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Solid phase synthesis of hydantoins by thermal cyclization and screening of reaction conditions using APOS 1200

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Summary

A novel strategy for solid-phase synthesis of hydantoins with high optical purity is described using a thermal pH-neutral cyclization and simultaneous release from resin. Hereby even hydantoins bearing a pH-sensitive side chain (protection) are available. The reaction conditions are well screened applying the parallel organic synthesizer APOS 1200.

Abbreviations: AA, amino acid; AC, 4-hydroxymethyl-3-methoxyphenoxyacetic acid; Boc, t-butyloxycarbonyl; DMF, N,N-dimethylformamide; Fmoc, 9-fluorenylmethyloxycarbonyl; FT-ATR-IR, Fourier transform attenuated total reflexion infrared spectroscopy; PHB, para hydroxybenzyl linker; PEG, polyethyleneglycol; RAM, Rink amide linker; TG, TentaGel; THF, tetrahydrofuran; Trt, trityl; UV, ultraviolet.

Introduction

The parallel solid-phase synthesis of small molecules has been established to support the drug development process. Heterocycles are compounds of particular interest because they provide a scaffold that fixes pharmacophoric groups in a defined way [1].

Hydantoins, for example, cover a 5-membered rigid ring with different substitution pattern and have attracted much interest. Various solid-phase synthesis strategies have been described in the last years with a common feature: acidic or basic conditions are required for cyclization or cleavage [2a–i]. This may restrict a sequence if pH-sensitive side chains are desired and furthermore certain basic conditions [2i] promote complete racemisation of the chiral products. Here we describe the adaption and optimized scope of a solution-phase synthesis of hydantoins [3] via a thermal pH-neutral cyclization to the solid support. We use our novel synthesizer *APOS 1200*[1] to speed up the process of screening to find the optimal reaction conditions as well as to produce a representative 14-membered library.

Results and discussion

The reaction sequence which is outlined in Figure 1 includes 3 steps and starts with *TentaGel*[1] resins preloaded with an Fmoc amino acid. The Fmoc amino acid is either attached to the resin via an acid labile linker or directly to the hydroxyl-group of the TentaGel-S-OH resin to form a PEG-ester derivative. After Fmoc cleavage with piperidine an urea is formed by acylation with an isocyanate or isothiocyanate; finally the hydantoin is created by a thermal cyclization/cleavage step and protected hydantoin derivatives are obtained.

The reaction parameters of the final step, i.e. type of isocyanate, isothiocyanate, amino acid side chain,

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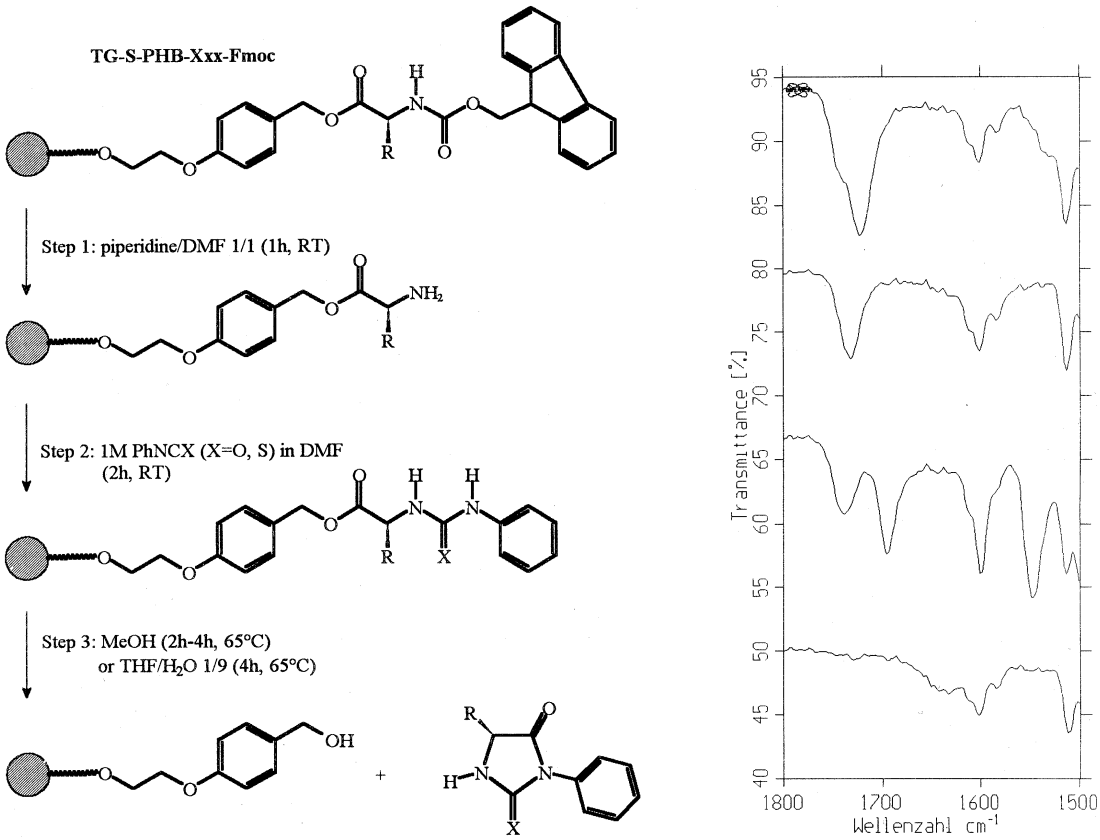


Figure 1. Reaction sequence of solid-phase synthesis of hydantoins by thermal cyclization and ATR-FT-IR spectra[4] of the corresponding resins.

Table 1. Influence of the substituent at the urea precursor for cyclization and release of 5-benzyl-hydantoins in water at 65 °C/4 h from the PHB-resin

Substituent of the N-terminus	Yield (%)
Propyl	<5
Cyclohexyl	<5
Phenyl[3]	80
4-NO_2-Phenyl	18
4-Cl-Phenyl	38
4-MeO-Phenyl	92

linkage and solvent for cyclization were examined in detail.

We observed that N-phenyl substituted urea derivatives of amino acids tend to cyclize easily under thermal conditions while with the propylurea derivatives the product is obtained in less than 5% yield. Side by side experiments indicate that thermal treatment of the Fmoc-Phe-PHB-resin in water hydrolyses the benzylester linkage partially which results in an amino acid leakage up to 5%. To investigate whether there is a steric or electronic influence to the cleavage/cyclization reaction, we prepared the corresponding cyclohexyl urea derivative. The cyclohexyl group has no resonance effect but is sterically almost as bulky as the phenyl group. Upon thermal treatment of the cyclohexylurea derivative cyclization and cleavage was observed in less than 5% yield. Obviously the mesomeric influence of the phenyl group is essential for high yields by thermal cleavage and cyclization. Next we considered substituted phenylurea precursors. While aromatic derivatives with an electron-withdrawing group (–Cl, –NO_2) showed reduced tendency to cyclize, the opposite was found for derivatives with an electron-donating group (–OMe, Table 1).

Furthermore, cyclization was found to be enhanced for phenylthioureas in comparison to corresponding

Table 2. Conditions for cyclization of 5-benzyl-3-phenyl-hydantoin at 65 °C (Table 5, No. 10) and ATR-FT-IR spectra[4] of the remaining resins. Chemical yields are based on the theoretical recovery of product starting from preloaded TG-S-PHB-Phe-Fmoc resin

Solvent Time of reaction	Yield (%)	Lane
H_2O 4 h	80	–
MeOH 2 h	79	A
MeOH 4 h	81	B
THF 4 h	6	C
THF/H_2O 9/1 4 h	11	D
THF/H_2O 1/1 4 h	73	E
THF/H_2O[3] 1/9 4 h	77	F
Dioxan/H_2O 1/1 4 h	69	G

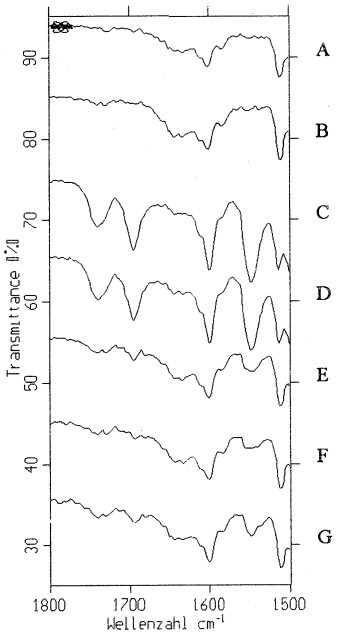
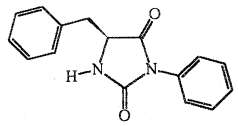
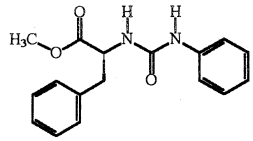


Table 3. Comparison of analytical methods for determination of the detected side product N-phenylcarbamyl-phenylalanine methyl ester during cyclization of 5-benzyl-3-phenyl-hydantoin (Table 1) in MeOH (65 °C, 4 h)

Structure	M ESI-MS calc.	HPLC- UV (220 nm) $[M+H]^+$ (%)	HPLC- UV (240 nm) R_t[a] (%)	HPLC- R_t[a] (%)	^{1}H-NMR (%)
	266	267 + 308 (90)	18.2 min (99)	18.2 min (90)	(93–94)
	298	299 (10)	21.6 min (1)	21.6 min (10	(6–7)

[a] Analytical HPLC: 250×4.6 Gromsil ODS2 column with 5 μm particle size; gradient elution 5%–95% acetonitrile containing 0.08% TFA for 40 min then 95% acetonitrile containing 0.08%TFA for 5 min; flow 1 ml/min.

Table 4. Determination of optical purities by GC-MS: Amount of detected D-Val dependent on cleavage/cyclization conditions

Solvent	Temperature (°C)	Reaction time (h)	D-Val
THF/H_2O 9/1	65	4	3.3
THF/HCl(2N) 9/1	65	4	3.4
THF/HCl(6N) 9/1	65	4	2.79
TFA/CH_2Cl_2 1/1	65	4	3.16

phenylureas as measured by FT-ATR-IR spectra of the remaining resin but was found to be similar concerning chemical yields. A partial Edman release of the thiohydantoin immediately after thiourea formation or during the following washing procedure cannot be excluded [2d, 5]. Comparison of the different amino acid side chains shows no effect to the cyclization reaction. Similar high purities and yields were obtained for a broad range of amino acids with different functionalities and protecting groups (Table 5). The resin-bound phenylurea derivative of proline seems to be sterically hindered and low yields are obtained (No. 11, Table 5) whereas the corresponding phenylthiourea derivative gave higher yields (No. 13, Table 5).

The reaction is furthermore influenced by the type of linkage. If the amino acid is fixed by an ester bond to commonly used acid-labile linkers as well as to TentaGel-S-OH via a PEG-ester linkage, the thermal cyclization proceeds. For example, the phenylurea derivative of the resin bound PEG ester of leucine was formed as described in Figure 1. Treatment of this resin with pure water at 65 °C/4 h yields the hydantoin in 87%. If the amino acid is fixed to the resin via an amide bond (e.g. TentaGel-S-RAM) no cyclization can be observed.

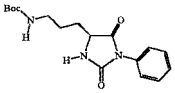
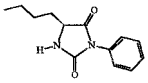
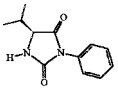
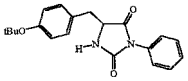
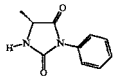
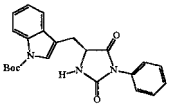
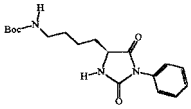
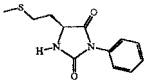
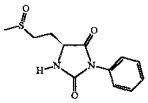
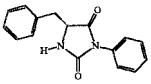
The solvent has substantial impact on this reaction and the advantage of TentaGel vs. polystyrene, its capability to a wide range of solvents including aqueous systems and water itself, was very useful in this case. High yields are achieved by using water for cleavage. The phenylurea derivative of the resin bound PHB ester of phenylalanine was formed as described in Figure 1 and treatment of this resin with pure water at 65 °C/4 h yields the hydantoin in 80%. Also MeOH (65 °C, 2–4 h) results in quantitative cleavage in most cases. A disadvantage of using MeOH is the formation of the N-phenylcarbamyl amino acid methyl ester as a side product. Investigation of the portion of formed methylester was between 1 and 10% depending on the chromatographic or spectroscopic method (Table 3).

However, the ratio of hydantoin methylester is nearly constant over the reaction time, indicating a side reaction rather than a general cleavage followed by cyclization. The dipolar aprotic solvent THF avoids the described formation of the side product but promotes the cyclization only to a small extent. THF/H_2O mixtures (1/1 and 1/9) combine high yields with high purities. Especially during this examination FT-ATR-IR spectroscopy [4] proved to be a useful and fast method for *on bead* reaction monitoring (Table 2).

From all these results the driving force of cyclization and concomitant release from resin is the formation of a 5 membered cyclic hydantoin starting with a hydrogen-bond assisted, nucleophilic attack of a phenyl substituted urea nitrogen to a carbonyl group of an ester in the β-position.

Finally the optical purities of the chiral products were determined. TG-S-PHB-L-Val-Fmoc was subjected to the general reaction sequence (Figure 1, Step 1 piperidine/DMF 1/1 and Step 2 1M PhNCO in DMF) and the hydantoin was cleaved under pH-neutral and various acidic conditions. The optical purities of the products were assessed using standard routines for amino acid analysis.[2] The partially hydrolyzed derivatives were analyzed by GC-MS. Independent from the applied reaction sequence that included either a cyclization at 65 °C/pH neutral conditions or at 65°C/acidic conditions, the same amount of D-valine was formed (Table 4).

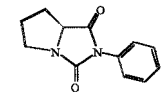
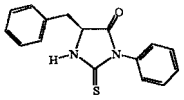
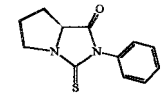
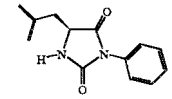
Table 5. Representative structures, calculated molecular weights (^{12}C, ^{1}H, ^{14}N, ^{16}O, ^{32}S), found mass peaks, chemical yields based on the theoretical recovery of product starting from preloaded TG-S-PHB-AA-Fmoc (No. 1–13) and TG-S-AA-Fmoc (No. 14), purities (analytical HPLC-UV detection at 220 nm) and retention times for a hydantoin library synthesized on APOS 1200 (No. 1–7: cleavage with MeOH, 65 °C, 2 h; No. 8 and 9: cleavage with THF/H_2 1/1, 65 °C, 4 h; No. 10–13: cleavage with THF/H_2O 1/9, 65 °C, 4 h; No. 14: cleavage with H_2O, 65 °C, 4 h)

No.	Structure	M calc.	HPLC-ESI-MS[a] $[M+H]^+$ Found	Chemical yield (%)	Purity (HPLC 220 nm) (%)	R_t[b] (min)
1		333	334	78	>99	19.1
2		232	233 274[e]	59	>99	18.9
3		218	260[e]	80	98	15.7
4		338	339 380[e]	77	96	22.1
5		190	232[e]	63	97	11.2
6		405	406 447[e]	79	95	26.3
7		347	348	89	99	20.2
8[c]		250	251 292[e]	81	97	15.6
9[d]		266	267 533[f]	83	95	9.6
10		266	267 308[e]	77	>99	18.2

Typical procedure

200 mg of preloaded Fmoc-AA resin (TG-S-PHB-AA-Fmoc, TG-S-AC-AA-Fmoc, TG-S-Trt-AA-Fmoc, TG-S-AA-Fmoc or TG-HL-AA-Fmoc; about 50–100 μmol eq.) preswollen in DMF are filled as a slurry into the glass reactors of APOS 1200. After draining, 1.5 ml of piperidine/DMF 1/1 is added. After 1 h at room temperature the resin is washed 12 times with 2 ml of DMF. 1.5 ml of isocyanate (1 M in DMF/15–30 eq.) is added and after 2 h at room temperature the acylation is quantitative as indicated by a ninhydrin test. The resin is washed 14 times with 2 ml of DMF, 3 times with 2 ml of CH_2Cl_2, 3 times with 2 ml of THF and finally 3 times with 2 ml of chosen solvent or solvent mixture for cyclization or alternat-

Table 5. (continued)

No.	Structure	M calc.	HPLC-ESI-MS[a] $[M+H]^+$ Found	Chemical yield (%)	Purity (HPLC 220 nm) (%)	R_t[b] (min)
11		216	217 258[e]	44	>99	14.8
12		282	283 324[e]	68	>99	20.7
13		232	233 274[e]	66	98	18.9
14		232	–[g]	87	>99	18.2

[a] HPLC-ESI-MS spectra were recorded on Finnigan TSQ 700 ESIF; Finnigan MAT GmbH, Bremen.
[b] Analytical HPLC: 250×4.6 Gromsil ODS2 column, 5 μm particle size; gradient elution 5%–95% acetonitrile containing 0.08% TFA for 40 min then 95% acetonitrile containing 0.08%TFA for 5 min; flow 1 ml/min.
[c] Distilled THF-free of peroxide, determined with Peroxide-Test, Merkoquant 10011, E. Merck, Darmstadt.
[d] Distilled THF, stored two months, 10–25 mg/l peroxide, determined with Peroxide-Test, Merkoquant 10011, E. Merck, Darmstadt.
[e] MeCN adduct.
[f] Dimer.
[g] Not measured.

ively dried under vacuum for storage. For cyclization and cleavage, 2 ml of pure solvent or solvent mixture is added to the preswollen resin or 2.5 ml is added to a dried resin. After 4 h at 65 °C the suspension is drained and the released product extracted from the resin (7 × 1 ml THF).

Conclusions

Hydantoins in high optical purity can be cleaved from solid supports by a thermal pH-neutral cyclization. The N-carbamyl amino acid precursor has to be attached via an ester bond to the resin and the terminal urea nitrogen has to be substituted by a phenyl group. Whereas electron rich substituents at the phenylgroup increase the yields, electron poor substituents decrease the yields of the final hydantoin derivative. The thermal cyclization proceeds best in protic solvents like H_2O or MeOH and, thereby, best from solid supports that are compatible with these solvents.

Notes

[1] *TentaGel* resins are polystyrene-polyoxyethylene copolymers; *APOS 1200*: *A*utomated *P*arallel *O*rganic *S*ynthesizer; Rapp Polymere GmbH, Ernst Simon Str. 9, D-72072 Tübingen, Germany.
[2] Determination of the optical purity:
a) 6N DCl/D_2O 110 °C/24 h
b) MeOD/DCl 110 °C/15 min
c) TFA
d) chromatography: ChirasilVal (20 m/⌀(0,28 μm/0,2 μm film), GC-MS massfiltre M 168.
[3] A sample was analyzed by ^{1}H-NMR spectroscopy (Bruker ACP 250, Bruker Analytik GmbH, Rheinstetten) without further purification: ^{1}H-NMR (250 MHz, $CDCl_3$, TMS): δ 2.91–3.00(dd, 1 H); 3.23–3.30(dd, 1 H); 4.32–4.36(m, 1 H); 5.59(br s, 1 H); 7.14–7.40(m, 10 H).
[4] FT-IR: Bruker Vektor 22 Spektrometer, Bruker Analytik GmbH, Rheinstetten; ATR unit: Golden Gate Single Reflection Diamond, Graseby Specac Limited, Kent, U.K.

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