 6M KOH, rt; (**d**) 30% TFA in CH_2Cl_2, rt.

standard manufacturer protocols. Thus, after the identification of the minimum binding sequence within the H5 peptide that interacts with mAb-E_2, alanine substitution was conducted applying combinatorial chemical techniques. This exercise allowed identifying a hexapeptide (*VSWFFE*) and a heptapeptide (*VSWFFED*) that bind mAb-E_2 with high affinity (IC_{50} = 6 and 30 nM) and inhibit the binding of E_2 to ER-α (IC_{50} of 100 μM) and ER-β (IC_{50} of 100-250 μM). The peptides described in this study may be useful in the development of new ER ligands.

ANDROGEN RECEPTOR

The androgen receptor (AR), a member of the superfamily of nuclear transcription factors that mediate the action of steroid hormones, has been identified and cloned as only one type of receptor. The crystal structures of the ligand-binding domains of AR and human progesterone receptor, in complex with the same ligand – metribolone (R1881) – have recently been determined [86,87]. However, antiandrogen drugs based on these crystallographic data have not yet been designed, and a better understanding of the molecular action mechanism of this receptor is needed. Presently, the best available androgen blockade therapy consists in the administration of an luteinizing hormone releasing hormone agonist (medical castration) in combination with a pure antiandrogen (flutamide, casodex, anandron) [88]. However, the relatively weak affinity of the available antiandrogens for the AR leaves the residual endogenous androgens (from testis and adrenals), free to interact with the AR and to potentially activate the growth of prostate cancer cells, which is especially harmful in the case of androgen-hypersensitive tumors. Thus, the development of a new generation of pure antiandrogens, with stronger affinity for the receptor, is much needed. In regard to this goal, as for the estrogen research field, combinatorial chemistry represents a valuable tool to provide a large number of compounds for studying structure-activity relationships toward the development of a pure antiandrogen or a selective androgen receptor modulator.

Steroidal Templates

Model libraries of C19 steroid derivatives were synthesized by Maltais *et al*. [89] in order to produce androgen receptor antagonists (Fig. (**17**)). Briefly, 16α-(azidoethyl)-5α-androstane-3α,17β-diol (**70**) was synthesized in solution and linked to solid support with the diethylsilylether linker before being diversified using the peptide *N*-Fmoc chemistry. Libraries with one level (10 members) and two levels of diversity (40 members) were generated following a parallel approach giving individual compounds **74** and **75**. On the other hand, compounds with three levels of diversity were obtained by a split-and-pool

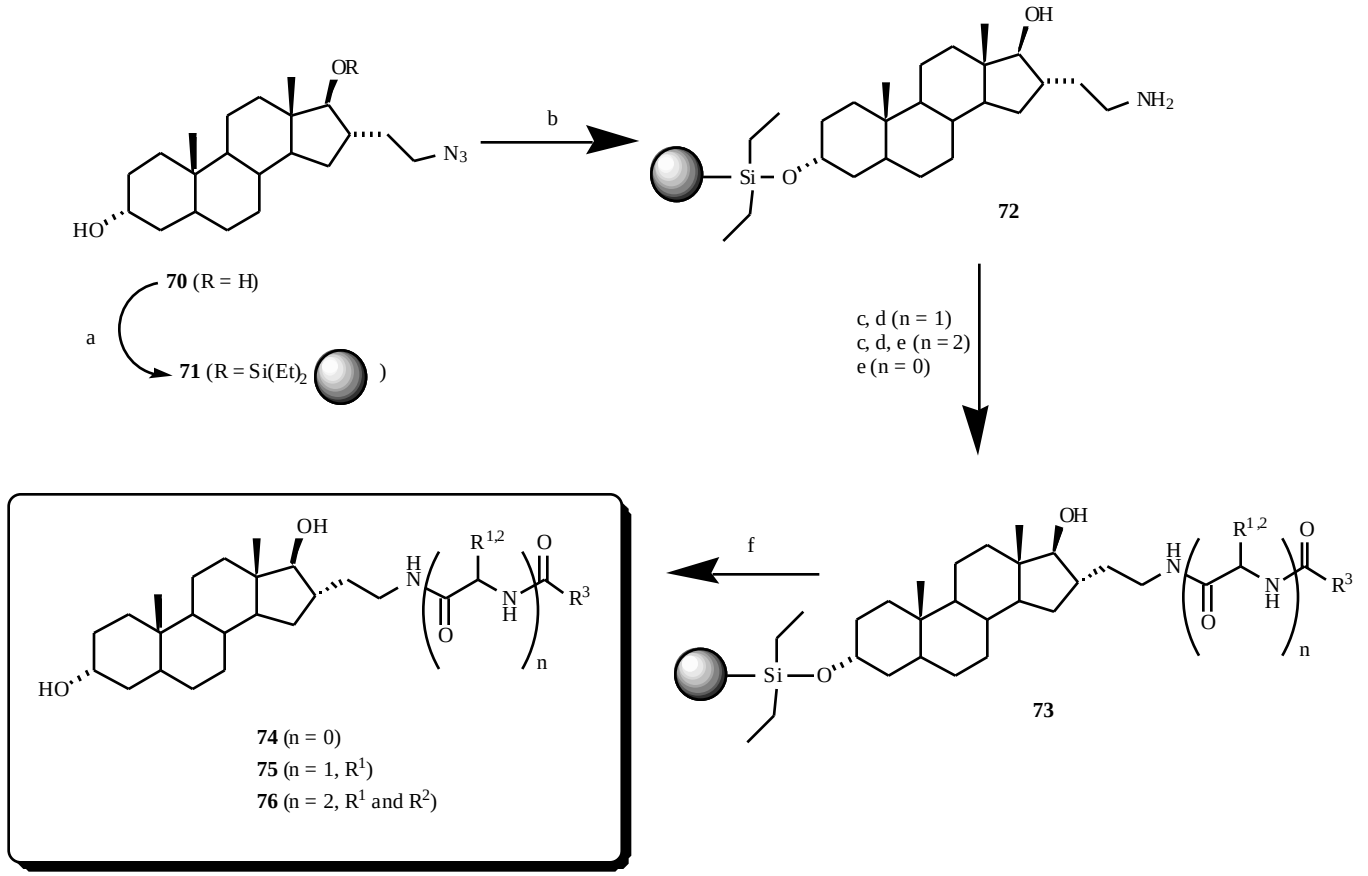

Fig. (17). SPOS of 16α-aminoethyl-*N*-substituted-androstane derivatives **74-76**. (**a**) *i.* Polystyrene butyldiethylsilane resin, 1,3-dichloro-5,5-dimethylhydantoin, CH_2Cl_2, rt; *ii* imidazole, CH_2Cl_2, rt; (**b**) $SnCl_2$:HSPh:TEA (1:4:5), THF, rt; (**c**) $R^{1,2}CH(NHFmoc)COOH$, PyBOP, HOBt, DIPEA, DMF, rt; (**d**) piperidine:CH_2Cl_2 (80:20), rt; (**e**) R^3COOH, PyBOP, HOBt, DIPEA, DMF, rt; (**f**) *i. p*-TSA (0.07M), CH_2Cl_2, rt; *ii.* $NaHCO_3$ washing step.

approach giving mixtures (15 pools) of general structure **76**. These libraries were assessed for AR-binding affinity as well as for proliferative and antiproliferative activities on Shionogi AR^+ cells. Although the screening revealed promising results, these peptidosteroids were found to be weak antiandrogens.

Non Steroidal Templates

Nilutamide (anandron) is a well-known androgen antagonist used in anticancer therapy. This drug was built around a small and rigid hydantoin heterocycle nucleus that represents a valuable scaffold for combinatorial chemistry. The preparation of a small library of fully substituted hydantoin compounds **80** was recently described by Lamothe *et al*. [90] (Fig. (**18**)). The methodology consisted in linking a series of amino acids to Rink resin (**77**) by formation of stable amide **78**, introducing a second level of diversity by synthesizing amidoureas **79**, and releasing hydantoin precursors from solid support before final cyclization under TFA conditions giving **80**. No biological results and/or androgen activities were reported in this article, but it is clear that such a methodology is of interest for the development of non-steroidal antiandrogens. Those interested in the broad applications of hydantoins arereferred to a recent review by Meusel and Gutschow [91] of recent developments in hydantoin chemistry, including a section on solid-phase organic synthesis.

CONCLUSION

The biosynthesis of steroid hormones (steroidogenesis) is a crucial process in the homeostasis of biological systems. In the broad series of steroids biotransformed from cholesterol, the sexual hormones, namely estrogens and androgens, represent key modulators of estrogen- and androgen-sensitive diseases, especially breast and prostate cancers. Through the study of steroidogenesis (Fig. (**2**)), promising therapeutic targets (receptors and enzymes) have been identified, allowing the development of agents able to modulate the effects of steroid hormones. The contribution of classic medicinal chemistry has been remarkable in this regard, as exemplified by the development of important drugs such as antiestrogens (tamoxifen, raloxifene, acolbifene), antiandrogens (casodex, cyproterone acetate), aromatase inhibitors (anastrozole, letrozole) and 5α-reductase inhibitors (finasteride). Our knowledge of steroid biosynthesis and of steroid receptors has however tremendously progressed in the past ten years. Indeed, a second estrogen receptor (ER-β) was recently identified and new isoforms of steroidogenic enzymes have been reported, thus adding to the number of potential therapeutic targets. Tissue distribution of steroid receptors and enzymes is also an important aspect of the biological response. These recently obtained and constantly developing data, combined with the new fields of proteomics and genomics, will increase the number of potential therapeutic targets.

In this context, the emergence of SPOS and combinatorial chemistry provides new efficient tools for the medicinal chemists working in drug development [92-97]. These new methodologies have already contributed to the development of steroidogenic enzyme inhibitors and receptors (ER and AR) agonists/antagonists, as reviewed in the present article. Although the contribution of SPOS to the field of steroidogenesis modulators can appear modest until now, the future is promising and this methodology should become essential for both lead identification and lead optimization processes. Furthermore, directed oriented

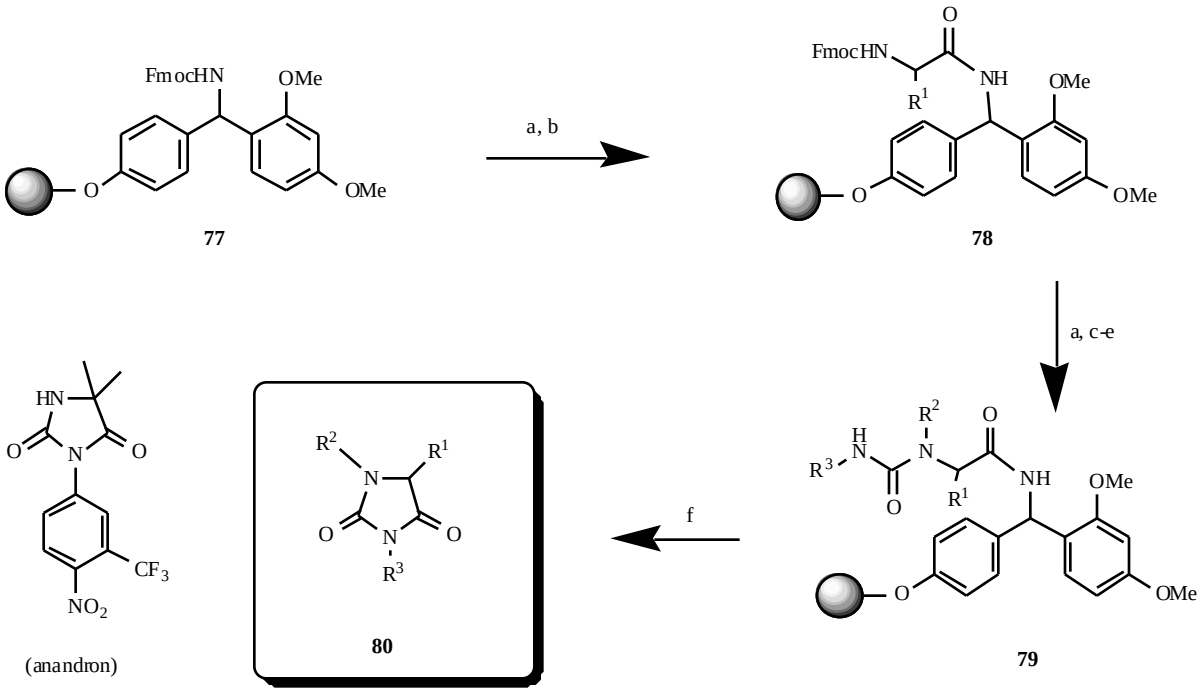

Fig. (18). SPOS of hydantoin derivatives **80**. (**a**) 20% piperidine in DMF, rt; (**b**) $R^1CH(NHFmoc)COOH$, DIPEA, BOP, NMP, rt; (**c**) R^2CHO, AcOH, CH_2Cl_2, rt; (**d**) $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt; (**e**) isocyanate (R^3NCO), CH_2Cl_2, rt; (**f**) TFA in CH_2Cl_2, Et_3SiH, rt or LiOH, THF/MeOH/H_2O, 50ºC.

synthesis (DOS) [98-101], a new emerging field, will certainly increase the scope of SPOS in the development of new drugs. Although in the developmental stage, the solid-phase synthesis of libraries through dynamic combinatorial chemistry (DCC) [102-105] could be revolutionary for the identification of therapeutic agents. Indeed, dynamic processes using an enzyme and SPOS have been reported to enhance the ability to detect the best inhibitor in a mixture of compounds [105]. The use of DOS and DCC in the development of estrogen and androgen modulators remains, however, an exciting challenge for medicinal and organic chemists.

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The Canadian Institutes of Health Research (CIHR) and Le Fonds de la Recherche en Santé du Québec (FRSQ) are gratefully acknowledged for operating grants and a fellowship, respectively. Careful reading of the manuscript by Sylvie Méthot is also greatly appreciated.

LIST OF ABBREVIATIONS

AcOH = acetic acid

BHT = 3,5-di-*t*-butyl-4-hydroxytoluene

BOP = benzotriazole-1-yl-oxy-tris-dimethylamino-phosphonium hexafluoro-phosphate

DBU = 1,8-diazobicyclo[5,4,0]undec-7-ene

DCC = dicyclohexylcarbodiimide

DEA = diethylamine

DIPC = diisopropylcarbodiimide

DIPEA = diisopropylethylamine

DMAP = 4-dimethylaminopyridine

DME = dimethoxyethane

DMF = dimethylformamide

DMSO = dimethyl sulfoxide

EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide

EtOH = ethanol

Fmoc = 9-fluorenylmethoxycarbonyl

HATU = 1,1,3,3-tetramethyluronium hexafluorophosphate

HBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate

HFIP = hexafluoro-2-propanol

HOBt = *N*-hydroxybenzotriazole

LHMDS = lithium hexamethyldisilazide

MeOH = methanol

NMP = *N*-methyl-pyrrolidinone

PPTS = pyridinium *p*-toluenesulfonate

p-TSA = *p*-toluenesulfonic acid

PyBOP = benzotriazol-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate

PyBroP = bromo-tris-pyrrolidinophosphonium hexafluorophosphate

TEA = triethylamine

TFA = trifluoroacetic acid

THF = tetrahydrofuran

TMOF = trimethyl orthoformate

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