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PARALLEL SOLID-PHASE SYNTHESIS OF A MODEL LIBRARY OF 7α-ALKYLAMIDE ESTRADIOL DERIVATIVES AS POTENTIAL ESTROGEN RECEPTOR ANTAGONISTS

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Abstract: The C17-THP derivative of 7α-(11-azidoundecanyl)-estradiol (**4**) was synthesized and coupled to an aminomethyl resin via a photolabile *o*-nitrobenzyl linker. Reduction of the azide by the Staudinger reaction to its corresponding amine followed by acylation using four activated NFmoc protected amino acids gave a first level of diversity. Subsequent deprotection of the Fmoc followed by a second acylation with five activated carboxylic acids produced, after photocleavage, a model library of twenty antiestrogen-related 7α-alkylamide estradiol derivatives in acceptable overall yields and very good purities.

Antiestrogens compete with endogenous estrogens for binding to estrogen receptors (ER), but do not activate any of the normal transcriptional estrogenic responses.[1] In addition to their promising clinical potential,[2-4] these compounds are useful tools in the study of the mode of action of estrogen receptors.[5,6] Recent advances in the comprehension on the molecular level of regulatory mechanisms of estrogen-sensitive genes have led to the concept of selective estrogen receptor modulators (SERMs).[7-9] These compounds produce antiestrogenic effects in certain tissues, while displaying estrogen-like activity in others. SERMs, along with pure antiestrogens, are promising drug candidates for the treatment of estrogen-sensitive diseases such as breast cancer and osteoporosis. It is noteworthy that differences between a complete agonist, an antagonist, and a modulator are minimal from a molecular standpoint. Moreover, the cellular context plays a predominant role in determining the biological functions of a given compound. Consequently, the rational design of SERMs or antiestrogens is a difficult task further complicated by the as yet absence of complete 3-D structures of the two types of estrogen receptors (α and β), although the structures of both the ligand binding domain[10-12] and the DNA binding domain[13] of ERα have been elucidated recently. Taking these limitations into account, a combinatorial synthetic approach becomes an attractive means of investigating the molecular determinants of the complex interactions between estrogen receptors, the ligand, and the other proteins implicated in biological responses. Herein, we report the development of a synthetic approach that could allow the rapid generation of several estradiol derivatives of antiestrogen-related structures.

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Pure steroidal antiestrogens, exemplified by ICI 164384 (Figure 1), were chosen as lead structures for the development of a solid-phase synthesis of antiestrogen-related compounds. Recently, we reported the solid-phase synthesis of N-alkyl 16β-(amidopropyl)-estradiols using the *o*-nitrobenzyl photolabile linker to attach the phenolic function to the polymer support.[14] The same linker strategy was used to perform the parallel synthesis of a model library of 7α-(alkylamide)-estradiol derivatives (compounds **33–52**).

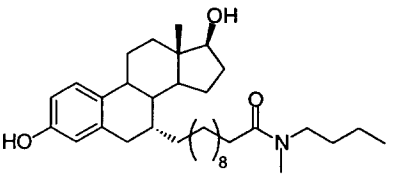
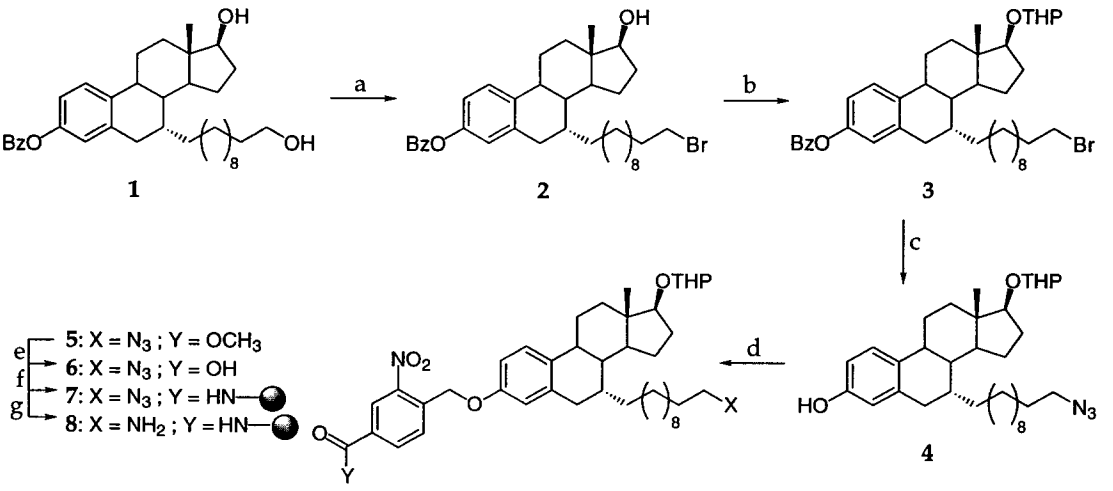


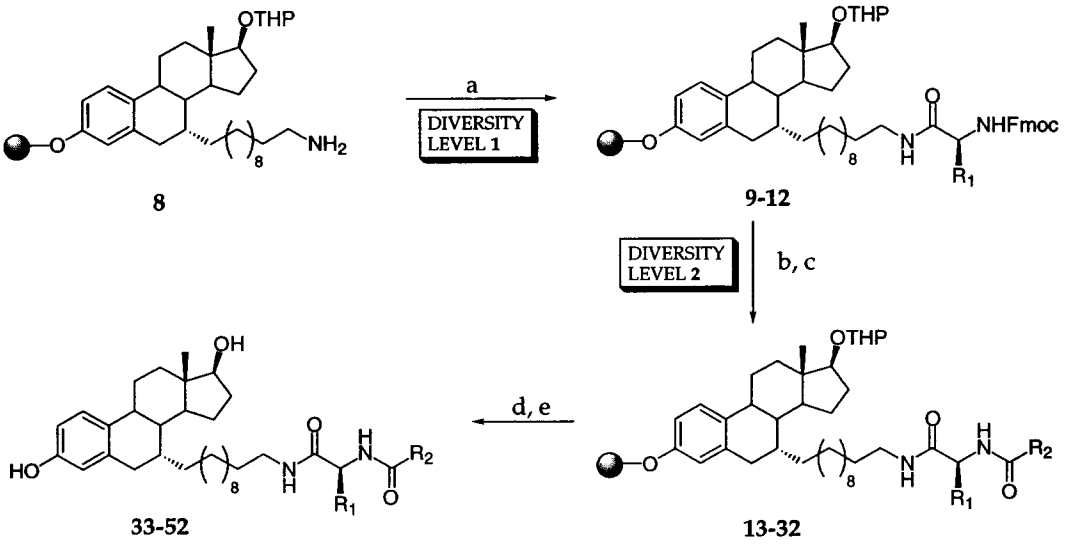
Figure 1. ICI 164384, a pure steroidal antiestrogen

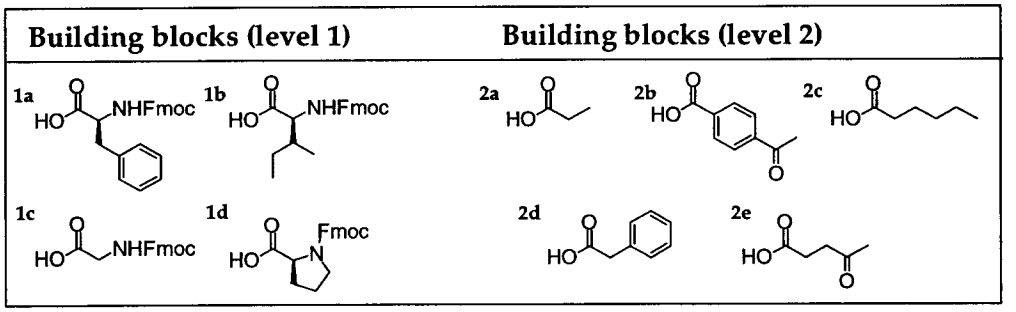
The estradiol derivative **4** was synthesized from intermediate **1**,[1] which was readily available in our laboratory (Scheme 1). Regioselective bromination of the primary alcohol gave **2** and the secondary 17β-alcohol was protected as a tetrahydropyranyl ether to provide **3**. Displacement of the primary bromide by an azide anion was accompanied by the cleavage of the benzoyl ester once the reaction was heated, thus resulting in the desired precursor **4**. This precursor was *O*-alkylated with the *o*-nitrobenzyl linker to give the methyl ester **5**, which was hydrolyzed to **6** prior to its attachment onto the aminomethyl resin to yield **7**. The presence of the steroid derivative on the solid support was monitored by FT-IR (N_3 stretch at 2095 cm^{-1}) and the completion of the coupling reaction was confirmed by a negative Kaiser test.[15] Azide **7** was reduced to the amine **8** according to an adapted procedure described by Vaultier and co-workers.[16,17]



Scheme 1. *Reagents and conditions:* (a) CBr_4, PPh_3, CH_2Cl_2, 0 °C (64%); (b) Dihydropyran, *p*-TSA, CH_2Cl_2, 25 °C (90%); (c) NaN_3, DMF, 80 °C (77%); (d) Cs_2CO_3, methyl 4-(bromomethyl)-3-nitro-benzoate,[18] CH_3CN/DMF (4:1), 25 °C (65%); (e) LiOH aq, THF, 25 °C (76%); (f) Aminomethyl resin (0.75 mmol/g), EDC, HOBt, DMF, 25 °C; (g) *i.* PPh_3, THF, 25 °C; *ii.* H_2O, 70 °C.

The resin **8** was split into 4 polypropylene solid-phase reactors[19] and each vessel was submitted to a standard N-Fmoc amino acid coupling to give the first level of diversity (Scheme 2, compounds **9–12**). Then, the Fmoc protecting group was removed and the resin was again split into 5 new vessels for a second acylation (compounds **13–32**). Acidic THP hydrolysis and photocleavage gave twenty final compounds **33–52**.[20] Acceptable overall yields and very good HPLC purities were obtained for all library members except for three compounds having levulinic acid (*2e*) as a building block (Table 1). All structures were confirmed by ^{1}H NMR spectroscopy and mass spectrometry. Compounds were tested after photocleavage without further purification.





Scheme 2. *Reagents and conditions:* (a) Fmoc-NHCHR$_1$-COOH, HBTU, HOBt, *i*-Pr$_2$EtN, DMF, 25 °C; (b) 20% piperidine, DMF, 25 °C; (c) R$_2$COOH, HBTU, HOBt, *i*-Pr$_2$EtN, DMF, 25 °C; (d) *p*-TSA, 1-butanol/ClCH$_2$CH$_2$Cl (1:1), 25 °C; (e) hv (350 nm), MeOH, 25 °C.

To validate our approach, each member of the model 7α-(alkylamide)-estradiol library was individually tested *in vitro* to assess its ability to inhibit estrogen-induced proliferation of T-47D human breast cancer cells in culture (Figure 2).[21] As can be seen, all the newly synthesized compounds **33–52** displayed significant antiproliferative activities at 0.1 μM. At this concentration, several of the library members showed potencies close to that of the

Table 1. Chemical characterization of the 20-member model library of 7α-estradiol derivatives.

Compounds	R_1	R_2	Theoretical mass (g/mol)	Observed mass[a] (rel. int, %)	Overall yield (%)[b]	HPLC purity (%)[c]
33	**1a**	**2a**	644.5	643.5 (100)	26	95
34	**1a**	**2b**	734.5	733.5 (100)	14	92
35	**1a**	**2c**	686.5	687.6 $[M+H]^+$	19	86
36	**1a**	**2d**	706.5	705.3 (100)	17	94
37	**1a**	**2e**	686.5	685.6 (100)	12	44
38	**1b**	**2a**	610.5	609.4 (100)	17	93
39	**1b**	**2b**	700.5	699.8 (100)	27	91
40	**1b**	**2c**	652.5	651.4 (100)	23	94
41	**1b**	**2d**	673.0	673.9 $[M+H]^+$	23	91
42	**1b**	**2e**	652.5	651.7 (100)	24	61
43	**1c**	**2a**	554.4	553.5 (100)	22	92
44	**1c**	**2b**	644.4	643.6 (100)	23	89
45	**1c**	**2c**	596.5	595.4 (100)	27	88
46	**1c**	**2d**	616.4	615.4 (100)	27	86
47	**1c**	**2e**	596.4	595.2 (72)	21	66
48	**1d**	**2a**	594.4	593.4 (100)	31	94
49	**1d**	**2b**	684.5	683.3 (100)	29	88
50	**1d**	**2c**	636.5	635.5 (100)	33	90
51	**1d**	**2d**	656.5	655.5 (100)	38	95
52	**1d**	**2e**	636.5	635.9 (100)	26	92

[a] LRMS $[M-H]^-$; Recorded on API-150ex apparatus equipped with a turbospray ion source. [b] Calculated for the sequence of 7 steps (from **6** to **33–52**) using the manufacturer loading of AM resin (NovaBiochem, 0.75 mmol/g). [c] HPLC (Waters); UV detector at 205 nm; Nova Pak C18 column (150 mm x 3.9 mm); Flow rate: 1 mL/min.

antiestrogen ICI 164384. Interestingly, the most potent compounds of each series of amino acids (level 1) did not belong to the same category of carboxylic acids (level 2). For example, alkyl substituents such as propionyl (***2a***) and levulinyl (***2b***) groups were most effective with compounds that possessed Phe (***1a***) or Ile (***1b***) as amino acid building blocks. However, for Gly- or Pro-containing compounds (***1c***, ***1d***, respectively), aromatic substituents such as the phenylacetyl (***2d***) or *p*-acetylbenzoyl (***2b***) groups were the better suited. In fact, compounds built with these aromatic groups in combination with a prolyl residue displayed the most antiestrogenic activities among the twenty new derivatives. Furthermore, no stimulation of basal T-47D cells proliferation was observed in absence of estradiol for any of the library members tested at 0.1 μM (data not shown).

In conclusion, these results illustrated the power of the described solid-phase synthesis to more rapidly identify promising lead compounds when compared with conventional methods of medicinal chemistry. The possibilities afforded by the described solid-phase synthesis could be further extended by additional levels of diversity, especially through the use of amino acids. This efficient parallel solid-phase synthesis is also compatible for use in encoded split-and-pool combinatorial synthesis, which could be used to generate a larger number of estradiol derivatives. Biological screening of these larger libraries should prove valuable when determining structure-activity relationships with different biological targets including estrogen receptors and steroidogenic enzymes. In

our knowledge, this is the first study targeted to the rapid generation of phenolic steroid derivatives, which are interesting drug candidates.

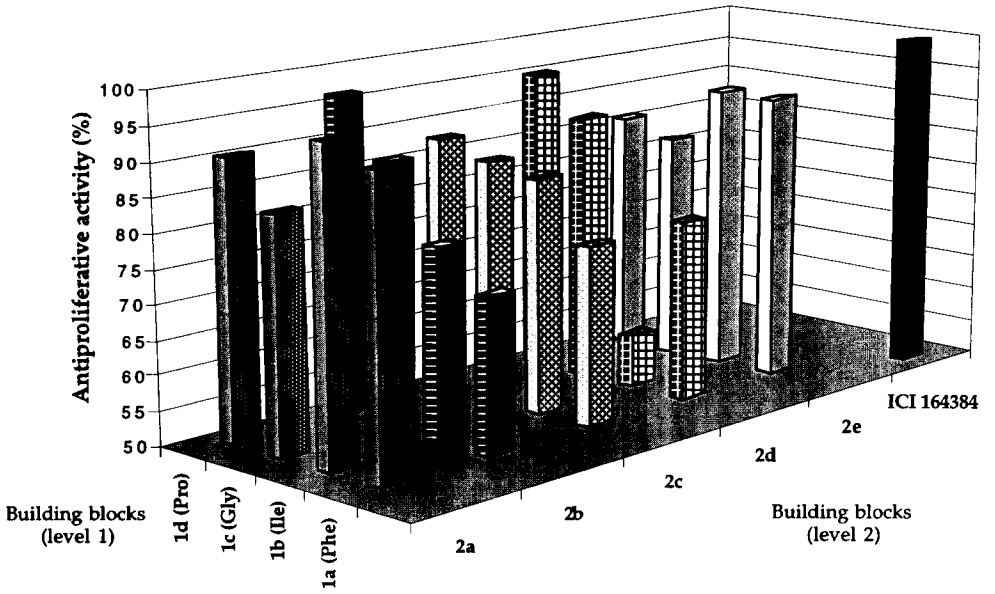


Figure 2. Effect of 0.1 μM of the 20-member 7α-(alkylamide)-estradiol derivatives model library on estradiol (0.1 nM)-induced proliferation of human breast cancer cell line T-47D. Antiestrogenic activity is expressed as percentage of inhibition of estradiol-mediated cell growth, which was calculated as follows: % inhibition = 100 x {1 - [(μg DNA drug treated - μg DNA control)/(μg DNA E_2 treated - μg DNA control)]}. Results are expressed in mean ± SEM of triplicates.

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19. Reactions were performed in 10 mL fritted polypropylene PD-10 columns (Pharmacia Biotech 17-0435-01) equipped with nylon 3-way stopcocks (Biorad 732-8107).
20. *Typical procedure*: To a sample of resin **7** (1.8 g; 1.3 mmol) suspended in 25 mL of dry THF in a round-bottom flask was added PPh_3 (1.8 g; 6.7 mmol) and the mixture was vortexed in absence of light at room temperature. After 24 h, water (7 mL) was added and the suspension was gently heated at 70 °C for an additional 16 h. The resulting resin was filtered, washed successively with THF (5 x 10 mL), DMF (5 x 10 mL), CH_2Cl_2 (5 x 10 mL), MeOH (3 x 10 mL), and dried under vacuum for 16 h to afford 1.8 g of resin **8**. The Kaiser test was clearly positive and the azide stretch has disappeared in the FT-IR spectrum. The resin **8** was splitted in 4 portions of 400–500 mg and each portion was submitted to the following coupling reaction: In a dry round-bottom flask containing a solution of Fmoc-$HNCHR_1$-COOH (3 equiv.; 0.9 mmol) dissolved in DMF (3 mL) was added successively HBTU (3 equiv.; 0.9 mmol) and HOBt (3 equiv.; 0.9 mmol). The activation was allowed to stir 15 min at 0 °C before the addition of diisopropylethylamine (6 equiv.; 1.8 mmol) and the resulting solution was transferred via a syringe to a suspension of amino resin **8** in 2 mL of DMF. The resulting suspension was vortexed for 1 h at 25 °C and the resin was filtered, washed with DMF (5 x 3 mL) and CH_2Cl_2 (5 x 3 mL). The resins **9–12** (450 mg) were suspended in 2 mL of piperidine/DMF (2:8) and vortexed for 15 min at 25 °C. The resin was filtered and suspended again in 2 mL of piperidine/DMF (2:8). After 1 h of mixing, the resins were filtered again and washed with DMF (10 x 3 mL). Each resin was splitted again in 5 portions of 80–90 mg. The resulting resin was treated with 1.2 mL of a 0.17 M of the activated carboxylic acid prepared as above. After 90 min of mixing at 25 °C, the resins were filtered, washed with DMF (5 x 2 mL), CH_2Cl_2 (5 x 2 mL), MeOH (5 x 2 mL), and dried for 16 h under vacuum. Sample resins **13–32** were suspended separately in 2 mL of a 0.07 M of *p*-TSA in a mixture of 1,2-dichloroethane/1-butanol (1:1) for 24 h at room temperature. Then, each sample was filtered and washed with DMF (5 x 3 mL), CH_2Cl_2 (5 x 3 mL), MeOH (5 x 3 mL), and dried 16 h under vacuum. The dried corresponding resins (60–80 mg) were suspended in 1.5 mL of oxygen-free MeOH and irradiated at 350–365 nm with a UVP high intensity longwave lamp (B-100AP model) for 24 h at 25 °C. Then, resins were filtered and washed with CH_2Cl_2 (4 x 2 mL) and MeOH (4 x 2 mL) to give, after evaporation of solvents, 5–12 mg of the desired compounds **33–52**.
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