ACS Combinatorial Science

Letter

pubs.acs.org/acscombsci

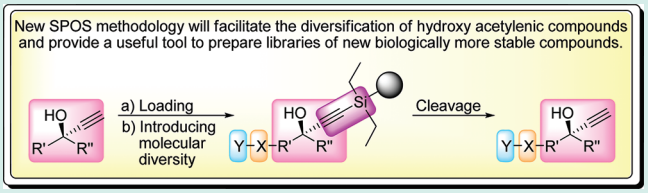
New Diethylsilylacetylenic Linker for Parallel Solid-Phase Synthesis of Libraries of Hydroxy Acetylenic Steroid Derivatives with Improved Metabolic Stability

Amélie Talbot, René Maltais, and Donald Poirier*

Laboratory of Medicinal Chemistry, CHUQ (CHUL) Research Center, 2705 Laurier Boulevard, Quebec, QC, G1V 4G2, Canada

Supporting Information

ABSTRACT: Acetylenic tertiary alcohols are well-known to be compounds that are biologically more stable than their corresponding secondary alcohols. The linkage of an acetylenic compound to a polymer support and further introduction of molecular diversity was found to be an interesting way to generate libraries of hydroxy acetylenic derivatives and thus potentially improve their biological properties. For the first time, we describe the loading of an ethynyl steroid to a polystyrene-diethylsilane resin and its uses for the solid-phase synthesis of a model library of 21 steroid derivatives. Two levels of molecular diversity were introduced by successive addition of amino acids and carboxylic acids, and hydroxy acetylenic steroids were then released by an acidic treatment in high yield and purity without further purification step.



KEYWORDS: *linker, solid-phase synthesis, library, hydroxy acetylenic compounds, steroids*

It is well-known that 17β-hydroxy-steroids that possess an ethynyl group at position 17α are much more stable drugs than analogues without the ethynyl group.[1] Indeed, the transformation of alcohol is reduced since the presence of ethynyl group hinders the oxidation of 17β-hydroxy[1−3] or the glucuronidation and its elimination in urine.[4,5] With this in mind, some pharmaceutical compounds were synthesized having the hydroxy acetylenic group to increase their in vivo stability and consequently their biological potency.[6,7] One of the best known is the synthetic ethinylestradiol, which is the main estrogen used in oral contraceptives[8,9] and the most prescribed drug worldwide.[9] Steroidal compounds like levonorgestrel, norethisterone, norethynodrel, desogestrel, etonogestrel, lynestrenol, and gestodene are all hydroxy acetylenic steroid derivatives used as progestin drugs while quinestrol and mestranol are used as estrogenic drugs.[2] More recently, some hydroxy acetylenic compounds, steroidal or nonsteroidal, were synthesized and reported to possess numerous interesting biological properties. In fact, steroidal compounds could be used for treating autoimmune conditions and related diseases like sclerosis, ulcerative colitis or arthritis,[10] metabolic and pulmonary disorders[11] and a variety of cancers[12] while nonsteroidal compounds possess inhibitory activities against rennin,[13] act like androgen receptor ligands[14] and are used for treatment of different cancers.[15−17] Another interesting hydroxy acetylenic compound is tibolone, a synthetic 19-norpregnane with estrogenic, progestogenic and androgenic activities that is able to treat multiple psychological and physiological menopausal symptoms as well as to confer beneficial preventive health benefits.[18]

In general, the ethynyl group was introduced by an addition to a carbonyl group at the end of the chemical synthesis. This strategy is however greatly limited by the incompatibility of the other chemical functionalities on the molecule. It would therefore be advantageous to develop new methodologies to introduce the ethynyl group or to use new approaches that would better exploit this chemical functionality. The linkage of an acetylenic compound to a polymer support and further introduction of molecular diversity seemed to us an interesting alternative that has not yet been used, to generate libraries of hydroxy acetylenic derivatives and thus potentially accelerate the discovery of new biologically more stable or active compounds (Figure 1). Focusing on this new approach, we

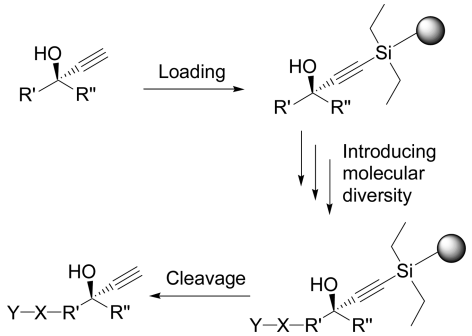


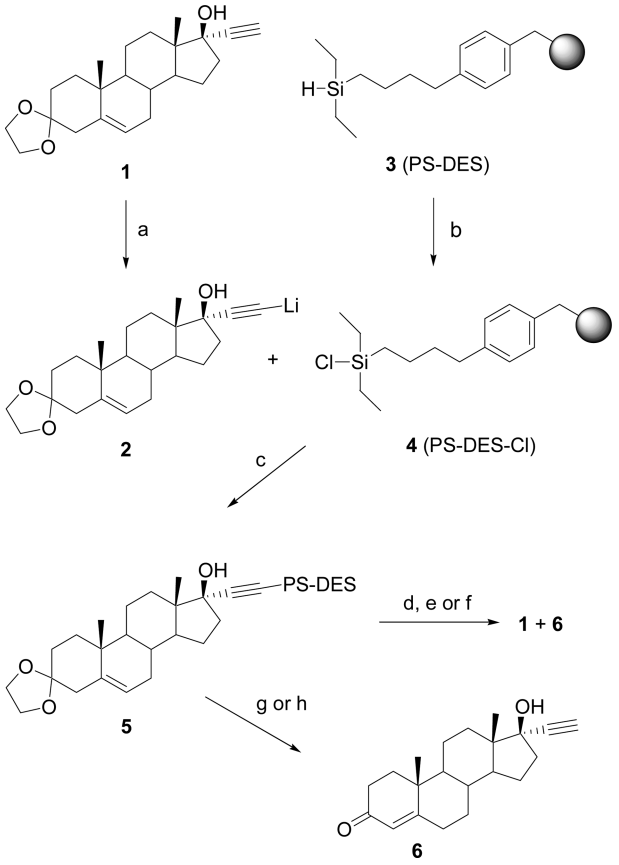
Figure 1. Diethylsilylacetylene as a new linker for solid-phase organic synthesis of diversified compounds.

Received: March 14, 2012
Revised: May 9, 2012
Published: May 15, 2012

described for the first time the loading of a 17α-ethynyl-steroid to a polystyrene-diethylsilane resin and its uses for the solid-phase synthesis of a model library of steroid derivatives.

Trimethylsilylacetylene is a useful reagent for introducing an ethynyl group on a ketone.[19] After removal of the silyl group by hydrolysis, the generated acetylenic compound is obtained in very good yield. Based on this, we hypothesized that the polystyrene-diethylsilane (PS-DES) resin could be a suitable solid support to link and release an acetylenic compound after introducing molecular diversity. To optimize the reaction conditions for the loading and cleavage steps, we first used steroid **1** as a model compound (Scheme 1). For coupling

Scheme 1. Loading of Protected Ethisterone (1) on PS-DES Resin 3 and Its Release As Compounds 1 and 6 or Compound 1 Only[a]



[a]Reagents and conditions: (a) MeLi, THF, 0 °C to rt, 75 min; (b) 1,3-Dichloro-5,5-dimethylhydantoin, DCM, rt, 1 h; (c) THF, rt, 4−16 h; (d) 10% TBAF in DCM (v/v), rt; (e) 6% HF-pyridine in DCM (v/v), rt; (f) 50% TFA in DCM (v/v), rt; (g) HCl/MeOH/DCM 2:9:9 (v/v), rt; (h) HCl/MeOH/DCM 1:9:30 (v/v), rt.

assays, the ethynyl steroid **1**[20] was first activated by formation of the organolithium **2** and then reacted with PS-DES-Cl resin **4**, which was generated in situ by a treatment of PS-DES resin **3** with 1,3-dichloro-5,5-dimethylhydantoin. For this loading step, different reaction times and steroid concentrations (equivalents) were tested (Figure 2). With two equivalents of **1**, versus one equivalent of PS-DES resin **3**, the loading yield of **5** as calculated by the increase of the resin weight was of 22%, 40%, and 61% for reaction times of 4, 8, and 16 h, respectively. The coupling yield of **5** increased to 72% and 71% when three and four equivalents of **1** were used, respectively.

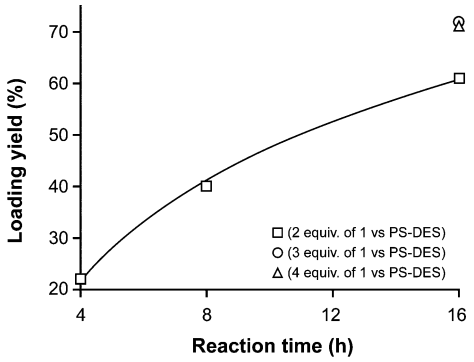


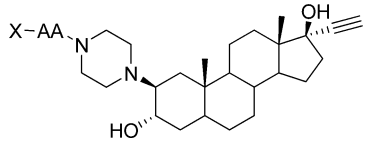
Figure 2. Loading yield (weight increase) for the coupling of **1** to PS-DES resin **3** in function of reaction time and steroid equivalent number. To acetylenic compound **1** in dry THF was added MeLi and the mixture was added at room temperature to PS-DES-Cl resin **4** generated from **3** and swollen in THF. After the reaction time, the resin **5** was filtered, washed, dried during 16 h, and weighed.

After these loading tests, we decided to use a 16 h reaction time and two equivalents of acetylenic compound for our next coupling experiments. However, before doing that, it was necessary to determine the conditions to release the hydroxy acetylenic compound. We first placed resin **5** in the presence of 20% piperidine in DCM and K_2CO_3 5% in MeOH to verify the linker's stability under basic conditions. Fortunately, the diethylsilyl acetylenic linker was stable under these conditions and thus opened the door to perform Fmoc chemistry. After this verification, the cleavage of **1** or **6** from resin **5** was tested using five different conditions. When cleavage was performed using a solution of 10% TBAF in DCM, a solution of 6% HF-pyridine in DCM or a solution of 50% TFA in DCM, both compounds **1** and **6** were obtained indicating an incomplete deprotection of acetal group. However, in the assay with a solution of HCl/MeOH/DCM (2:9:9) or (1:9:30), steroid **6** was the only compound obtained. Thus, to obtain a single product and promote the swelling and the cleavage of the desired product, the condition containing HCl and the highest ratio in DCM (HCl/MeOH/DCM (1:9:30), rt, 18 h) was chosen for our next cleavage experiments. A loading test under optimized conditions followed by a cleavage under the optimal acid conditions confirmed the recovery of the hydroxy acetylenic compound **6** in good yield (55%), thus suggesting the usefulness of the new linker for generating libraries.

After we established that a hydroxy acetylenic compound can be loaded to a solid support and released in acid conditions, we were interested in using this linker to synthesize a library of diversified compounds. This library was designed based on our previous structure−activity relationship (SAR) results that identified a series of 2β-piperazino derivatives of 5α-androstane-3α,17β-diol showing interesting antiproliferative activity on HL-60 cancer cells.[21,22] The presence of an acetylenic group at position 17α was also found to improve both in vitro antiproliferative activity and in vivo bioavailability in some cases.[12] Twenty one aminosteroids with two levels of diversity (3 × 7) were then targeted as potentially interesting inhibitors of human cancer cell growth (Table 1). Thus, one amino acid (pyridylalanine), not easily workable in solution chemistry, and two amino acids (proline and phenylalanine), producing the best cytotoxic effect, were selected for introducing the first level of molecular diversity. From previous SAR results,[21,22] we also selected seven carboxylic acids as

dx.doi.org/10.1021/co300034y | ACS Comb. Sci. 2012, 14, 347−351

Table 1. Molecular Formula, Experimental Mass (M + H), Quantity, and HPLC Purity of the 21 Library Members Generated with the New Diethylsilylacetylenic Linker



Carboxylic acid (X) / **Amino acid (AA)**	2-Naphthoic acid	3-Acetyl benzoic acid	Quinaldic acid	Isovaleric acid	Cyclohexyl carboxylic acid	Cyclohexyl acetic acid	Phenyl acetic acid
L-Proline	**A1**[a]	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**
	$C_{41}H_{53}N_3O_4$	$C_{39}H_{53}N_3O_5$	$C_{40}H_{52}N_4O_4$	$C_{35}H_{55}N_3O_4$	$C_{37}H_{57}N_3O_4$	$C_{38}H_{59}N_3O_4$	$C_{38}H_{53}N_3O_4$
	652.40 g/mol	644.45 g/mol	653.35 g/mol	582.45 g/mol	608.45 g/mol	622.45 g/mol	616.40 g/mol
	19 mg	17 mg	7 mg	9 mg	15 mg	12 mg	16 mg
	95%	90%	97%	91%	95%	87%	92%
L-Phenyl-alanine	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**
	$C_{45}H_{55}N_3O_4$	$C_{43}H_{55}N_3O_5$	$C_{44}H_{54}N_4O_4$	$C_{39}H_{57}N_3O_4$	$C_{41}H_{59}N_3O_4$	$C_{42}H_{61}N_3O_4$	$C_{42}H_{55}N_3O_4$
	703.40 g/mol	694.40 g/mol	703.40 g/mol	632.45 g/mol	658.45 g/mol	672.45 g/mol	666.45 g/mol
	21 mg	18 mg	14 mg	5 mg	16 mg	17 mg	17 mg
	66% [81%][b]	81% [80%][b]	86% [81%][b]	61% [83%][b]	34% [83%][b]	57% [80%][b]	83% [81%][b]
L-Pyridyl-alanine	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**
	$C_{44}H_{54}N_3O_4$	$C_{42}H_{54}N_3O_5$	$C_{43}H_{53}N_4O_4$	$C_{38}H_{56}N_4O_4$	$C_{40}H_{58}N_4O_4$	$C_{41}H_{60}N_4O_4$	$C_{41}H_{54}N_4O_4$
	704.40 g/mol	695.40 g/mol	704.40 g/mol	633.45 g/mol	659.45 g/mol	673.45 g/mol	667.40 g/mol
	17 mg	19 mg	20 mg	16 mg	18 mg	19 mg	18 mg
	90%	89%	85%	90%	88%	88%	92%

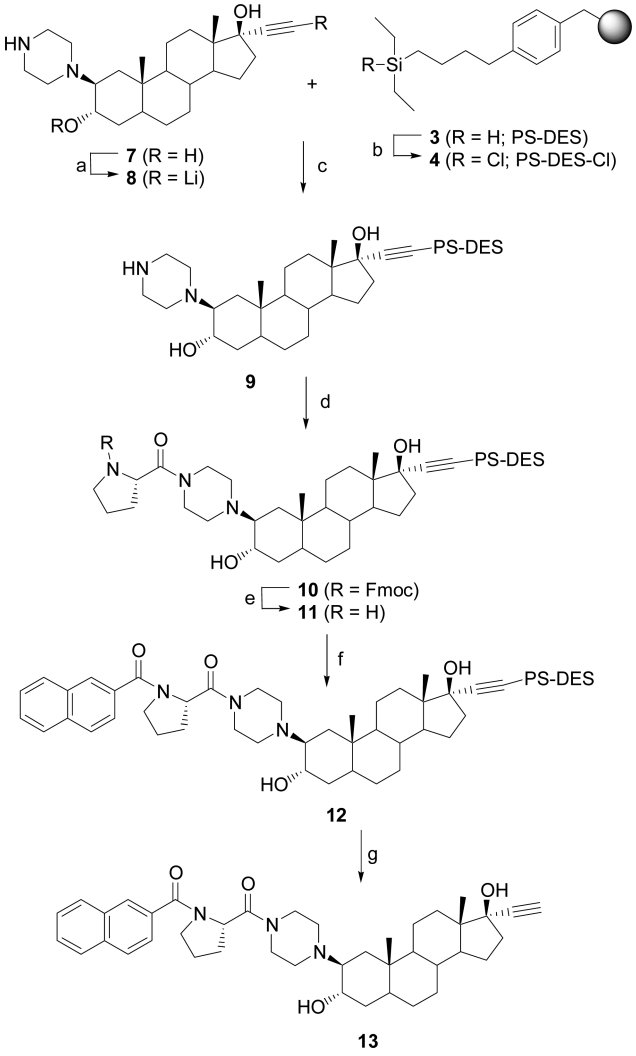
[a]This library member corresponds to compound **13**. [b]Because of an unidentified impurity which absorbs strongly in UV detection, the purity of compounds **B1**-**B7** was assessed using mass detection [data in square brackets].

second level of molecular diversity. Before the start of the library synthesis, the aminosteroid **13** (corresponding to **A1** library member) was first synthesized to investigate the compatibility of the linker with the sequence of reactions reported in Scheme 2. The preparation of this model library member was performed in two parts: steroid **7** was initially synthesized in solution using the method we previously reported[12] and then used for the solid-phase synthesis. In order to optimize the coupling of steroid **8** with the activated resin **4**, we tested the influence of MeLi stoichiometry on loading efficacy (Figure 3). We have found that dry resin weights increased by 5%, 54%, and 53% when 1.05, 2.05, and 3.05 equivalents of MeLi were used respectively to the steroid **7** (1 equivalent). The condition involving 2.05 equivalents of MeLi was chosen for the synthesis of the model and library members. Thus, MeLi was added to acetylenic compound **7** and the mixture reacted at room temperature with chlorosilyl resin **4**, swollen in THF and previously generated in situ from PS-DES resin **3**, to provide resin **9** in 54% yield by weight increase. The IR spectra of resin **9** showed the presence of the characteristic OH and NH bands attributed to the 2β-piperazine-3α-OH-androstane steroid backbone. In the next step, a *N*-Fmoc-proline was added to resin **9** giving **10** as confirmed by the IR spectra of resin **10** clearly showing the characteristic carbamate and amide bands. The Fmoc protecting group was then removed with a solution of 20% piperidine in DCM to give resin **11**, which showed in IR the disappearance of the carbamate band. The 2-naphthoic acid was introduced by an amidation step to obtain the resin **12**. Finally, the amino steroid **13** was released from resin **12** in very good purity and yield with a solution of HCl/MeOH/DCM (1:9:30). The IR, ^{1}H NMR, ^{13}C NMR, and MS analyses confirmed the introduction of diversity elements and the presence of the hydroxy acetylenic functional group.

The model library of 21 aminosteroids (Table 1) was carried out using the same strategy (Scheme 3) as for the synthesis of model compound **13**. Resin **9** was split in three portions and the first level of diversity was introduced by acylation of resin **9** with three selected Fmoc-protected amino acids (AA) to give the resins **14**. The Fmoc-protecting group was then removed and each batch of resins **15** was split in seven parts. The second level of molecular diversity was then introduced by an amidation step using seven carboxylic acids. At the end of this second step of diversification, the cleavage of the steroid

dx.doi.org/10.1021/co300034y | *ACS Comb. Sci.* 2012, 14, 347−351

Scheme 2. Synthesis of the Aminosteroid 13 Using the New Diethylsilylacetylenic Linker[a]



[a]Reagents and conditions: (a) MeLi, THF, 0 °C to rt, 75 min; (b) 1,3-Dichloro-5,5-dimethylhydantoin, DCM, rt, 1 h; (c) THF, rt, 16 h; (d) Fmoc-Pro-OH, PyBOP, HOBt, DIPEA, DMF, rt, 3 h; (e) 20% Piperidine in DCM (v/v), rt, 1 h; (f) 2-Naphthoic acid, PyBOP, HOBt, DIPEA, DMF, rt, 3 h; (g) HCl/MeOH/DCM 1:9:30 (v/v), rt, 18 h.

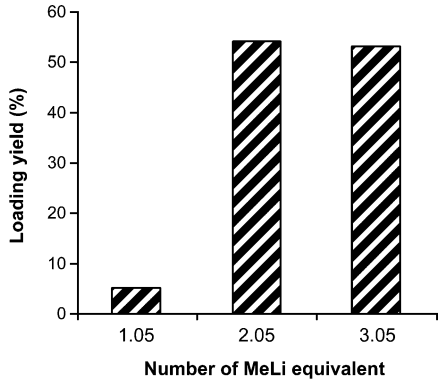
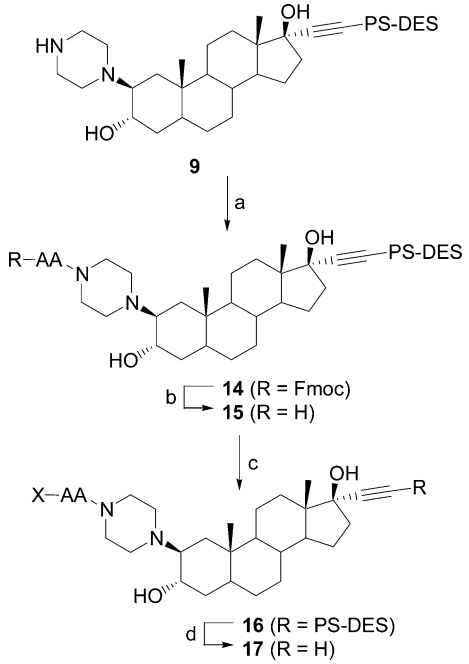


Figure 3. Loading yield (weight increase) for the coupling of steroid **7** to PS-DES resin **3** as a function of MeLi equivalents. To acetylenic compound **7** in dry THF was added MeLi and the mixture added at room temperature to PS-DES-Cl resin **4** generated from **3** and swollen in THF. After the reaction time, the resin **9** was filtered, washed with DCM, dried during 16 h and weighed.

Scheme 3. Strategy for the Synthesis of Aminosteroid Library Members[a]



[a]Reagents and conditions: (a) Fmoc-amino acid, PyBOP, HOBt, DIPEA, DMF, rt, 2 × 3 h; (b) 20% Piperidine in DCM (v/v), rt, 1 h; (c) Carboxylic acid, PyBOP, 6-Cl-HOBt, DIPEA, DMF, rt, 5 h; (d) HCl/MeOH/DCM 1:9:30 (v/v), rt, 22 h. See Table 1 for all amino acids and carboxylic acids used as building blocks.

derivatives **17** was performed from resins **16** as described before for the resin **12**. All library members showed a major one spot by thin-layer chromatography (TLC) and NMR spectra as well as the mass spectra confirmed the appropriate structures. By HPLC, the average purity of the final compounds was 87−97% for the **A1**−**A7** compounds, 80−83% for the **B1**−**B7** compounds and 85−92% for the **C1**−**C7** compounds. The lower purity for the compounds **A6**, **B1**−**B7**, and **C3** was caused by the formation of a byproduct (9−15%) corresponding to the elimination of the ethynyl group thus providing the C17-ketone derivative. Experimental mass, quantity and HPLC purity of 21 library members generated with the new diethylsilylacetylenic linker were given in Table 1. In addition to these data, ^{1}H NMR spectra of the 21 library members also confirmed the expected structures. Interestingly, the bioavailability expressed as plasmatic concentration of library member **A1** in rats (AUC_{0-12h} = 1338 ng·mL/h) was found superior to that obtained with the corresponding unacetylated compound (AUC_{0-12h} = 429 ng·mL/h).[12] This result clearly demonstrates the usefulness of this linker strategy to rapidly give access to compounds with improved metabolic stabilities.

In summary, we developed a new diethylsilylacetylenic linker to accelerate and facilitate the development of compounds possessing an ethynyl group. The preparation of a model compound using this new linker was successfully achieved by solid-phase synthesis. Following this first result, a library with two levels of molecular diversity was generated providing 21 aminosteroids of very good purity and suitable for screening of

dx.doi.org/10.1021/co300034y | ACS Comb. Sci. 2012, 14, 347−351

biological activity. The new diethylsilylacetylenic linker proved to be an efficient tool for the solid-phase synthesis of libraries of hydroxy acetylenic compounds.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for the synthesis of model compound **13** (A1) and all library memebrs. ^{1}H NMR spectra for library members **A1**−**A7**, **B1**−**B7**, and **C1**−**C7**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 1 (418) 654-2296. Fax: 1 (418) 654-2761. E-mail: Donald.Poirier@crchul.ulaval.ca.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to the Canadian Institutes of Health Research and the Quebec Breast Cancer Foundation for financial support. We would like to thank Marie-Claude Trottier for NMR, MS, and HPLC analyses, as well as Micheline Harvey for careful reading of this manuscript.

■ ABBREVIATIONS

AA, amino acid; AcOH, acetic acid; ByBOP, (benzotriazol-1-yloxy)tripyrrolidinophosphoniumhexa-fluorophosphate; 6-Cl-HOBt, 6-chloro-1-hydroxybenzotriazole; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; h, hour; HOBt, 1-hydroxybenzotriazole hydrate; HPLC, high-performance liquid chromatography; IR, infrared spectroscopy; MS, mass spectrometry; NMR, nuclear magnetic resonance; Pal, pyridyl-alanine; Phe, phenylalanine; Pro, proline; PS-DES, polystyrene diethylsilyl resin; rt, room temperature; TBAF, tetra-*n*-butylammonium fluoride; TLC, thin-layer chromatography; TFA, trifluoroacetic acid; THF, tetrahydrofuran

■ REFERENCES

(1) Moshet, C.; Hollender, J. Microbial degradation of steroid hormones in the environment and technical systems. Term Paper, Swiss Federal Institude of Technology, Institute of Biogeochemistry and Polluant Dynamics (IBP-ETH), Universitaetsstrasse 16, CH-8092 Zurich, 2009.

(2) Kuhl, H. Pharmacology of estrogens and progestogens: influence of different routes of administration. *Climateric* **2005**, *8* (Suppl 1), 3−63.

(3) De Mes, T. Z. D. Fate of estrogens in biological treatment of concentrated black water. PhD-Thesis Wageningen University, Wageningen, The Netherlands, 2007.

(4) Ebner, T.; Remmel, R. P.; Burchell, B. Human bilirubin UDP-glucuronosyltransferase catalyses the glucuronidation of ethinylestradiol. *Mol. Pharmacol.* **1993**, *43*, 649−654.

(5) de Wildt, S. N.; Kearns, G. L.; Leeder, J. S.; van der Anker, J. N. Glucuronidation in humans—Pharmacogenetic and developmental aspects. *Clin. Pharmacokinet.* **1999**, *36*, 439−452.

(6) Huber, M. M.; Ternes, T. A.; Von Gunten, U. Removal of estrogenic activity and formation of oxidation products during ozonation of 17α-ethinylestradiol. *Environ. Sci. Technol.* **2004**, *38*, 5177−5186.

(7) Thorpe, K. L.; Cummings, R. I.; Hutchinson, T. H.; Scholze, M.; Brighty, G.; Sumpter, J. P.; Tyler, C. R. Relative potencies and combination effects of steroidal estrogens in fish. *Environ. Sci. Technol.* **2003**, *37*, 1142−1149.

(8) O'Grady, D. Biodegradation of 17α-ethinylestradiol in wastewater. PhD-Thesis McGill University, Montréal, 2007.

(9) Williams, C. L.; Stancel, G. M. Estrogens and progestins. In *The Pharmacological Basis of Therapeutics*, 9th ed.; Gilman, A. G., Goodman, L. S., Hardman, G., Limbird, L., Eds.; McGraw-Hill,: New York, 1996; 1411−1440.

(10) Frincke, J. M.; Reading, C.; Auci, D.; Ahlem, C. N. Preparation of androstenes and androstanes for treating autoimmune conditions and related diseases. U.S. Patent 20080153792, Jun 26, 2008.

(11) Flores-Riveros, J.; Frincke, J. M.; Reading, C.; Stickney, D.; Ahlem, C. N. Drug screening and treatment methods. U.S. Patent 20080146532, Jun 19, 2008.

(12) Poirier, D.; Roy, J.; Maltais, R. Preparation of 2-(*N*-substituted pirerazinyl) steroid derivatives as anticancer agents. International Patent Application WO 2010060215, June 3, 2010; U.S. Patent Application 2011/0312926 A1, Université Laval, Qc, 121 pp.

(13) Al-Nadaf, A. H.; Taha, M. O. Discovery of new rennin inhibitory leads via sequential pharmacophore modeling, QSAR analysis, in silico screening and in vitro evaluation. *J. Mol. Graph. Model.* **2011**, *29*, 843−864.

(14) Burden, P. M.; Ai, T. H.; Lin, H. Q.; Akinci, M.; Costandi, M.; Hambley, T. M.; Johnston, G. A. R. Chiral derivatives of 2-cyclohexylideneperhydro-4,7-methanoindenes, a novel class of non-steroidal androgen receptor gigan: synthesis, X-ray analysis and biological activity. *J. Med. Chem.* **2000**, *43*, 4629−4635.

(15) Adema, A. D.; Bijnsdorp, I. V.; Sandvold, M. L.; Verheul, H. M.; Peters, G. J. Innovations and opportinities to improve conventional (deoxy)nucleoside and fluoropyrimidine analogs in cancer. *Curr. Med. Chem.* **2009**, *16*, 4632−4643.

(16) Bijnsdorp, I. V.; Schwendener, R. A.; Schott, H.; Fichtner, I.; Smid, K.; Laan, A. C.; Schott, S.; Losekoot, N.; Honeywell, R. J.; Peters, G. J. Cellular pharmacology of multi- and duplex drugs consisting of ethinylcytidine and 5-fluoro-2′-deoxyuridine. *Invest. New Drug.* **2011**, *29*, 248−257.

(17) Galmarini, C. M.; Popowycz, F.; Joseph, B. Cytotoxic nucleoside analogues: differents strategies to improve their clinical efficacy. *Curr. Med. Chem.* **2008**, *15*, 1072−1082.

(18) Kloosterboer, H. J.; Reed, M. J. Tibolone: a selective tissue estrogenic activity regulator (STEAR). *Maturitas* **2004**, *48* (Suppl. 1), S1−S42.

(19) Francis, C. L.; Williamson, N. M.; Ward, D. A. The synthesis of tetrahydroquinolines related to virantmycin. *Synthesis* **2004**, *16*, 2685−2691.

(20) Wuts, P. G. M.; Ritter, A. R. A novel synthesis of spironolactone. An application of the hydroformylation reaction. *J. Org. Chem.* **1989**, *54*, 5180−5182.

(21) Roy, J.; DeRoy, P.; Poirier, D. 2β-(*N*-Substituted piperazino)-5α-androstane-3α,17β-diols: parallel solid-phase synthesis and anti-proliferative activity on human leukemia HL-60 Cells. *J. Comb. Chem.* **2007**, *9*, 347−358.

(22) Roy, J.; Maltais, R.; Jegham, H.; Poirier, D. Libraries of 2β-(*N*-Substituted piperazino)-5α-androstane-3α,17β-diols: chemical synthesis and cytotoxic effects on human leukemia HL-60 cells and on normal lymphocytes. *Mol. Diversity* **2011**, *15*, 317−339.