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Synthesis of Spirohydantoins and Spiro-2,5-diketopiperazines via Resin-Bound Cyclic α,α-Disubstituted α-Amino Esters

George J. T. Kuster,[†] Leon W. A. van Berkom,[†] Mohamed Kalmoua,[†] Arnold van Loevezijn,[‡] Leo A. J. M. Sliedregt,[‡] Bart J. van Steen,[‡] Chris G. Kruse,[‡] Floris P. J. T. Rutjes,[†] and Hans W. Scheeren*[,†]

Institute for Molecules and Materials, Radboud University Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands, and Solvay Pharmaceuticals, Sector Discovery Weesp, P.O. Box 900, 1380 DA Weesp, The Netherlands

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A seven-step solid-phase synthesis of spirohydantoins **7** and an eight-step solid-phase synthesis of spiro-2,5-diketopiperazines **8** is reported. Key intermediate in the synthesis of both compound libraries is the resin-bound cyclic α,α-disubstituted α-amino ester **5**, which can be obtained after selective homogeneous reduction of the aliphatic nitro ester **4** using tin(II) chloride dihydrate. Nitro ester **4**, in turn, is synthesized by a high-pressure-assisted [4 + 2] cycloaddition of resin-bound nitro alkene **2** and butadiene **3**, whereas nitro alkene **2** is obtained by a Knoevenagel condensation of resin-bound nitro acetate with an imine. Novel spirohydantoins **7** are obtained by isocyanate coupling with the resin-bound amino ester **5**, followed by cyclization cleavage using a base. Novel spiro-2,5-diketopiperazines **8** are obtained by PyBOP coupling of a Fmoc-protected amino acid with resin-bound amino ester **5**, followed by Fmoc deprotection and an acid-assisted cyclization cleavage. After preparation of seven different resin-bound α,α-disubstituted α-amino esters **5**, a 7 × 8 compound library of spirohydantoins **7** was synthesized using eight different isocyanates, and a 7 × 8 compound library of spiro-2,5-diketopiperazines **8** was synthesized using eight different Fmoc amino acids.

Introduction

Incorporation of conformationally restricted amino acids into peptides can lead to new insights in receptor−ligand interactions and the process of peptide folding and might, as well, lead to new compounds suitable for therapeutic studies.[1,2] For this reason, the development of new methodologies for the synthesis of $c^{\alpha,\alpha}$-disubstituted α-amino acids can be of great value for the area of proteonomics. Interestingly, when occurring in their cyclized form (e.g. hydantoin, 2,5-diketopiperazine), the unnatural $c^{\alpha,\alpha}$-disubstituted α-amino acid derivatives themselves show a wide range of therapeutic activities, which turns the spirohydantoin and spirodiketopiperazine substructure into attractive cores for library synthesis (Figure 1).[3]

The spirohydantoin derivatives **1a** and **1b** show antidiabetic and antiepileptic[4] activities, respectively. Derivatives **1c**[5,6] and **1d**[7] can act as new psychotropic agents (antidepressants, anxiolytics, antipsychotics). SpiroDKP derivatives **1e**[8,9] have shown neuroprotection and antiproliferative activity. Derivatives **1f**[10] and **1g**[11] have shown to be useful in preventing inflammatory diseases and immune and allergic diseases.

Recently, we reported the synthesis of resin-bound nitro alkenes and their use in the synthesis of resin-bound, $c^{\alpha,\alpha}$-disubstituted, α-amino esters which were converted into cyclic 2-arylethylamines and amino alcohols.[12] The resin-bound nitro alkenes were reacted as dienophiles in a high-pressure-assisted Diels−Alder reaction with 2,3-dimethylbutadiene. Previously, it was demonstrated that high pressure can be applied as an excellent technique for solid-phase cycloadditions.[12,13] High-pressure conditions eliminate the need for stirring and heating, which can cause damage to the resin, and it eliminates the use of a catalyst, which deposits residues in the polymeric matrix. High pressure also allows the use of building blocks (dienes, dienophiles) of relatively low reactivity or building blocks that are sensitive toward heat or (Lewis acid) catalysts, thus enlarging the pool of building blocks and substituents enormously.[13a]

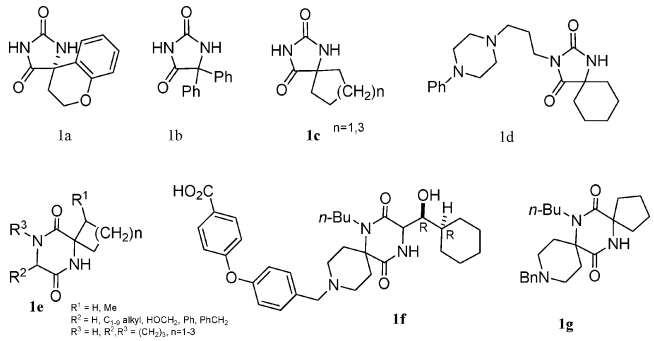

Figure 1. Overview of spirohydantoin and spiro-2,5-diketopiperazine (DKP) derivatives (**1b**−**f**) and their therapeutic effects.

* Corresponding author. Phone: +31 (0)24 365 3389. E-mail: j.scheeren@science.ru.nl.
† Radboud University Nijmegen.
‡ Solvay Pharmaceuticals.

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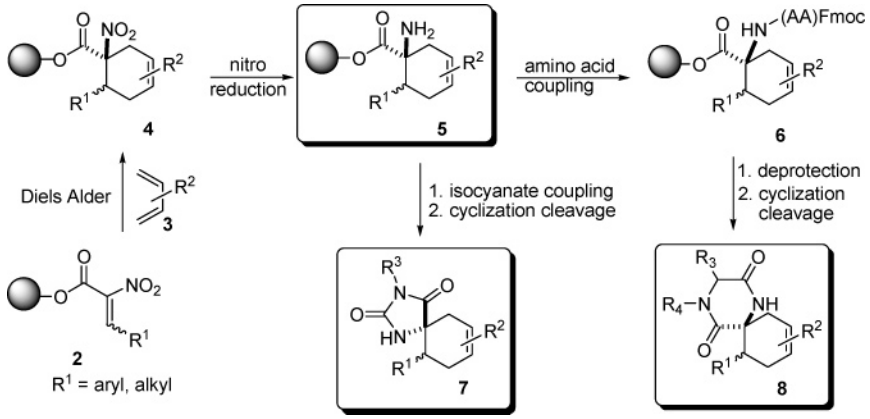
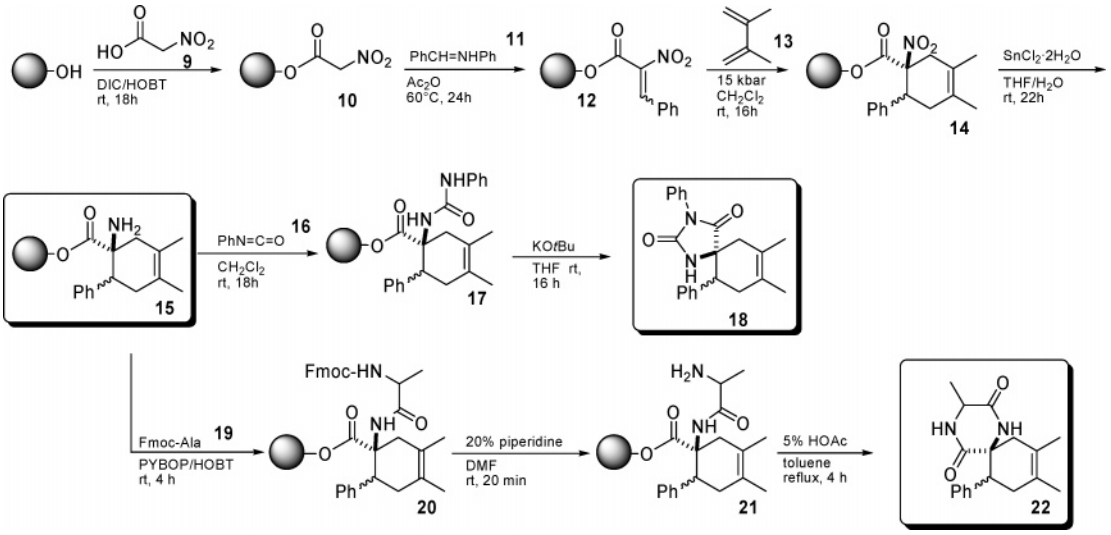
Scheme 1. General Synthetic Route toward Novel Spirohydantoins (**7**) and Spiro-2,5-diketopiperazines (**8**)



Scheme 2. Synthetic Route toward Spirohydantoin **18** and Diketopiperazine **22**



In this paper, the application of resin-bound $c^{\alpha,\alpha}$-disubstituted α-amino ester **5** in the synthesis of novel spirohydantoin derivatives **7** and novel spiro-2,5-diketopiperazine (spiroDKP) derivatives **8** is demonstrated (Scheme 1). The central feature in the synthesis is a high-pressure-assisted solid-phase Diels−Alder reaction of butadiene **3** with a resin-bound nitro alkene **2**. Resin-bound $c^{\alpha,\alpha}$-disubstituted α-amino ester **5** was obtained after selective reduction of nitro ester **4**. The reaction of an isocyanate with amino ester **5** followed by cyclization yielded spirohydantoin derivatives **7**.[14]

Coupling of an Fmoc-protected amino acid to amino ester **5** gave resin-bound dipeptide **6**, after which Fmoc-deprotection and an acid-assisted cyclization cleavage step yielded spiro-2,5-diketopiperazine (DKP) derivatives **8**.

Both reaction sequences allow the introduction of functional groups on the spirohydantoin and spirodiketopiperazine core structures by application of different aldehydes (R^1), dienes (R^2), isocyanates (R^3), or amino acids (R^3/R^4).[15]

Results and Discussion

Solid-Phase Synthesis of Spirohydantoin 18 and Spiro-2,5-diketopiperazine 22. Nitroacetic acid **9** was esterified to the Merrifield-OH resin using diisopropylcarbodiimide and *N*-hydroxybenzotriazole to give resin-bound nitro acetate **10** (Scheme 2).[16] Next, the resin-bound nitro alkene **12** was prepared via the Babievskii modification of the Knoevenagel condensation.[17] Schiff base **11** and resin-bound nitro acetate were heated in acetic anhydride (60 °C for 24 h) to give nitro alkene **12**.[18] Solution-phase studies of this reaction have shown that there is no selectivity in the condensation, resulting in an approximately 1:1 mixture of the *E* and *Z* isomers. IR analysis showed a shift of the C═O vibration from $\nu \sim 1752$ to $\nu \sim 1736$ cm^{-1} and the NO_2 vibration from $\nu \sim 1563$ to $\nu \sim 1536$ cm^{-1}. After a high-pressure-assisted Diels−Alder reaction with 2,3-dimethylbuta-1,3-diene **13** at 15 kbar, cycloadduct **14** was obtained.[19] Disappearance of the C═C vibration ($\nu \sim 1643$ cm^{-1}) and a shift of the NO_2 vibration to $\nu \sim 1552$ cm^{-1} indicated complete conversion of the nitro alkene. The aliphatic nitro group of cycloadduct **14** was reduced using a 1.0 M solution (10 equiv) of tin(II) chloride dihydrate in THF/H_2O 10/1 according to an optimized procedure.[20] Since the Knoevenagel adduct was formed as an ∼1:1 mixture of *E*/*Z* isomers, the amino ester **15** existed as a mixture of all possible diastereoisomers. The amine **15** was converted to urea derivative **17** by treatment with phenylisocyanate **16** at room temperature. Cyclization was performed using potassium *tert*-butoxide in tetrahydrofuran and yielded spirohydantoin derivative **18** in a purity of 97% and a yield of 74−95%.[21,22] IR analysis of the resin after the cleavage reaction showed complete disappearance of the C═O vibration, which

indicated complete conversion in the cyclization step. Logically, spirohydantoin **18** was isolated as a mixture of diasteroisomers (1.5:1).

To obtain the spiro-2,5-diketopiperazine derivative **22**, Fmoc-protected alanine **19** was coupled to the resin-bound amino ester **15** with PyBOP/HOBT, yielding resin-bound dipeptide **20**. Fmoc deprotection in 20% piperidine in DMF was followed by a cyclization cleavage step with 5% HOAc in refluxing toluene and yielded a diastereomeric mixture of spiro-2,5-diketopiperazine (DKP) derivatives **22** in high purity and 69% overall yield.

Library Synthesis. To validate the developed solid-phase methodology for the synthesis of spiroDKPs and spirohydantoins with different R groups (substituted aromats, hetero aromats, and alkyl groups in combinaton with several amino acids and isocyanates; see Scheme 1), a compound library for each class of structures was prepared. A series of seven aldehydes, **A**−**G**, were chosen to obtain seven different resin-bound, $c^{\alpha,\alpha}$-disubstituted, α-amino esters **5**, which were each coupled to a selection of eight isocyanates **a**−**h** to give a 7 × 8 matrix of different hydantoins.[23] For the setup of the DKP library, the seven different resin-bound amino esters **5** were coupled with a selection of eight Fmoc-protected amino acids.

First, a batch of polymer supported nitro acetate **10** was prepared by reaction of hydroxymethylene resin with nitroacetic acid **9**. After formation of the Schiff bases **11A**−**G** from the aldehydes **A**−**G**, the batch of polymer-supported nitro acetate was divided into seven portions. Each portion was reacted with a different Schiff base, **A**−**G**, to give seven different resin-bound nitro alkenes, **12A**−**G**. The α,*β*-unsaturated nitro esters **12A**−**G** were reacted with 2,3-dimethylbuta-1,3-diene at high-pressure conditions (15 kbar) to give cycloadducts **14A**−**G**, which were next reduced using tin(II) chloride to give a series of seven resin-bound amino esters, **15A**−**G**. These amino esters were each divided into two portions, of which one was used in the hydantoin library synthesis and one in the DKP library synthesis.

Each batch of the seven resin-bound $c^{\alpha,\alpha}$-disubstituted α-amino esters, **15A**−**15G** was reacted with all selected isocyanates **16a**−**h**, and in a separate experiment, each batch of the seven resin-bound amines **15A** to **15G** was reacted with all selected amino acids **19a**−**h**. After cleavage from the resin, the yield of each of the spirohydantoins **18Aa**−**Gh** and each of the spiroDKPs **22Aa**−**Gh** was determined gravimetrically, and the purity was determined by LC/MS (equipped with an ELSD and UV-detector; see Table 1).

From the results displayed in Table 1, it was concluded that most hydantoins from the library were obtained in moderate to good overall yields and in high purities (>90%). Substituents on the phenyl ring (R^1 position: electron-rich (**18B**, **18D**) or -poor (**18C**)) were allowed, as well as replacement of the phenyl group with an alkyl substituent (**18E**). The results of derivatives **18F** and **18G** showed that alkylated hetero aromatic substituents were also suitable for the developed route toward spirohydantoins.[24] Furthermore, it can be concluded from the results of Table 1 that the isocyanate coupling resulting in the introduction of substituent R^2 has been successful with all chosen derivatives, although in some cases, saponification of the ethyl ester of derivatives **18Xh** was observed.

Table 1. Analysis of the Spirohydantoin Library

			MW			
entry	R^1	R^2	calcd	found MH^+	yield %[a]	purity %[b]
18Aa	Ph	Ph	346.4	346.8	90	99
18Ab	Ph	4-CNPh	371.4	372.3[c]	89	93
18Ac	Ph	4-MeOPh	376.5	377.2	84	99
18Ad	Ph	4-MeCOPh	388.5	389.8	53	94
18Ae	Ph	4-Me_2NPh	389.5	390.2	81	96
18Af	Ph	*c*-hexyl	352.5	353.6	77	97
18Ag	Ph	CH_2═$CHCH_2$	310.4	311.6	75	99
18Ah	Ph	EtO_2C	342.4	271.6[d]	(12)	(97)
18Ba	4-MeOPh	Ph	376.5	377.2	54	99
18Bb	4-MeOPh	4-CNPh	401.5	420.5[c]	(47)	(95)
18Bc	4-MeOPh	4-MeOPh	406.5	407.1	58	98
18Bd	4-MeOPh	4-MeCOPh	418.5	419.6	27	96
18Be	4-MeOPh	4-Me_2NPh	419.5	420.2	59	98
18Bf	4-MeOPh	*c*-hexyl	382.5	383.3	51	99
18Bg	4-MeOPh	CH_2═$CHCH_2$	340.4	340.9	50	99
18Bh	4-MeOPh	EtO_2C	372.4	301.5[d]	(30)	(91)
18Ca	4-CNPh	Ph	371.4	372.3	34	90
18Cb	4-CNPh	4-CNPh	396.4	397.5	32	90
18Cc	4-CNPh	4-MeOPh	401.5	402.2	47	96
18Cd	4-CNPh	4-MeCOPh	413.5	414.7	14	91
18Ce	4-CNPh	4-Me_2NPh	414.5	415.3	54	95
18Cf	4-CNPh	*c*-hexyl	377.5	378.7	31	95
18Cg	4-CNPh	CH_2═$CHCH_2$	335.4	354.6[c]	(38)	(86)
18Ch	4-CNPh	EtO_2C	367.4			
18Da	3,4-(OCH_2O)Ph	Ph	390.4	391.1	51	98
18Db	3,4-(OCH_2O)Ph	4-CNPh	415.4	434.6[c]	44	95
18Dc	3,4-(OCH_2O)Ph	4-MeOPh	420.5	421.0	60	98
18Dd	3,4-(OCH_2O)Ph	4-MeCOPh	432.5	433.5	17	90
18De	3,4-(OCH_2O)Ph	4-Me_2NPh	433.5	434.5	62	98
18Df	3,4-(OCH_2O)Ph	*c*-hexyl	396.5	397.5	44	98
18Dg	3,4-(OCH_2O)Ph	CH_2═$CHCH_2$	354.4	355.5	55	98
18Dh	3,4-(OCH_2O)Ph	EtO_2C	386.4	315.4[d]	(25)	(95)
18Ea	*c*-hexyl	Ph	352.5	353.2	64	97
18Eb	*c*-hexyl	4-CNPh	377.5	378.7[c]	53	95
18Ec	*c*-hexyl	4-MeOPh	382.5	383.2	63	97
18Ed	*c*-hexyl	4-MeCOPh	394.5	395.4	29	94
18Ee	*c*-hexyl	4-Me_2NPh	395.5	396.0	63	99
18Ef	*c*-hexyl	*c*-hexyl	358.5	359.4	50	95
18Eg	*c*-hexyl	CH_2═$CHCH_2$	316.4	317.5	57	99
18Eh	*c*-hexyl	EtO_2C	348.4	277.4[d]	(33)	(99)
18Fa	MeN-2′-pyrrole	Ph	349.4	350.7	18	98
18Fb	MeN-2′-pyrrole	4-CNPh	374.4	375.7	16	75
18Fc	MeN-2′-pyrrole	4-MeOPh	379.5	380.4	17	98
18Fd	MeN-2′-pyrrole	4-MeCOPh	391.5	392.5	8	23
18Fe	MeN-2′-pyrrole	4-Me_2NPh	392.5	393.6	18	96
18Ff	MeN-2′-pyrrole	*c*-hexyl	355.5	356.5	16	94
18Fg	MeN-2′-pyrrole	CH_2═$CHCH_2$	313.4	314.2	16	95
18Fh	MeN-2′-pyrrole	EtO_2C	345.4	-	-	-
18Ga	MeN-3′-indole	Ph	399.5	400.5	39	94
18Gb	MeN-3′-indole	4-CNPh	424.5	425.5[c]	29	93
18Gc	MeN-3′-indole	4-MeOPh	429.5	430.9	42	88
18Gd	MeN-3′-indole	4-MeCOPh	441.5	442.4	24	75
18Ge	MeN-3′-indole	4-Me_2NPh	442.6	443.9	40	90
18Gf	MeN-3′-indole	*c*-hexyl	405.5	406.7	36	91
18Gg	MeN-3′-indole	CH_2═$CHCH_2$	363.5	364.5	44	99
18Gh	MeN-3′-indole	EtO_2C	395.5	324.6[d]	24	98

[a] Overall yield after cyclization cleavage. [b] Based on ELSD analysis; % area. [c] $MH^+ + 19$ [NH_4^+]. [d] $MH^+ - 72$ [$EtOC(O)^-$].

The overall yield of the reactions varied between 8 and 90%. The purity of 88% of the derivatives **18A**−**G** was higher than 90%. Varying ratios of the two diastereoisomers were encountered in different reactions. For all products, the anticipated molecular mass was detected with MS, except for some of the **18Xh** derivatives, of which sometimes

Table 2. Analysis of the Spiro-2,5-diketopiperazine library

entry	R^1	R^2	R^3	MW calcd	MW found, MH^+	yield, %[b]	purity, %[c]
22Aa	Ph	H	H	284.4	285.2	45	98
22Ab	Ph	CH_3	H	298.4	299.3	53	99
22Ac	Ph	CH_2Ph	H	374.5	375.6	68	99
22Ad	Ph	3-indoleCH_2	H	413.5	414.7	46	98
22Ae	Ph	$-(CH_2)_3N-$		324.4	325.0	63	99
22Af	Ph	$CH(CH_3)_2$	H	327.4	328.6	40	98
22Ag	Ph	$CH(OH)CH_3$	H	328.4	329.2	31	95
22Ah	Ph	$-(CH_2)_3NHBoc$	H	441.6	442.9	67	95
22Ba	4-MeOPh	H	H	314.4	315.4	32	93
22Bb	4-MeOPh	CH_3	H	328.4	329.2	36	99
22Bc	4-MeOPh	CH_2Ph	H	404.5	405.6	40	99
22Bd	4-MeOPh	3-indoleCH_2	H	443.5	444.7	22	99
22Be	4-MeOPh	$-(CH_2)_3N-$		354.4	355.1	44	99
22Bf	4-MeOPh	$CH(CH_3)_2$	H	357.5	357.4	23	99
22Bg	4-MeOPh	$CH(OH)CH_3$	H	358.4	359.4	14	98
22Bh	4-MeOPh	$-(CH_2)_3NHBoc$	H	471.6	472.7	39	98
22Ca	4-CNPh	H	H	309.4	349.6		
22Cb	4-CNPh	CH_3	H	323.4	329.3		
22Cc	4-CNPh	CH_2Ph	H	399.5	400.4	45	52
22Cd	4-CNPh	3-indoleCH_2	H	438.5	439.7	32	39
22Ce	4-CNPh	$-(CH_2)_3N-$		349.4	350.7	53	70
22Cf	4-CNPh	$CH(CH_3)_2$	H	351.4	352.5	29	40
22Cg	4-CNPh	$CH(OH)CH_3$	H	353.4	354.4	27	10
22Ch	4-CNPh	$-(CH_2)_3NHBoc$	H	466.6	467.9	43	65
22Da	3,4-(OCH_2O)Ph	H	H	328.4	329.3	31	99
22Db	3,4-(OCH_2O)Ph	CH_3	H	342.4	343.2	35	99
22Dc	3,4-(OCH_2O)Ph	CH_2Ph	H	418.5	419.3	44	99
22Dd	3,4-(OCH_2O)Ph	3-indoleCH_2	H	457.5	458.8	23	99
22De	3,4-(OCH_2O)Ph	$-(CH_2)_3N-$		368.4	369.0	46	99
22Df	3,4-(OCH_2O)Ph	$CH(CH_3)_2$	H	370.4	370.9	22	99
22Dg	3,4-(OCH_2O)Ph	$CH(OH)CH_3$	H	372.4	373.5	14	99
22Dh	3,4-(OCH_2O)Ph	$-(CH_2)_3NHBoc$	H	485.6	486.7	38	99
22Ea	*c*-hexyl	H	H	290.4	291.2	31	99
22Eb	*c*-hexyl	CH_3	H	304.4	305.5	30	99
22Ec	*c*-hexyl	CH_2Ph	H	380.5	381.4	38	99
22Ed	*c*-hexyl	3-indoleCH_2	H	419.6	420.5	24	90
22Ee	*c*-hexyl	$-(CH_2)_3N-$		330.5	331.6	30	99
22Ef	*c*-hexyl	$CH(CH_3)_2$	H	332.5	333.4	21	99
22Eg	*c*-hexyl	$CH(OH)CH_3$	H	334.5	335.9	12	98
22Eh	*c*-hexyl	$-(CH_2)_3NHBoc$	H	447.6	448.6	33	99
22Fa	MeN-2′-pyrrole	H	H	287.4			
22Fb	MeN-2′-pyrrole	CH_3	H	301.4	302.5	17	54
22Fc	MeN-2′-pyrrole	CH_2Ph	H	377.5	378.8	22	50
22Fd	MeN-2′-pyrrole	3-indoleCH_2	H	416.5	417.8	14	5
22Fe	MeN-2′-pyrrole	$-(CH_2)_3N-$		327.4	328.6	19	95
22Ff	MeN-2′-pyrrole	$CH(CH_3)_2$	H	329.4	330.5	14	10
22Fg	MeN-2′-pyrrole	$CH(OH)CH_3$	H	331.4			
22Fh	MeN-2′-pyrrole	$-(CH_2)_3NHBoc$	H	444.6	445.5	17	50
22Ga	MeN-3′-indole	H	H	337.4	338.5	15	99
22Gb	MeN-3′-indole	CH_3	H	351.4	352.6	22	99
22Gc	MeN-3′-indole	CH_2Ph	H	427.5	428.8	25	99
22Gd	MeN-3-′indole	3-indoleCH_2	H	466.6	467.5	16	90
22Ge	MeN-3′-indole	$-(CH_2)_3N-$		377.5	378.7	21	99
22Gf	MeN-3′-indole	$CH(CH_3)_2$	H	379.5	380.7	15	99
22Gg	MeN-3′-indole	$CH(OH)CH_3$	H	381.5	382.6	12	95
22Gh	MeN-3′-indole	$-(CH_2)_3NHBoc$	H	494.6	495.9	21	98

[a] Additional analysis of both libraries by ^1H NMR confirmed the results obtained by LC/MS analysis and gave more insight in purity and diastereomeric product ratios.[26] [b] Overall yield after cyclization cleavage. [c] Based on ELSD analysis.

hydrolysis/R^2-elimination was detected (i.e., **18Ah**, **18Bh**, **18Dh**, **18Eh**, **18Gh**).

As displayed in Table 2, most spiro-2,5-diketopiperazines were obtained in moderate to good overall yields and in high purities (>90%). Electron-rich (**22B**, **22D**), -poor (**22C**), and neutral (22A) aromatic substituents were allowed, as well as aliphatic (**22E**) substituents. Derivatives with *p*-cyanophenyl substitution (**22C**) sometimes gave low purities (**22Cc**−**d**) or no product formation (**22Ca**−**b**). The methyl-2′-pyrrole substituent (**22F**) gave much lower purities and yields, as compared to the derivatives in the hydantoin library (**18F**), whereas on the other hand, the methyl-3′-indole (**22G)** gave excellent purities but, again, relatively low yields. Furthermore, it can be concluded from the results of Table 2 that the amino acid coupling resulting in the introduction of substituents R^2-R^3 has been successful: a range of

different functionalities (aliphatic, aromatic, hetero aromatic, hydroxy, Boc-protected amine)[25] were compatible with the outlined route. The overall yield varied between 12 and 68% (with the exception of **22Ca**−**b**, **22Fa**, and **22Fg**). The purity of 77% of the derivatives **22A**−**G** was higher than 90%. Again, in all cases, varying ratios of the four possible diastereoisomers were observed.

Conclusion

The synthesis of the novel spirohydantoin **18** and spiroDKP **22** on the solid phase was explored and optimized. Key steps in the synthesis were the Knoevenagel condensation, the high-pressure-assisted cycloaddition reaction, and the selective nitro reduction of the cycloadduct with tin(II) chloride, yielding the resin-bound $c^{\alpha,\alpha}$-disubstituted α-amino ester. The high-pressure-assisted cycloaddition represents one of the first applications of a high-pressure reaction on the solid phase. Tin(II) chloride was selected to realize a selective reduction of the aliphatic and sterically hindered resin-bound nitro ester **14** to resin-bound amino ester **15**. Optimal reduction parameters were selected after an extensive optimization experiment (automated parallel solution chemistry) using different solvents, temperatures and concentrations. The resin-bound amino ester **15** was coupled with an isocyanate, and a subsequent potassium *tert*-butoxide-mediated cyclization cleavage reaction yielded the desired novel spirohydantoin **18** in good purity and yield. To obtain spiroDKP **22**, the resin-bound amino ester **15** was coupled with Fmoc-protected alanine. After Fmoc deprotection and an acetic acid-assisted cyclization cleavage reaction, the desired novel spiroDKP **22** was obtained in good purity and yield.

The methodology for the solid-phase synthesis of spirohydantoin **18** and spiroDKP **22** was successfully applied in the synthesis of a spirohydantoin library and a spiroDKP library. The novel products were obtained in good purities and in reasonable to good yields, showing that the synthetic route allowed the use of building blocks (imines, isocyanates, Fmoc-amino acids) with a wide diversity in substituents pattern. Derivatives with *N*-methylpyrrole and 4-cyanophenyl substituents gave in general lower yields or purities, which indicates that for some derivatives, an alternative synthetic route should be developed.[27]

In summary, an efficient, solid-phase synthesis toward a spirohydantoin and a spiro-2,5-diketopiperazine library has been developed. Due to the cyclization cleavage protocol, mostly clean product mixtures of diastereomers have been isolated after a seven- and eight-step synthesis on the solid phase.

Experimental Section

General Remarks. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AC-300 spectrometer in $CDCl_3$ using tetramethylsilane as internal standard.

Solid-Phase Reactions. Hydroxymethylene resins (100−200 mesh cross-linked with 1% divinylbenzene, 1.0−2.0 mmol/g) were purchased from Aldrich. Solid-phase reactions were conducted in regular flasks and were agitated by gentle (interval) stirring with a magnetic stirrer bar. The standard protocol for workup involved filtration of the reaction mixture over a P3 glass filter, followed by extensive washing of the resin using several swell and squeeze cycles (dichloromethane/methanol). After washing, the resin was dried in a vacuum oven at 70 °C and 400 mbar. IR spectra of products or intermediates were recorded only after washing and drying of the resin with an Anadis Thermo Mattson IR300 spectrometer.

Parallel Synthesis. All parallel experiments were performed at Solvay Pharmaceuticals in Weesp using the Automated Molecular Assembly Plant for addition, agitation, heating, filtration, extraction, and evaporation. LC/MS measurements were carried out using a Sciex API 150EX (single quadrupole), coupled to two Perkin-Elmer micropumps (series 200) and equipped with a Perkin-Elmer 785A UV spectrophotometer operating at 254 nm and a Sedex 55 ESL detector. MS parameters, APCI method: positive scanning area of 150−750 step 1. (2 scans/s; 350 °C, 2 μA on the corona needle). LC analysis: 2.8 min/cycle (flushed with eluent A; 2.5 min eluent B; 0.3 min eluent A). Eluent A = 95% water 5% acetonitrile with 10 mmol ammonium acetate and 0.25% formic acid; eluent B = 100% acetonitrile with 0.25% formic acid. RP column: Waters Xterra MS column, 30 × 4.6 mm, C_{18}; dp of 2.5 μm.

The overall yield of a multistep solid-phase reaction sequence after cleavage of the product from the resin was determined using the following equation,

$$\text{yield} = (m \times M/r \times C_i) \times 100\%$$

where m is the mass of the cleaved product (g), M is the molecular weight of cleaved product (mmol), r is the mass of amine functionalized resin used for the amino acid coupling (g), and C_i is the initial loading of the resin (mmol/g)).

Nitroacetic Acid (6). Potassium hydroxide (224 g, 3.99 mol) was dissolved in water (100 mL) in a three-necked, round-bottomed flask equipped with a funnel, condenser, and magnetic stirrer. The solution was stirred rapidly, and nitromethane (61 g, 1.00 mol) was added dropwise. During the addition of nitromethane, the reaction mixture turned yellow and started to reflux. After some time, suddenly a thick suspension of dipotassium nitroacetate was formed. After addition of all nitromethane, the reaction mixture was heated to reflux for 1 h, after which the reaction mixture was cooled to room temperature and filtered. The filtrate was washed with cooled methanol (5 °C), and the product was dried under vacuum to yield the dipotassium nitroacetate (68.6 g) as small, yellow-white crystals. To a solution of the dipotassium nitro acetate (68.6 g) in ice−water (100 mL), a solution of 37% hydrogenchloride/water (180 g) was added dropwise at such a rate that the temperature of the reaction mixture did not exceed 0 °C. Next, the reaction mixture was saturated with an excess of sodium chloride and extracted four times with diethyl ether, and the combined organic layers were dried (sodium sulfate), filtered, and evaporated to give a yellow oil. Chloroform was added, and the solvent was evaporated again. Upon cooling, the off-white oil slowly

crystallized. Nitroacetic acid (20.8 g) was obtained as thick white needles. Analytical data were in accordance with literature.[28]

Esterification of Nitroacetic Acid (9) to Hydroxymethylene Resin 10. To a cooled (−10 °C) suspension of hydroxymethylene resin (24.7 g; 1.0−2.0 mmol/g), nitroacetic acid **9** (15.7 g, 149 mmol) and *N*-hydroxybenzotriazole (0.5 g, 3.7 mmol) in dry tetrahydrofuran (200 mL) and 1,3-diisopropylcarbodiimide (20.6 g, 163 mmol) in dry tetrahydrofuran (100 mL) was added at such a rate that the temperature of the reaction mixture did not exceed 0 °C. After addition of the coupling reagent, the reaction mixture was stirred at room temperature for 18 h (interval stirring: 15 min/h). The reaction mixture was filtered, and the resin was washed with alternating dimethylformamide and methanol (two cycles), next with alternating methanol and dichloromethane (two cycles), and finally, with methanol (two times). IR 1753 (C═O), 1561 (as NO_2) cm^{-1}.

Proof of Principle: Solid-Phase Synthesis of Spirohydantoin 18 and Spiro-2,5-diketopiperazine 22. Formation of Resin-Bound Nitro Alkene 12 Using the Babievskii-Modified Knoevenagel Condensation. A suspension of the Merrifield-bound nitro acetate **10** (2.50 g) and the imine *N*-benzylideneaniline (15.0 mmol) in acetic anhydride (15 mL) was stirred at 60 °C for 24 h (interval stirring: 15 min/h). The reaction mixture was filtered, and the resin was washed with alternating dimethylformamide and methanol (two cycles) and next with alternating methanol and dichloromethane (two cycles). IR 1734 (C═O), 1642 (C═C), 1536 (as NO_2) cm^{-1}.

Solid-Phase Diels−Alder Cycloaddition Reaction to Give 14. A suspension of resin **12** (1.78 g), 2,3-dimethylbuta-1,3-diene (2.94 g), and 75 mg of 5-*tert*-butyl-4-hydroxy-2-methylphenylsulfide [29] in dichloromethane in a 15.0-mL Teflon vessel was reacted at 15 kbar and 30 °C for 16 h. The reaction mixture was filtered, and resin **14** was washed with methanol and THF then with alternating methanol and dichloromethane (three cycles). IR 1741 (C═O), 1553 (as NO_2) cm^{-1}.

Solid-Phase Reduction of Cycloadduct 14 to Give 15. A suspension of resin **14** (1.98 g) in a 1.0 M solution of tin(II) chloride dihydrate in tetrahydrofuran/water (9/1, 25 mL) was stirred at room temperature for 22 h (interval stirring: 15 min/h). The reaction mixture was filtered, and the resin was washed with DMF (3×); then alternating methanol and triethylamine/tetrahydrofuran (1/10, two cycles); and finally, alternating with methanol and dichloromethane (two cycles). Note: Before the resin was washed with base (when it was still the HCl salt of the resin-bound amine) it swelled in methanol. After washing with the base, it shrinks in methanol.

Coupling of Phenylisocyanate 16 with Resin-Bound Amine 15. To a suspension of the resin **15** (344 mg, 0.688 mmol) in dichloromethane (3 mL) was added phenylisocyanate **16** (112 μL, 1.02 mmol), and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was filtered, and the resin was washed with alternating methanol and dichloromethane (three cycles). IR 1727, 1597, 1493, 1446, 1195, 754 cm^{-1}.

Cleavage of Hydantoin 18 from Hydroxymethylene Resin 17. To a suspension of the resin **17** (369 mg) in dry tetrahydrofuran (3 mL) was added potassium *tert*-butoxide (193 mg, 5.0 equiv), and the reaction mixture was stirred for 16 h at room temperature. The reaction was quenched with 1 M ammonium chloride/water (3 mL), and next, the reaction mixture was filtered. The resin was washed with alternating dichloromethane and methanol (three cycles). The filtrate was concentrated to dryness, and next, dichloromethane and 1 M ammonium chloride/water were added. The organic layer was isolated, and the water layer was extracted twice more with dichloromethane. The combined organic layers were dried (magnesium sulfate), filtered, and evaporated in vacuo to give hydantoin **18** (97.5 mg) in 97% purity (GC analysis; diastereomeric ratio of 1.5/1). The diastereomeric hydantoins **18a** and **18b** were separated using column chromatography (EtOAc/heptane, 1/3) and were obtained as white solids in 74−95% yield.

7,8-Dimethyl-3,10-diphenyl-1,3-diazaspiro[4.5]dec-7-ene-2,4-dione (18a). Isolated as a white solid. An analytical sample was obtained by crystallization from ethanol/heptane. The stereochemistry was unambiguously determined using X-ray analysis. mp (ethanol/heptane) 174 °C. GC t_R = 16.2 min. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.66 (s, 3H), 1.70 (s, 3H), 2.18 (d, 1H, J = 17.1 Hz), 2.28 (dd, 1H, J = 5.7 Hz, 17.7 Hz), 2.55−2.65 (m, 1H), 3.04 (d, 1H, J = 17.1 Hz), 3.38 (dd, 1H, J = 6.0 Hz, 12.3 Hz), 6.66 (dd, 2H, J = 8.1 Hz, 2.4), 6.75 (s, 1H), 7.19−7.34 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.14, 19.36, 34.73, 41.22, 45.89, 65.24, 121.96, 125.77, 126.38, 127.92, 128.24, 128.55, 128.80, 128.91, 131.27, 138.04, 156.25, 174.33. HRMS: calcd for M^+ ($C_{22}H_{22}N_2O_2$), 346.1681; found, 346.1683. IR (film) 1774, 1709, 1601, 1502, 1411, 1286, 1204, 1130, 910, 772, 733, 703 cm^{-1}.

7,8-Dimethyl-3,10-diphenyl-1,3-diazaspiro[4.5]dec-7-ene-2,4-dione (18b). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68 (s, 3H), 1.70 (s, 3H), 2.19−2.30 (m, 1H), 2.40 (d, 1H, J = 17.7 Hz), 2.49 (d, 1H, J = 17.4 Hz), 2.98−3.09 (m, 2H), 6.69 (s, 1H), 7.03−7.06 (m, 2H), 7.16−7.40 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.12, 19.19, 35.81, 41.18, 48.20, 63.89, 120.95, 126.41, 126.70, 127.85, 128.23, 128.56, 128.77, 129.05, 131.44, 138.65, 155.66, 173.58. HRMS: calcd for M^+ ($C_{22}H_{22}N_2O_2$), 346.1681; found, 346.1683. IR (film) 1714, 1498, 1411, 1182, 906, 733, 703 cm^{-1}.

Coupling of Fmoc-L-alanine 19 with Resin-Bound Amine 15. To a suspension of the resin **15** (506 mg) and 697 mg (2.24 mmol) of Fmoc-L-alanine in dimethylformamide, 303 mg (2.24 mmol) of HOBT, and 1168 mg (2.24 mmol) of PYBOP were added. Finally, 579 mg (4.48 mmol) of DIPEA was added, and the reaction mixture was stirred for 4 h at room temperature (interval stirring: 15 min/h). The resin was filtered and washed with with alternating dimethylformamide and methanol (two cycles) and, next, with alternating methanol and dichloromethane (two cycles).

Fmoc Deprotection of Resin 20. A 10-mL portion of a solution of 20% piperidine in dimethylformamide was added to the resin on the glass filter. During 20 min, the slurry was stirred three times, after which the solvents were filtered off. The resin was washed with alternating dimethylforma-

mide and methanol (two cycles) and, next, with alternating methanol and dichloromethane (two cycles).

Synthesis of Spiro-2,5-diketopiperazine 22 by Cyclization Cleavage of Resin 21. A 15-mL portion of a solution of 5% acetic acid in toluene was added to the resin, and the mixture was refluxed for 4 h. Then solvents were filtered off, and the resin was washed with 10 mL of a solution of 5% acetic acid in toluene and methanol (two cycles) and dichloromethane. The collected filtrate was evaporated to dryness and yielded 58 mg (0.195 mmol) of the 4 diastereomeric *spiro*-DKPs of **22** as an off-white solid. GC/MS and ^{1}H NMR analysis confirmed the formation of four diastereomers of (**22**) in a 2:2:3:3 ratio. The diastereomers were separated by silica gel chromatography using EtOAc/heptane (4:1) as eluent.

Diastereomer 1. GC/MS: $m/z = 298$ (MH^+), 158, 143. ^{1}H NMR (300 MHz, decoupled, $CDCl_3/CD_3OD$): 7.29−7.21 (5H, m, Ph), 3.02 (1H, dd, $J = 5.3, 9.8$, H-11), 2.84−2.74 (1H, m, H-10), 2.66−2.55 (2H, m, H-7, H-3), 2.30 (1H, dd, $J = 4.9, 17.3$, H-10′), 2.16 (1H, d, $J = 17.9$, H-7′), 1.74 (3H, s, CH_3), 1.71 (3H, s, CH_3), 1.18 (3H, d, $J = 6.9$, CH_3).

Diastereomer 2. GC/MS: $m/z = 298$ (MH^+), 158, 143. ^{1}H NMR (300 MHz, decoupled, $CDCl_3$): 7.29 (5H, s, Ph), 6.22 (1H, s (br), NH), 5.40 (1H, s (br), NH), 3.38 (1H, dd, $J = 6.0, 12.3$, H-11), 3.24 (1H, d, $J = 17.2$, H-7), 2.77 (1H, q, $J = 6.9$, H-3), 2.63−2.52 (1H, m, H-10), 2.25 (1H, dd (br), $J = 5.2, 18.2$, H-10′), 2.05 (1H, d, $J = 11.7$, H-7′), 1.72 (3H, s, CH_3), 1.69 (3H, s, CH_3), 1.21 (3H, d, $J = 6.9$, CH_3).

Diastereomer 3. GC/MS: $m/z = 298$ (MH^+), 158, 143. ^{1}H NMR (300 MHz, decoupled, $CDCl_3$): 7.28 (5H, s, Ph), 6.27 (1H, s (br), NH), 5.76 (1H, s (br), NH), 3.88 (1H, dq, $J = 1.8, 7.0$, H-3), 3.48 (1H, dd, $J = 6.2, 12.2$, H-11), 3.16 (1H, d (br), $J = 16.8$, H-7), 2.57−2.46 (1H, m, H-10), 2.24 (1H, dd, $J = 6.3, 18.4$, H-10′), 2.16 (1H, d (br), $J = 17.3$, H-7′), 1.72 (3H, s, CH_3), 1.68 (3H, s, CH_3), 0.63 (3H, d, $J = 7.0$, CH_3).

Diastereomer 4. GC/MS: $m/z = 298$ (MH^+), 158, 143. ^{1}H NMR (300 MHz, decoupled, $CDCl_3$): 7.30−7.21 (5H, m, Ph), 7.00 (1H, s (br), NH), 6.31 (1H, s (br), NH), 3.92 (1H, dq, $J = 2.5, 7.0$, H-3), 2.98−2.86 (2H, m, H-10, H-11), 2.70 (1H, d (br), $J = 17.8$, H-7), 2.35 (1H, d (br), $J = 16.2$, H-10′), 2.16 (1H, d (br), $J = 16.9$, H-7′), 1.73 (3H, s, CH_3), 1.69 (3H, s, CH_3), 0.80 (3H, d, $J = 7.0$, CH_3).

Library Synthesis. Synthesis of Schiff Bases 11A−G. 11A. A mixture of aniline (2.00 mL, 22.0 mmol) and benzaldehyde (2.45 mL, 24.2 mmol) was stirred for 1 h at room temperature. Diethyl ether (30 mL) and anhydrous magnesium sulfate were added to the reaction mixture, and the reaction mixture was stirred at room temperature for another 20 h. Next, the reaction mixture was filtered and evaporated to dryness to give *N*-benzylideneaniline (3.98 g, 100%). The spectral properties of the product are in accordance with literature.[30]

11B. A mixture of the *p*-anisaldehyde (3.41 g, 25.0 mmol), aniline (2.42 g, 26.0 mmol), and magnesium sulfate in diethyl ether (6 mL) was stirred at room temperature for 19 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness to give the imine **11B** in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.86 (s, 3H), 6.95 (d, 1H, $J = 6.9$ Hz), 7.12−7.24 (m, 3H), 7.29−7.39 (m, 2H), 7.82 (d, 1H, $J = 6.9$ Hz), 8.34 (s, 1H).

11C. To a suspension of 4-cyanobenzaldehyde (6.63 g, 50.6 mmol) and sodium sulfate (35 g) in dichloromethane (5 mL) at room temperature was added dropwise a solution of aniline (4.96 g, 53.4 mmol) in dichloromethane (5 mL). After 3 h, the reaction mixture was filtered and concentrated to dryness to give **11C** in quantitative yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.19−7.35 (m, 3H), 7.37−7.49 (m, 2H), 7.77 (d, 2H, $J = 6.7$ Hz), 8.02 (d, 1H, $J = 6.7$ Hz), 8.50 (s, 1H).

11D. A suspension of piperonal (3.76 g, 25.1 mmol), aniline (2.43 g, 26.1 mmol), and magnesium sulfate in diethyl ether (6 mL) was stirred at room temperature for 19 h. According to NMR, the conversion was 80%. The reaction mixture was diluted with tetrahydrofuran (10 mL) and heated to reflux for 22 h. The reaction mixture was filtered and the filtrate was evaporated to dryness to give **11D** in 94% purity. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.01 (s, 2H), 6.85 (d, 1H, $J = 7.8$ Hz), 7.13−7.27 (m, 4H), 7.29−7.42 (m, 2H), 7.50 (s, 1H), 8.30 (s, 1H).

11E. To a suspension of cyclohexanecarboxaldehyde (2.85 g, 25.4 mmol) and sodium sulfate (35 g) in acetonitrile (10 mL) at −40 °C was added dropwise a solution of aniline (2.45 g, 26.3 mmol) in acetonitrile (20 mL). The reaction mixture was stirred at −40 °C for 45 min and then at room temperature for 10 min. The reaction mixture was filtered and concentrated to dryness to give **11E**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.12−2.18 (m, 10H), 2.25−2.48 (m, 1H), 6.95−7.40 (m, 5H), 7.69 (d, 1H, $J = 5.0$ Hz).[31]

11F. A suspension of 1-methylpyrrole-2-carboxaldehyde (2.70 g, 25.0 mmol), aniline (2.40 g, 25.8 mmol) and magnesium sulfate in diethyl ether (6 mL) was stirred for 16 h at room temperature. To the reaction mixture, 4-Å mol sieves were added, and the reaction mixture was kept at −20 °C for 4 days. The reaction mixture was filtered and evaporated in vacuo to give the Schiff base **11F**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.05 (s, 3H), 6.20 (dd, 1H, $J = 2.5$ Hz, 3.8 Hz), 6.68 (dd, 1H, $J = 1.9$ Hz, 3.8 Hz), 6.79 (dd, 1H, $J = 1.9$ Hz, 2.5 Hz), 7.10−7.22 (m, 3H), 7.31−7.41 (m, 2H), 8.28 (s, 1H).

11G. A suspension of 1-methylindole-3-carboxaldehyde (4.00 g, 25.2 mmol), aniline (2.40 g, 25.8 mmol), and magnesium sulfate in dichloromethane/diethyl ether (6 mL) was stirred for 16 h at room temperature. According to NMR analysis, the conversion was 55%. The reaction mixture was diluted with ethyl acetate and 4-Å mol sieves was added, and it was stirred at room temperature for 4 days. After filtration and evaporation of solvents, the Schiff base **11G** was obtained in 91% purity. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.85 (s, 3H), 7.09−7.45 (m, 8H), 7.54 (s, 1H), 8.43−8.52 (m, 1H), 8.63 (s, 1H).

General Procedure for Synthesis of Nitro Alkenes 12A−G. The flasks **A**−**G** were loaded with polymer-supported nitro acetate (2.50 g, 5 mmol). To each flask **A**−**G** was added 15 mL of acetic anhydride and 15 mmol of the corresponding Schiff base **11A**−**G**. The reaction mixtures **A**−**G** were stirred at 60 °C for 24 h (interval stirring: 15

Table 3. Typical IR Peaks of Resins **14A**−**g** and **15A**−**g**

	ν (cm^{-1})						
	A	B	C	D	E	F	G
14	1747	1744	1749	1744	1745	1741	1739
	1553	1552	1554	1553	1549	1554	1552
15	1727	1727	1732	1726	1728	1721	1720

min/h). The reactions were filtered, and the resins were washed with alternating dimethylformamide and methanol (two cycles) and next with alternating methanol and dichloromethane (two cycles). The resins **12A**−**G** were dried at 70 °C and 400 mbar.

General Procedure for the Cycloaddition Reaction. The Teflon vessels (15 mL) **A**−**G** were loaded with the polymer-supported nitro alkenes **12A**−**G** (2.80 g). To each vessel **A**−**G** was added 2,3-dimethylbuta-1,3-diene (3.20 g, 39 mmol) and 5-*tert*-butyl-4-hydroxy-2-methylphenylsulfide (100 mg).[29] The vessels **A**−**G** were filled with dichloromethane, closed, and placed at 15 kbar, room temperature, for 16 h. The reaction mixtures were filtered, and the resins were washed with alternating warm dimethylformamide and methanol (two cycles) and next with alternating methanol and dichloromethane (two cycles). The resins **14A**−**G** were dried at 70°C and 400 mbar. IR data of resins **14A**−**G** are listed in Table 3.

General Procedure for Reduction. A suspension of the resins **14A**−**G** (∼3 g) in 25 mL of a 2.0 M solution of tin(II) chloride dihydrate (50 mmol, ∼10 equiv) in THF/H_2O, 10:1, was stirred at room temperature for 22 h. The reactions were filtered, and the resins were washed with tetrahydrofuran, dimethylformamide (3×), and methanol (2×). To desalt the formed amine, the resin was alternating washed with 20% triethylamine in dichloromethane and methanol (two cycles), then with alternating methanol and dichloromethane (two cycles). The resins **15A**−**G** were dried at 70 °C and 400 mbar. IR data of resins **15A**−**G** are listed in Table 3.

Automated AMAP Synthesis of the Hydantoins. Coupling with Isocyanates and Cyclization Cleavage. Distribution. Before the resins **15A**−**G** were distributed over the reactors, the masses of the empty reactors of the 8 × 10 matrix were measured. The reactors of the columns **A**−**G** were loaded with ∼100 mg of the polymer-supported amines **15A**−**G**. The mass of every reactor was again measured, and the amount of resin in every reactor was determined.

Reaction with Isocyanate. For the eight rows (**a**−**h**) in the 8 × 7 matrix, eight stock mixtures containing a 2.0 M solution of different isocyanates in dichloromethane was prepared. Row a, phenyl isocyanate; row b, 4-cyanophenyl isocyanate; row c, 4-methoxy isocyanate; row d, 4-acetylphenyl isocyanate; row e, 4-(dimethylamino)phenyl isocyanate; row f, cyclohexyl isocyanate; row g, allyl isocyanate; row h, ethyl isocyanatoformate. To each of the seven reactors in a particular row, a−h, was added the appropriate stock solution (2 mL), and the 8 × 7 matrix was agitated using an orbital shaker (30 s every 5 min) at 30 °C for 16 h. The solvent was removed, and the resin in each reactor was washed successively with 2.0-mL portions of dimethylformamide (2×); methanol (2×); alternating dimethylformamide and methanol (two cycles); methanol and dichloromethane (two cycles); and finally, with tetrahydrofuran.

Cyclization Cleavage. To each of the reactors in the 8 × 7 matrix was added a 2.0 M solution of potassium *tert*-butoxide in tetrahydrofuran (2 mL/reactor), and the 8 × 7 matrix was agitated using an orbital shaker (30 s every 5 min) at 30 °C for 18 h. The reactors were evaporated to dryness, and to each reactor, dichloromethane (2.0 mL) was added. The 8 × 7 matrix was agitated for 15 min at room temperature, and the dichloromethane layers were transferred to an 8 × 7 collection block. Again, dichloromethane (2.0 mL) was added, and the procedure was repeated (two cycles). The organic layers in the 8 × 7 collection block were evaporated in vacuo, and to all reactors, a 1.0 M solution of ammonium chloride in water (2 mL) was added. The water layer was extracted twice with dichloromethane. The organic layers were collected in another 8 × 7 collection block, and the reactors were evaporated to dryness to obtain the first cleaved hydantoins. Next, tetrahydrofuran (2 mL) was added to the remaining resin. The reactors were agitated for 15 min at room temperature, and the tetrahydrofuran layers were transferred to an 8 × 7 collection block. Again, tetrahydrofuran was added to the resins, and the procedure was repeated. The tetrahydrofuran was evaporated to dryness, and dichloromethane (2 mL) and 1.0 M ammonium chloride in water (2.0 M) were added. The dichloromethane layer was transferred to an 8 × 7 collection block, and the water layer was extracted again with dichloromethane. The dichloromethane layers were evaporated to dryness to obtain the second part of the cleaved hydantoins. For analysis and gravimetric yield determination, both batches of hydantoins were combined.

Automated AMAP Synthesis of the Spiro-2,5-diketopiperazines. Coupling of Fmoc-Protected Amino Acids with Resin-Bound Amine (15). The weights of the empty reaction vials were determined. After dispensing ∼100 mg of the resin-bound amine **15** over the reaction vials, the exact weight of the filled vials was determined.

Stock solutions (1.0 M) of the Fmoc-protected amino acids Glu, L-Ala, L-Phe, L-Try, L-Pro, L-Val, L-Thr, L-Orn in dimethylformamide were prepared, and 2.45 mL of DIPEA was added to each stock solution. Stock solutions (1.0 M) of 1-hydroxybenzotriazole and PYBOP (benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate) were prepared.

To each reactor, 830 μL of Fmoc-amino acid/DIPEA stock solution was added, then 600 μL of HOBT stock solution, followed by 620 μL of PyBOP stock solution, was added. The reaction vials were shaken on an orbital shaker at 25 °C for 17 h (interval shaking: 30 s/5 min). After removal of reaction liquids, the resins were washed with dimethylformamide and methanol (three cycles) and dimethylformamide.

Fmoc Deprotection of Resins. To each reactor, 2 mL of a 20% piperidine solution in dimethylformamide was added. After shaking the reactors at 25 °C for 30 min, the liquids were removed, and the resins were washed with dimethylformamide, methanol, tetrahydrofuran, methanol, tetrahydrofuran, methanol, dichloromethane, and methanol.

Synthesis of Spiro-2,5-diketopiperazines by Cyclization Cleavage of Resins. To each reactor, 3 mL of a 5% acetic acid solution in toluene was added. After shaking the reactors at 105 °C for 16 h (interval shaking: 30 s/5 min), the reaction liquids were collected in preweighted vials. To each reactor, 3 mL of a 5% acetic acid solution in toluene was added, and the reactors were shaken for 15 min at 25 °C, after which the solution was transferred to the preweighted vials. After evaporation of the solvents, the weights of the vials and the amount of cleaved product were determined.

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Supporting Information Available. To illustrate the ^{1}H NMR analysis of the two compound classes, several ^{1}H NMR spectra of the diastereomeric compounds mixtures are available as Supporting Information (compounds **18Aa**, **18Af**, **18Bd**, **18De**, **18Dg**, and **18Fc**, and **22Aa**, **22Bd**, **22Bg**, **22Db**, **22Dh**, **22Ef**, and **22Ge**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) In this study, 2,3-dimethylbutadiene was selected as a (symmetric) model diene. Application of other (asymmetric) dienes is currently part of our research.

(16) In the IR spectrum, both a strong C═O vibration ($\nu \sim 1754$ cm^{-1}) and an antisymmetric nitro vibration ($\nu \sim 1563$ cm^{-1}) were observed. The absence of O−H vibration signals in the IR was indicative for full conversion of the Merrifield-OH resin into nitro acetate resin **10**. Reported IR data of Merrifield−nitro acetate: 1563 and 1752 cm^{-1} from: Sylvian, C.; Wagner, A.; Mioskowski, C. *Tetrahedron Lett.* **1999**, *40*, 875.

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(18) At first, resin-bound nitro alkene **12** was prepared from polymer-supported nitro acetate **10** and benzaldehyde using microwave irradiation (350 W, 20 min; see ref 13b). However, detailed studies indicated incomplete conversion yielding a mixture of the desired α,β-unsaturated nitro ester **12** and the intermediate Henry adduct. For complete conversion, additional heating of the reaction mixture in acetic anhydride (250 W, 20 min) was necessary. IR analysis showed the C═O vibration at $\nu \sim 1736$ cm^{-1} and the NO_2 vibration at $\nu \sim 1536$ cm^{-1}, in agreement with the spectral properties of methyl (*Z*/*E*)-2-nitro-3-phenylprop-2-enoate.

(19) Although this cycloaddition can be carried out at atmospheric pressure in refluxing toluene, the use of high pressure was preferred in view of shorter reaction times, higher yields, avoiding high temperatures, stirring, and the use of large excesses of 2,3-dimethylbutadiene. Comparison of high

pressure versus reflux conditions: 4-methoxybenzyl (*E*/*Z*)-2-nitro-3-phenyl-2-propenoate reacted with 2,3-dimethylbutadiene and yielded 73% of the cycloadduct after 48 h of reflux in toluene. At 15 kbar, the same cycloadduct was obtained in 86% after 16 h at room temperature.

(20) Optimal reduction conditions were determined from a parallel experiment in which solvents (ethyl acetate, tetrahydrofuran, dimethylformamide), temperature (25 and 50 °C), concentration of tin(II) chloride (0.5−3.0 M), and amount of tin(II) chloride (5.0−15.0 equiv) were varied. In general, conversion to the amine **15** proceeded better at higher tin(II) chloride concentration. At 50 °C, poorer results for the reduction in ethyl acetate were observed, whereas higher conversions were observed in dimethylformamide. A reaction in tetrahydrofuran at room temperature seemed preferable in combination with concentrations of 1.0 M (10 equiv) and 1.5 M (5 equiv) $SnCl_2 \cdot 2H_2O$. Addition of 10% H_2O (recently reported by Willert, M.; Benito, J. M.; Meldal, M. *J. Comb. Chem.* **2003**, *5*, 91−101) resulted in a slight improvement of the yield.

(21) Cyclization attempts with triethylamine (in tetrahydrofuran or ethylene glycol dimethyl ether) or sodium hydride in tetrahydrofuran failed. The stereochemistry of one of the diastereomers of hydantoin **18** was confirmed with X-ray analysis.

(22) The reported yield is after six reaction steps and is based on an initial loading of the hydroxymethylene resin of 1.0−2.0 mmol/g.

(23) In principle, this matrix might be extended into a third dimension by application of a series of dienes; however, for the synthesis of the library as described in this report, just one diene was used.

(24) Attempts to introduce 3′-pyridyl, 4′-pyridyl, and 2′-pyrrole substituents at the R^1 position were unsuccessful. There are indications that the Knoevenagel reaction merely failed using imines with these substituents (according to IR and gravimetrical analysis).

(25) Although for all derivatives **22Xh** the MH^+ signal was found, the MH^+ signal of the Boc-deprotected derivative was dominant in the LC/MS analysis.

(26) ^1H NMR analysis of the spirodiketopiperazine derivatives was carried out in 5% AcOH-d_3 in $CDCl_3$. ^1H NMR analysis of the spirohydantoin derivatives was carried out in DMSO-d_6.

(27) Derivatives with 2′-, 3′-, or 4′-pyridyl substituents either were not formed or were formed in very low yields and purities when following the described synthetic route; unpublished results.

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