Design of Peptido-Steroid Libraries for Development of New Therapeutic Agents: I. Solid-Phase Synthesis

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INTRODUCTION

- We were interested in diversifying different steroid scaffolds taking advantage of the automated Fmoc-peptide chemistry on solid support.
- Our chemical challenge was to find the appropriate linkers for the coupling of different functionnalized steroids (ketone, alcohol and phenol) on polystyrene resin and to perform the Fmoc-peptide chemistry for the synthesis of steroid derivatives in good yields and acceptable purities for biological assays.
- We can now report the solid phase synthesis of peptido-steroid libraries bearing a small peptide unit (2-3 amino acids) for targeting several kinds of biologically relevant steroids including:
  - C2, C3, C7 and C11 peptido-steroids with an androstane (C19 steroid) nucleus
  - C7-, C16- and C17-peptido-steroids with an estradiol (C18 steroid) nucleus

CONCLUSION

- Four different linkage strategies, compatible with Fmoc chemistry, were used to achieve the solid phase synthesis of model libraries of peptido-steroids at various positions of the steroid scaffold (2β, 3β, 7α, 11β, 16β and 17α).
- The recommended linkers and resins are:
  - silyl ether linker (PS-diethylsilyl-resin) for steroidal alcohol
  - ketal linker (PS-1-glycerol-resin) for steroidal ketone
  - sulfamate linker (PS-trityl chloride resin) for steroidal phenol
- Good crude yields of peptido-steroids were obtained for the synthesis of different libraries with acceptable purity levels for screening, without further purification.
- Our lab in now ready to extend these model libraries to a more voluminous size thanks to the recent acquisition of a fully automated peptide synthesizer from aapptec.

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(3) **(a)** Tremblay, M. R.; Poirier, D. *Journal of Combinatorial Chemistry* (2000), 2, 48-65.
(b) Tremblay, M. R.; Poirier, D. *Tetrahedron Letters* (1999), 40, 1277-1280.

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- Poirier, D.; Maltais, R. *M. Rev. Med. Chem.* (2006), 6, 37-52.

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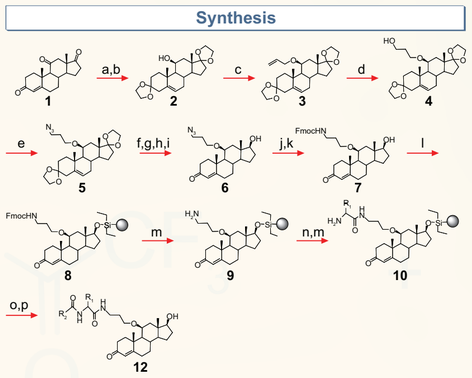
RESULTS

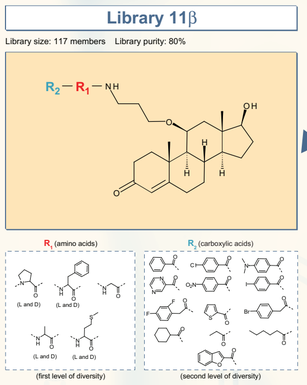
Synthesis



Reagents and conditions: (a) TMOF, p-TSA, ethylene glycol, benzene, rt (90%) (b) $LiAlH_4$, THF, rt (70%); (c) NaH, allyl bromide, $(nBu)_4NI$, DMF, rt (80%); (d) 1) 9-BBN, H_2O_2, THF, rt 2) NaOH, rt (80%); (e) PPh_3, DIAD, $(Ph)_2P(O)N_3$, THF, 0°C to rt (70%); (f) HCl 3M / acetone (9:1), rt (90%); (g) TMOF, p-TSA, ethylene glycol, benzene, rt.; (h) $NaBH_4$, MeOH, rt (90%); (i) HCl 3N / acetone (60%, 3 steps); (j) PPh_3, THF, H_2O, rt; (k) Fmoc-OSu, $NaHCO_3$, dioxane-H_2O (40%, 2 steps); (l) Cl-Si-$(CH_2CH_3)_2$-PS, CH_2Cl_2, imidazole, CH_2Cl_2, rt; (m) piperidine: CH_2Cl_2 (8:2), rt; (n) N-Fmoc-L-amino acid (FmocNHCH(R_3)COOH), PyBOP, HOBt, DIPEA, DMF, rt; (o) carboxylic acid (R_2COOH), PyBOP, HOBt, DIPEA, DMF, rt; (p) HCl 2M / MeOH (20%) / THF, rt.

Library 11β

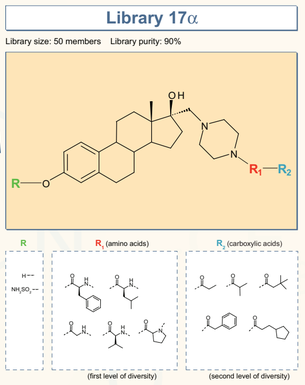
Library size: 117 members Library purity: 80%



(first level of diversity) (second level of diversity)

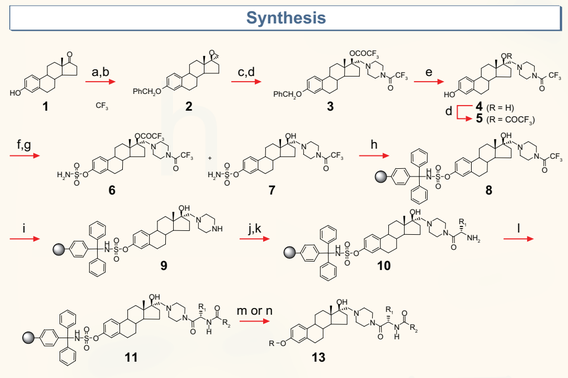
Library 17α

Library size: 50 members Library purity: 90%



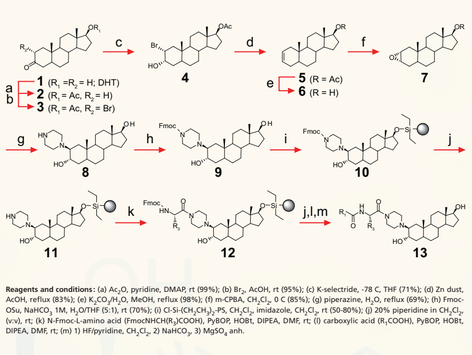
(first level of diversity) (second level of diversity)

Synthesis



Reagents and conditions: (a) $PhCH_2Br$, Cs_2CO_3, CH_3CN reflux; (b) NaH, $Me_3S^+I^-$, DMSO (76 %, 2 steps); (c) piperazine, EtOH, 55-60°C; (d) DMAP, $(CF_3CO)_2O$, CH_2Cl_2, 0 °C (76 %, 2 steps); (e) 20% $Pd(OH)_2/C$, H_2, MeOH-EtOAc (3:1, v/v) (46% of 4 and 39% of 5); (f) H_2NSO_2Cl, DBMP, CH_2Cl_2 (78 %, crude); (g) flash, silica gel, hexanes: acetone (70 : 30) (h) trityl chloride resin, DIPEA, CH_2Cl_2, rt, 12 h; (i) NaOH 3N, THF, rt, 3 h; (j) PyBrOP, HOBt, R_1-CH(NHFmoc)COOH, DIPEA, DMF, rt, 3 h; (k) 20 % piperidine/CH_2Cl_2, rt, 1 h; (l) PyBOP , R_2-COOH, DIPEA, DMF, rt; 3 h; (m) 5 % TFA/CH_2Cl_2, rt, 4 h; (n) piperazine, THF, 45-50 °C, 3 h.

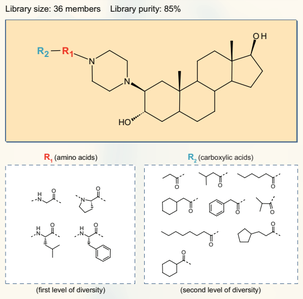
Synthesis



Reagents and conditions: (a) Ac_2O, pyridine, DMAP, rt (99%); (b) Br_2, AcOH, rt (95%); (c) K-selectride, -78 C, THF (71%); (d) Zn dust, AcOH, reflux (83%); (e) K_2CO_3/H_2O, MeOH, reflux (98%); (f) m-CPBA, CH_2Cl_2, 0 C (85%); (g) piperazine, H_2O_2, reflux (69%); (h) Fmoc-OSu, $NaHCO_3$ 1M, H_2O/THF (5:1), rt (70%); (i) Cl-Si-$(CH_2CH_3)_2$-PS, CH_2Cl_2, imidazole, CH_2Cl_2, rt (50-80%); (j) 20% piperidine in CH_2Cl_2, (v:v), rt; (k) N-Fmoc-L-amino acid (FmocNHCH(R_3)COOH), PyBOP, HOBt, DIPEA, DMF, rt; (l) carboxylic acid (R_1COOH), PyBOP, HOBt, DIPEA, DMF, rt; (m) 1) HF/pyridine, CH_2Cl_2, 2) $NaHCO_3$, 3) $MgSO_4$ anh.

Library 2β

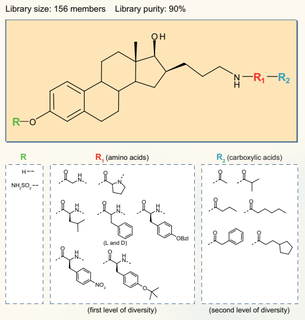
Library size: 36 members Library purity: 85%



(first level of diversity) (second level of diversity)

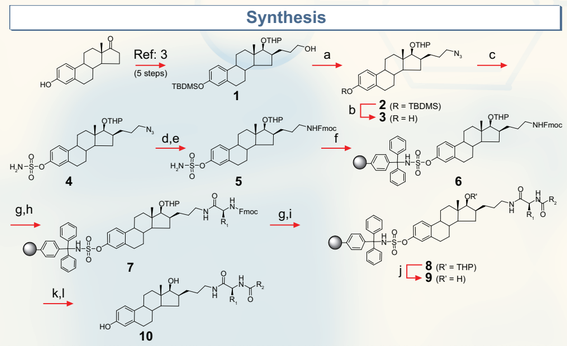
Library 16β

Library size: 156 members Library purity: 90%

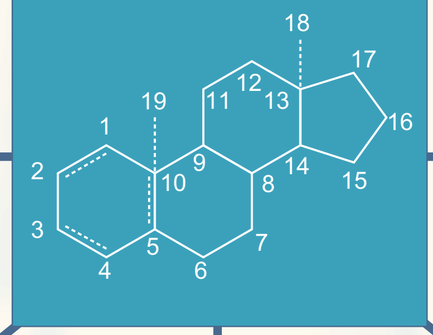


(first level of diversity) (second level of diversity)

Synthesis



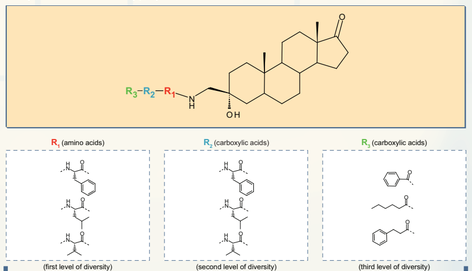
Reagents and conditions: (a) PPh_3, DIAD, $(C_6H_5O)_2P(O)N_3$, THF, rt (90%); (b) TBAF, THF, 0°C (94%); (c) NH_2SO_2Cl, DBMP, CH_2Cl_2, rt; (d) 5% (w/w) Pd $(OH)_2$/ C, EtOAc-MeOH (1:9, v/v); (e) $NaHCO_3$, FmocOSu, THF: H_2O (3:1, v/v), rt (68%, 3 steps); (f) trityl chloride resin, CH_2Cl_2, rt (70%); (g) 20% piperidine/CH_2Cl_2, rt; (h) PyBrOP, HOBt, R_1CH(NHFmoc)COOH, DIPEA, DMF, rt; (i) PyBOP, R_2COOH, DIPEA, DMF, rt; (j) 0.07 M p-TSA in 1-butanol: $ClCH_2CH_2Cl$ (1:1), rt; (k) 30% HFIP / CH_2Cl_2, rt.; (l) piperazine, THF, 45-50°C.



Libraries of peptido-steroids at various positions of steroid scaffold

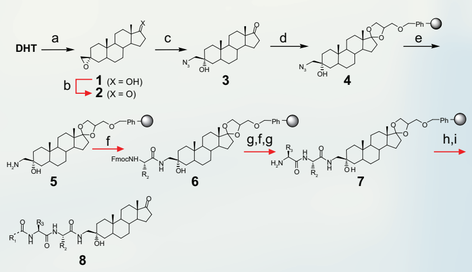
Library 3β

Library size: 27 members Library purity: 88%



(first level of diversity) (second level of diversity) (third level of diversity)

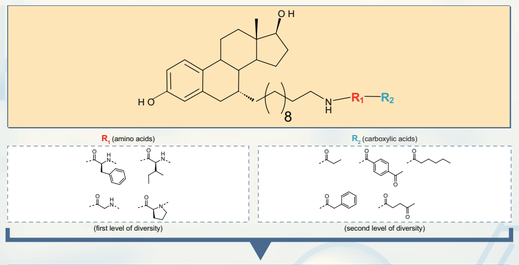
Synthesis



Reagents and conditions: (a) NaH, Me_3SOI, DMSO (70%); (b) TPAP, NMO, mol. sieve, CH_2Cl_2 (95%); (c) NaN_3, HBO_3, DMF, 100°C (75%); (d) polymer-bound glycerol (1.0 mmol/g), TMOF, $Sc(OTf)_3$, toluene, rt; (e) SnCl$_2$ (0.2 M), PhSH (0.8 M), Et_3N (1.0 M); (f) (FmocNHCH(R_2 or R_3)COOH)-L-amino acid, PyBOP, HOBt, DIPEA, DMF, rt; (g) piperidine:CH_2Cl_2 (2:8), rt; (h) carboxylic acid , PyBOP, HOBt, DIPEA, DMF, rt; (i) HCl 2.0 N in dioxane (containing 1% of H_2O), rt.

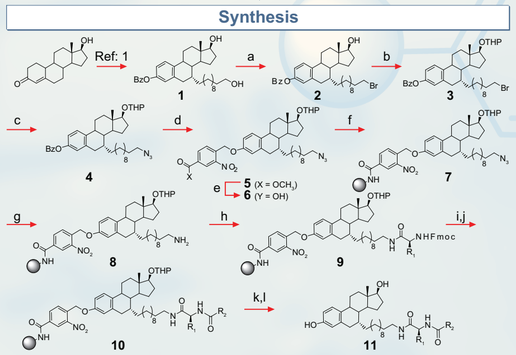
Library 7α

Library size: 20 members Library purity: 85%



(first level of diversity) (second level of diversity)

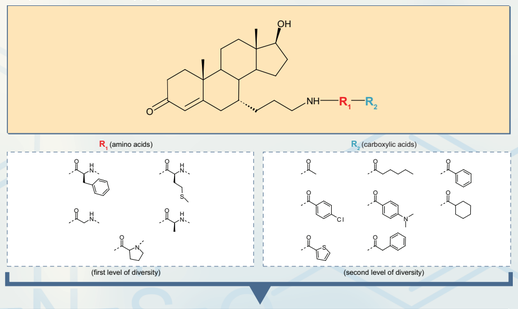
Synthesis



Reagents and conditions: (a) CBr_4, PPh_3, CH_2Cl_2, 0°C (64%); (b) Dihydropyran, p-TSA, CH_2Cl_2, rt (90%); (c) NaN_3, DMF, 80°C (77%); (d) Cs_2CO_3, methyl 4-(bromomethyl)-3-nitro-benzoate, CH_3CN/DMF (4:1), rt (65%); (e) LiOH aq, THF, rt (76%); (f) aminomethyl resin, EDC, HOBt, DMF, rt; (g) i. PPh_3, THF, rt; ii. H_2O, 70°C; (h) Fmoc-NHCHR$_1$-COOH, HBTU, HOBt, DIPEA, DMF, rt; (i) 20% piperidine, DMF, rt; (j) R_2COOH, HBTU, HOBt, DIPEA, DMF, rt; (k) p-TSA, 1-butanol/$ClCH_2CH_2Cl$ (1:1), rt; (l) hv (350 nM), MeOH, rt.

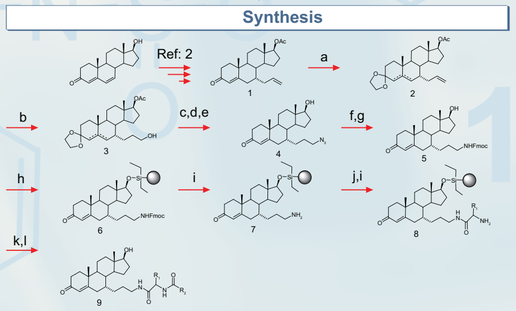
Library 7α

Library size: 32 members Library purity: > 80%



(first level of diversity) (second level of diversity)

Synthesis



Reagents and conditions: (a) ethylene glycol, p-TSA, toluene, reflux (80%); (b) 9-BBN, H_2O_2, NaOH, rt (90%); (c) PPh_3, $(Ph)_2P(O)N_3$, DIAD, THF, 0°C to rt; (d) HCl 3M / acetone (1:1); (e) K_2CO_3, MeOH, 50°C (65%, 3 steps); (f) PPh_3, H_2O, rt; (g) Fmoc-OSu, $NaHCO_3$, dioxane-H_2O (60%, 2 steps); (h) Cl-Si-$(CH_2CH_3)_2$-PS, CH_2Cl_2, imidazole, CH_2Cl_2, rt; (i) piperidine : CH_2Cl_2 (8:2), rt; (j) N-Fmoc-amino acid (FmocNHCH(R_1)COOH), PyBOP, HOBt, DIPEA, DMF, rt; (k) carboxylic acid (R_2COOH), PyBOP, HOBt, DIPEA, DMF, rt; (l) HCl 2M / MeOH (20%) / THF, rt.