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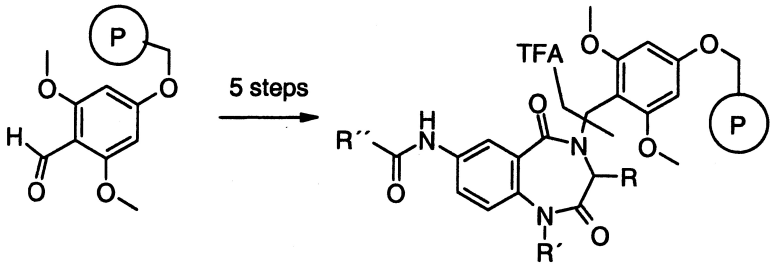

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Solid-Phase Synthesis of 7-Acylamino-1,4-benzodiazepine-2,5-diones

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A method for the synthesis of polymer-bound 7-acylamino-benzodiazepine-2,5-diones is described. The amino group of an α-amino acid is linked to polystyrene or TentaGel resin via reductive amination of polymer-bound 4-alkoxy-2,6-dimethoxybenzaldehyde. Acylation with unprotected 5-nitroanthranilic acid is followed by base-catalyzed ring closure. Reduction of the nitro group yields enantiomerically pure 7-aminobenzodiazepin-2,5-dione attached via the N-4 atom to the resin. Acylation of the amino group on the aromatic ring with acid chlorides in *N*-methylpyrrolidone (no DMF, no base!) followed by cleavage from the resin using TFA/Me_2S/water (90:5:5) provides the acylated benzodiazepinones in 52−69% (PS resin) and 41−48% (TG resin) yield (based on the theoretical loading) and >70% purity (HPLC, 210 nm). Using Fmoc-protected tyrosine fluoride in NMP gives the amino acid-coupled benzodiazepinones in 24% (PS resin) and 31% (TG resin) yield.

Introduction

Since the pathogenesis of allergic diseases is associated with elevated levels of immunoglobulin E (IgE), we developed a high-throughput reporter gene assay in a human B-cell line to screen for low molecular weight IgE inhibitory compounds.[1] Monitoring the IL-4-driven IgE-germline promoter activity (IgE-GLP) the (*S*)-3-cyclohexylmethyl-7-(*N*-α-decanoyltyrosyl)amino-benzodiazepine-2,5-dione **1** (Figure 1) was identified as a potent inhibitor of IgE synthesis in human B-cells. Benzodiazepines are well-known *β*-turn mimetics;[2] however, 7-acylaminobenzodiazepin-2,5-diones of type **1** have not been described before. Explorative hit derivatization in solution quickly revealed the importance of the 7-acylamino group for the inhibition of IgE synthesis. Furthermore, we could readily establish that the NH group in position 4 of the benzodiazepindione ring is key for inhibition of IgE synthesis and may not be substituted, thereby offering an ideal resin attachment point.[3] This important aspect, together with the three to four (protected amino acids) points of variation motivated us to move from a classical solution-phase-based lead optimization to a combinatorial approach utilizing solid-phase chemistry. Our solid-phase strategy for the synthesis of *N*-acyl benzodiazepine-2,5-diones requires ring formation on solid support and attachment to the support via the N-4 atom of diazepinedione ring. Although such protocols have been published,[4] the synthesis of 7-amino-benzodiazepine-2,5-diones and the acylation thereof have never been described, either in solution or on solid support. Here, we report on the development of a solid-phase synthesis of 7-acylamino-1,4-benzodiazepin-2,5 diones based on Ellman's route[4] with the focus on the acylation of resin-bound 7-aminobenzodiazepine-2,5-dione.

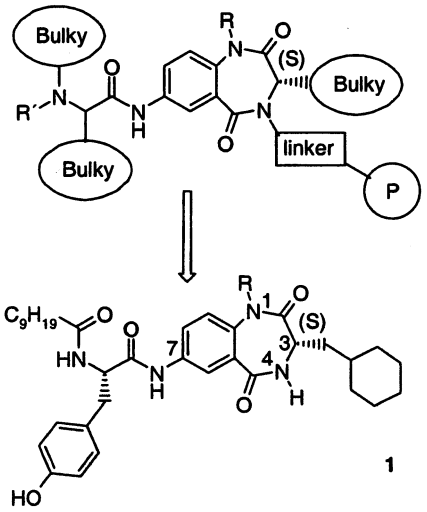

Figure 1. Structural requirements for IgE inhibition as the basis for the selection of the solid-phase synthesis strategy.

Results and Discussion

4-Hydroxy-2,6-dimethoxybenzaldehyde[5] was attached to chloromethylpolystyrene and TentaGel S Br resin using sodium hydride in DMF (Scheme 1). The load of the functionalized resins **2** was determined as 1.16 ± 0.03 (PS) and 0.25 ± 0.01 (TG) mmol/g by a photometric method.[6] The resins **2** were loaded with the starting amino acid ((*S*)-cyclohexylalanine methyl ester) using the racemization free reductive amination procedure.[4] Progress of the reaction was monitored on-bead by the disappearance of the CHO band at 1681 cm^{-1} with FT-IR. Acylation of PS-**3** with 5-nitroanthranilic acid was accomplished using EDC·HCl in NMP. The acylation protocol had to be carried out three times for complete conversion (PS-**4**: first, 27%; second, 66%; third, 102% of theoretical loading). The acylation of TG-**3** was repeated twice; however, in total, only 52% of the theoretical loading was obtained**.** Aiming at shorter reaction times,

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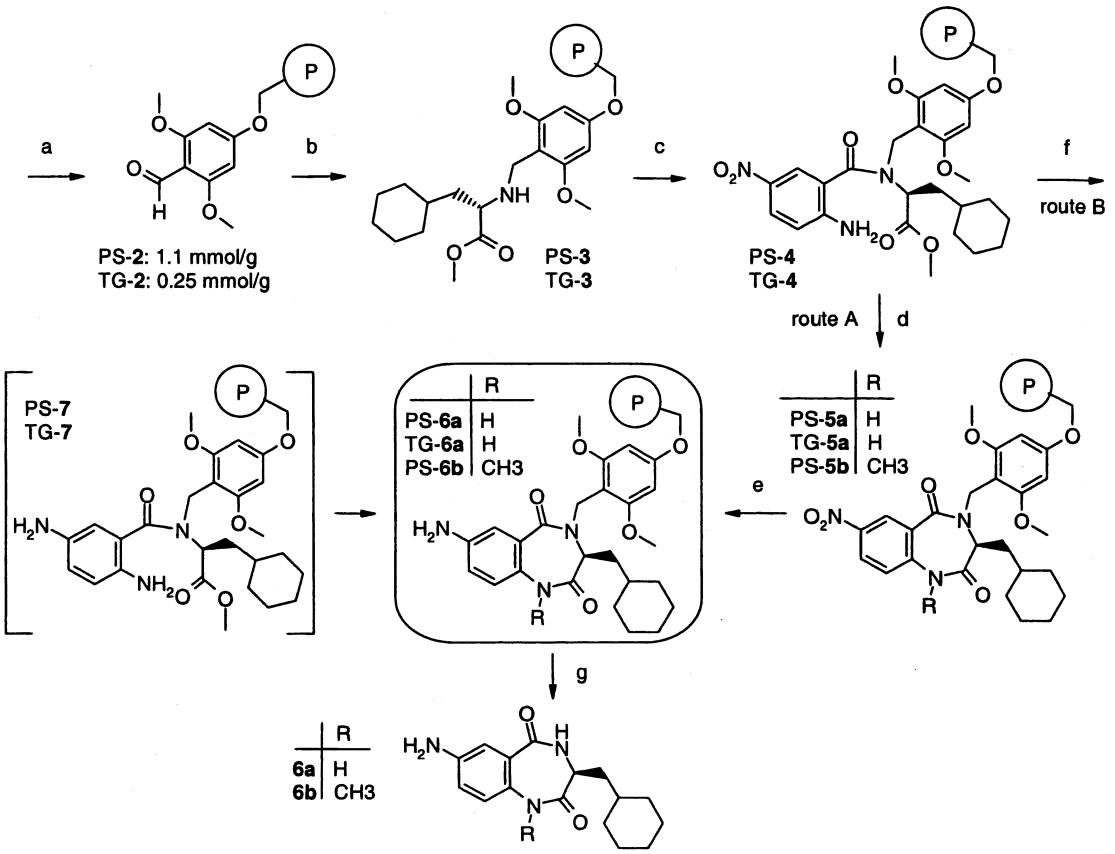
Scheme 1[a]



[a] Conditions: (a) (i) 2,6-dimethoxy-4-hydroxy-benzaldehyde, DMF, NaH, 1 h, ≤ 32°C; (ii) chloromethylpolystyrene (1.35 mmol/g) or TentaGel S Br resin (0.28 mmol/g) preswollen in DMF, 44 h, 50 °C; (b) (i) $NaBH(OAc)_3$, DMF/AcOH (99:1), 0.5 h, rt; (ii) (*S*)-cyclohexylalanine methyl ester hydrochloride, 2 h, rt; (c) (i) EDC·HCl, NMP, 0.5 h, rt; (ii) 5-nitroanthranilic acid, 20 h, rt; this procedure was repeated twice; (d) (i) acetanilide, THF, BuLi, 0.5 h, −78 °C; (ii) DMF, rt; (iii) PS-**4**/TG-**4**, 67 h, rt, (iv) CH_3I or CH_3COOH (R = H), 1 h, rt; (e) $SnCl_2$, DMF, 20 h, 50 °C; (f) $SnCl_2$, DMF/H_2O (93:7), 70 h, 80 °C; (g) TFA/Me_2S/H2O (90:5:5), 16 h, rt.

alternative acylation procedures were investigated. Using isatoic acid anhydride with/without base in DMF or NMP at room temperature and 55 °C gave lower loadings of PS-**4**, even when carried out repeatedly (data not shown).

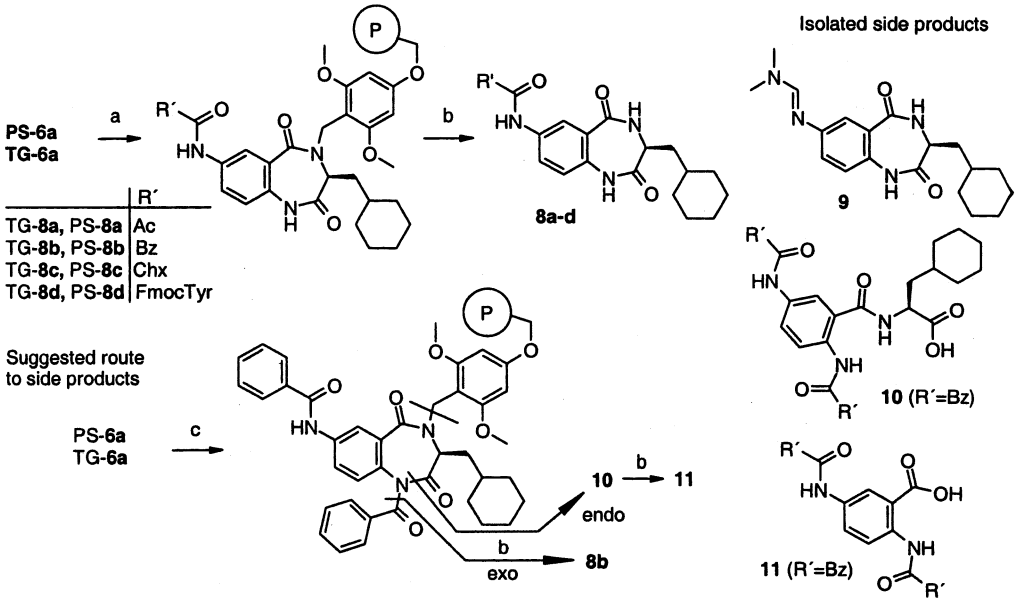
On-bead lactamization to the benzodiazepindione ring and further transformation to **6** was performed following two alternative procedures. Base-induced cyclization of PS/TG-**4** using lithium acetanilide in THF/DMF and subsequent addition of acetic acid or methyl iodide produced PS/TG-**5a**,**b** (Scheme 1, route A). Reduction of the nitro group with $SnCl_2$ in DMF/water at 50 °C gave the desired polymer-bound 7-aminobenzodiazepindiones PS/TG-**6a**,**b**. Shorter reaction times or performing the reaction at room temperature resulted in incomplete reduction.[7] Alternatively, reduction of PS/TG-**4** with $SnCl_2$ yielded the air- and light-sensitive diamines PS/TG-**7**, which lactamized at 80 °C to the polymer-bound benzodiazepindione PS/TG-**6a** (Scheme 1, route B). Route B was optimized, achieving the reduction and lactamization in one step. Cyclization at temperatures lower than 80 °C resulted in incomplete cyclization. For quality control, small amounts of **6a**,**b** were cleaved from the resin using TFA/Me_2S/water (90:5:5) (**6a**,**b** are sensitive to air oxidation!). Table 1 lists the content (purity) in the crude cleavage mixture before chromatographic purification and the overall yields of **6** (defined as found loading divided by theoretical loading). Using the Merrifield polystyrene resin (PS-**4**) and route A (base-catalyzed lactamization), **6a** and **b** were obtained in five steps with an overall purified yield of 55 and 41%, respectively (purity in the crude cleavage mixture: 86 and 78%). The identical procedure applied on TG-**4** (route A) allowed the isolation of **6a** with a purity of 86% and an overall yield of 42%. Route B gave **6a** in a slightly reduced yield (44% for four steps) and purity (73%) from the polystyrene resin. When the synthesis was carried out on TentaGel along route **B**, **6a** was obtained in even lower yield (30%); however, the purity in the crude cleavage mixture (87%) was superior to that obtained with the polystyrene resin (73%). The enantiomerical excess of **6a**

Table 1. Yields and Purity of **6a**,**b** Prepared via Routes A and B

route	resin	product	purity[a] %	yield[b] %	enantiomeric excess[c] [%]
A	PS	**6a**	86	55	97.8
A	PS	**6b**	78	41	nd
A	TG	**6a**	86	42	nd
B	PS	**6a**	73	44	97.0
B	TG	**6a**	87	30	nd

[a] RP-HPLC area % (210 nm) of the product in the crude cleavage cocktail. [b] Yield: measured loading of **6** divided by theoretical loading calculated on the basis of the specified loading for chloromethyl polystyrene (1.35 mmol/g) and TentaGel S Br resin (0.28 mmol/g). [c] Optical purity determined by chiral capillary electrophoresis.

Scheme 2[a]



[a] Conditions: (a) see Table 2 for reagent and conditions; (b) TFA/Me_2S/H2O (90:5:5), 16 h, rt; (c) benzoyl chloride, DMF, DIEA, 16 h rt.

Table 2. Acylation of **6a** (Prepared via Route A) with Acetyl Chloride (**8a**), Benzoyl Chloride (**8b**), and Cyclohexylcarbonyl Chloride (**8c**)

resin	solvent	base	product	purity[a] %	yield[b] %	educt[c] %
PS-**6a**	DMF	DIEA	**8a**	64	53	1
TG-**6a**			**8a**	92	40	<1
PS-**6a**	DMF	DIEA	**8b**	28	21	2
TG-**6a**			**8b**	36	14	<1
PS-**6a**	DMF	DIEA	**8c**	27	13	1
TG-**6a**			**8c**	41	5	<1
PS-**6a**	NMP	no base	**8a**	86	69	1
TG-**6a**			**8a**	100	48	1
PS-**6a**	NMP	no base	**8b**	74	59	2
TG-**6a**			**8b**	92	47	1
PS-**6a**	NMP	no base	**8c**	70	52	1
TG-**6a**			**8c**	91	41	1

[a] Area % (210 nm) of the product in the crude cleavage cocktail. [b] Yield: measured loading of **8** divided by theoretical loading calculated on the basis of the specified loading for chloromethyl polystyrene (1.35 mmol/g) and TentaGel S Br resin (0.28 mmol/g). [c] Measured loading of educt divided by theoretical loading.

obtained via route A and B was assessed as better than 97% via capillary electrophoresis (see Experimental Section for details). We judged the yield and overall purity of polymer bound **6** to be sufficient to explore the acylation conditions for the conversion of polymer bound **6** to **8**.

Acylation of polymer bound **6** proved to be more difficult than expected. Reaction of PS/TG-**6a** with acetyl chloride/DIEA in DMF followed by cleavage from the resin (TFA/Me_2S/water 90/5/5) gave **8a** as the major HPLC product in 53% (PS) and 40% (TG) yields (Scheme 2, Table 2). However, when the less reactive and sterically more demanding benzoyl chloride and cyclohexylcarbonyl chloride were used for acylation of PS-**6a**, only 21% **8b** and 13% **8c** were obtained after treating the resins with the cleavage cocktail. The low yield was not due to incomplete acylation (<2% educt after acylation), but rather was due to several byproducts' dominating the HPLC traces of the crude cleavage mixtures. Acylation of the TentaGel resin (TG-**6a**) gave even worse results. The major byproduct in the reaction of PS/TG-**6a** with benzoyl chloride was identified as the product of the reaction of DMF with **6a,** the *N*,*N*-dimethylamidine **9** (Scheme 2). The amidine **9** was also detected as a byproduct in the reactions of PS-**6a** with acetyl chloride and cyclohexylcarbonyl chloride, albeit to a lesser extent. The formation of amidines from aromatic amines, DMF, and sulfonyl chlorides has been described before; however, acyl chloides were described as bad catalysts for the amidine formation, so far.[8] Using *N*-methylpyrrolidone (NMP) instead of DMF for the acylation of PS/TG-**6a** with benzoyl chloride/Huenig's base prevented the formation of **9**; however, two side products still dominated the reaction mixture. Subsequent isolation and characterization identified the side products as the ring-opened structures **10** and **11** (Scheme 2). As a result of the fact that the corresponding acetylated side products were not observed when the same resin batch PS-**6a** was reacted with acetyl chloride/Huenig's base, we concluded that **10** and **11** must arise from further reaction of PS-**8b** and not from the acylation of the ring-open intermediate PS/TG-**7**. To investigate this surprising observation, we monitored the acylation of **6a** in solution under identical conditions (10-fold excess of benzoyl chloride) used for the on-bead reaction. This experiment revealed that the amide bonds N-1 and N-4 get acylated, as well (on resin only N-1). Depending on the sterical and electronic properties of the acyl moiety, incubation with the cleavage cocktail (TFA/Me_2S/water 90/5/5) could lead to the cleavage of the exo (no side product) or endo imide bond (open-ring side products) (Scheme 2). Furthermore it could be shown in solution that **10** is hydrolyzed to **11** under the cleavage conditions used, while **8b** was stable under these conditions. Having established a sound rationale for the side product formation, for example, the acylation of N-1 and subsequent ring opening, we tried less stringent acylation conditions. Performing the acylation under acidic conditions in NMP

Table 3. Acylation of **6a** with FmocTyr(OtBu)X

resin	reagents	purity **8d**[a] %	yield **8d**[b] %	educt[c] %
PS-**6a**	FmocTyr(OtBu)F	56	26	8
TG-**6a**		82	37	4
PS-**6a**	FmocTyr(OtBu)OH/DIC	60	33	9
TG-**6a**		74	35	3
PS-**6a**	FmocTyr(OtBu)OH/PyBroP/DIEA	55	33	8
TG-**6a**		63	24	4
PS-**6a**	FmocTyr(OtBu)OH/HATU/DIEA	56	32	8
TG-**6a**		76	37	4

[a] Area % (210 nm) of the product in the crude cleavage cocktail. [b] Yield: measured loading of **8d** divided by theoretical loading calculated on the basis of the specified loading for chloromethyl polystyrene (1.35 mmol/g) and TentaGel S Br resin (0.28 mmol/g). [c] Measured loading of educt divided by theoretical loading.

gave the best results (Table 2). All yields improved significantly, especially those for **8b** and **8c**. Although the TentaGel-supported reactions gave somewhat lower yields, the purities of the crude product after cleavage from the resin were superior to those obtained with the polystyrene resin.

Having established the optimal acylation conditions for polymer-bound **6a** with acid chlorides, we investigated the acylation of **6a** with amino acids to give **8d** (Table 3). We chose Fmoc-protected tyrosine *O-tert*-butyl ester preactivated as its flouride[9] and three complementary in situ activation protocols for the coupling procedures. Using NMP as the solvent, all four condensation procedures yielded **8d** in comparable yields and purities. However, for the polystyrene supported reactions, yields did not exceed 33% (compared to 52−69% for the acid chlorides, Table 2), and the purities ranged between 55 and 61%. Although the reactions were allowed to run overnight with a 10-fold excess of reagents, 8−10% educt was still detected in the crude cleavage mixture. The yields obtained with the TentaGel resin were in the range of 24−37% and closer to the yields obtained with the acid chlorides (41−48% Table 2). The acylation of TG-**6a** with FmocTyr(OtBu)F without any base gave **8d** in 34% yield and superior purity (82%), as compared to the other acylation protocols. Noteworthy is the fact that on the TentaGel resin, the acylation with an amino acid fluoride worked again best without any base addition.

Experimental Section

Melting points were determined on a Kofler microscope and are uncorrected. TLC was performed using silicalgel 60 F_{254} (HPTLC, Merck Art. Nr. 5635). Visualization of spots was accomplished using UV light (254/360 nm) or with ninhydrine followed by heating. Analytical HPLC analyses were performed on a HP 1100 system with a multiwavelength detector using a Waters Xterra column (4.6 × 50 mm C_{18}, 3.5 μm) and a flow rate of 2 mL/min. The column was eluted with a linear gradient of 10−95% acetonitrile in water containing 0.1% TFA in 6 min. Preparative HPLC separations were performed on a Gilson system (322 pump, 215 liquid handler 155 dual wavelength detector) using a Waters Prep Nova-Pak cartridge (25 × 100 mm C_{18}, 6 μm) and a flow rate of 20 mL/min. The column was eluted with a linear gradient of 10−90% acetonitrile in water containing 0.1% TFA in 13 min. MS analyses were obtained on a HP 1100 system with direct flow injection into a Thermoquest (Finnigan) MS detector with electrospray ionization. Parallel syntheses were performed on a Quest 210 (Argonaut) in 5-mL reaction vessels or on a Tecan Combitec reaction block. Vacuum removal of solvents was achieved using an IR dancer (Hettlab) or a Genevac centrifuge. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 250 or Avance 400 instruments. Chemical shifts are reported in parts per million relative to TMS as internal standard. All coupling constants are reported in hertz. Optical rotation data were obtained using a Perkin-Elmer Polarimeter 241. On-bead IR spectra were recorded on a Vector 22 FT-IR spectrometer. Only characteristic bands are reported. Determination of enantiomerical exess was performed on an HP3DCE instrument. Capillary: fused silica, 23.5 cm, i.d. 75 μm; mobile phase: 10 mM Tris-buffer, adjusted to pH 2.25 using citric acid + 4.1 mg/mL crown ether; voltage: 20 kV; *T*: 20 °C; UV detection: 210 und 254 nm. Combustion analysis was performed at central scientific services at Novartis Basel. The quality of all solvents used were of pa quality and not further purified. DMF, THF, MeOH, und diethyl ether were dried over a molecular sieve (4 Å). Yields were determined by submitting 10−100 mg of resin to the general cleavage protocol followed by quantification of the compound using RP-HPLC with external standardizations and are reported as percentage of theoretical loading [millimoles per gram] of the resin on the basis of the loading specified by the supplier: Theoretical loading (f_t) = (f_0)/(1 + $f_0\Delta M_W \times 10^{-3}$) where f_0 is the specified loading and ΔM_W is the mass difference [grams per mole] per reaction.

Resins. Merrifield resin (chloromethyl polystyrene, 1% divinylbenzene; load, 1.35 mmol/g; 200−400 mesh), from Alexis Corporation (Läufelfingen, Switzerland); TentaGel S Br (load 0.28 mmol/g) obtained via RAPP Polymer GmbH (Tübingen, Germany). 2,6-Dimethoxy-4-hydroxybenzaldehyde was synthesized from 3,5-dimethoxyphenol in 2 steps (61% yield).[5]

Cleavage from the Resin, General Procedure. Resin dried to constant weight (10−100 mg) was suspended in TFA/Me_2S/water (90:5:5; 1 mL) and gently shaken overnight. Evaporation of the solvents in a vacuum was followed by the addition of acetonitrile/water 9:1 containing 0.1%TFA. After sonification for 30 min and centrifugation (10 000 rpm, 10 min), the clear supernatant was subjected to analytical or preparative HPLC. The yields for **4**−**8** were calculated using the 254-nm absorption in the analytical HPLC with external calibration.

Solid Support 2, PS-2.[4] 2,6-Dimethoxy-4-hydroxybenzaldehyde (4.06 g, 22.0 mmol) in DMF (170 mL) was treated with NaH (0.857 g of a suspension in white oil (60%), 21.3 mmol). After addition of DMF, preswollen Merrifield resin (8.30 g, 11.0 mmol), and gently stirring at 50 °C for 44 h, the salmon-colored resin was collected by filtration, washed with DMF (7×), DCM (7×), and MeOH (3×) and dried in a vacuum to constant weight. Yield 96% (5). On-bead IR spectrum: 1681 cm^{-1} (CHO), 1600 cm^{-1}, 1199 cm^{-1}.

TG-2. Analogously to PS-**2**, 2,6-dimethoxy-4-hydroxybenzaldehyde (1.53 g, 8.40 mmol), NaH (0.330 g of a

suspension in white oil (60%), 8.25 mmol) and TentaGel S Br (10.0 g, 2.80 mmol) gave after 69 h reaction time at 50 °C TG-**2**. Yield: 93%. On-bead IR spectrum: 1679 cm^{-1} (CHO), 1601 cm^{-1}, 1202 cm^{-1}.

Solid Support 3, PS-3. A solution of $NaHB(OAc)_3$ (4.23 g, 20.0 mmol) in DMF containing 1% AcOH (140 mL) was treated with DMF preswollen resin PS-**2** (8.50 g, 9.60 mmol) and gently stirred for 30 min, followed by the addition of (*S*)-2-amino-3-cyclohexylpropionic acid methyl ester hydrochloride (4.43 g, 20.0 mmol) as a solid. After stirring for 2 h at room temperature, the resin was collected by filtration and washed with MeOH (2×), DMF (7×), DCM (7×), and MeOH (3×). Yield: 100% based upon the disappearance of the CHO signal in the on-bead IR spectrum. 1735 cm^{-1} (COOMe), no CHO signal observed.

TG-3. As described above, using $NaHB(OAc)_3$ (1.78 g, 8.40 mmol) in 60 mL of DMF (containing 1% AcOH), reaction of TG-**2** (10.37 g, 2.80 mmol) and (*S*)-2-amino-3-cyclohexyl propionic acid methyl ester hydrochloride (1.86 g, 8.40 mmol, 3 equiv) provided TG-**3** in quantitive yield.

Solid Support 4, PS-4. *N*-Methylpyrrolidone (NMP) preswollen PS-**3** (9.34 g, 8.87 mmol) was treated with a suspension of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (8.51 g, 44.4 mmol, 5 equiv) in NMP (120 mL) and stirred for 30 min. 5-Nitroanthranilic acid (6.46 g, 35.5 mmol, 4 equiv) was added as a solid within 40 min. After complete addition, the suspension was gently stirred for 20 h at room temperature. The resin was collected by filtration from the deep yellow reaction solution and washed with NMP (5×). The acylation procedure was repeated twice until no free amino acid could be detected in the cleavage mixture by TLC using Ninhydrin staining. Finally, the resin was washed with DMF (10×), DCM (7×), and MeOH (3×) and dried to constant weight. On-bead IR spectrum: 1735 cm^{-1} (COOMe, C═O), 1316 cm^{-1} (NO_2, C—NO_2). A small portion of the resin was treated with the cleavage cocktail, yielding **4** with 87% purity (HPLC, 254 nm). Theoretical loading, 0.82 mmol/g; found loading, 0.84 ± 5% mmol/g. Prep-HPLC provided **4** as yellow crystals. mp 155−156 °C; $C_{17}H_{23}N_3O_5$ (349.39) calcd C, 58.44%; H, 6.64%; N, 12.03%. Found C, 58.38%; H, 6.61%; N, 11.99%. TLC R_f = 0.31 (toluene EE = 8:2), $[\alpha]^{20}_D = +3.7°$ (c = 0.993, $CHCl_3$). ^1H NMR ($CDCl_3$): δ (ppm) = 0.85−1.90 (m, 13H), 3.80 (s, 3H), 4.80 (dt, J = 8.1 Hz, J = 5.5 Hz, 1H), 6.48 (bs, 2H), 6.60 (d, J = 9.1 Hz, 1H), 6.78 (bd, J = 8.1 Hz, 1H, NH), 8.06 (dd, J = 9.1 Hz, J = 2.5 Hz, 1H), 8.39 (d, 2.5 Hz).

TG-4. Using the above procedure, TG-**3** (10.7 g, 2.78 mmol, 1 equiv) was acylated twice with 5-nitroanthranilic acid (2.04 g, 11.2 mmol, 4 equiv) using EDC·HCl (2.68 g, 14.0 mmol, 5 equiv) in NMP. After the final wash, a deeply yellow colored resin (TG-**4**) was obtained. Theoretical loading, 0.25 mmol/g; found loading, 0.13 mmol/g. On-bead IR spectrum: 1733 cm^{-1} (broad, COOMe, C═O), 1636 cm^{-1} (CONH, C═O), 1318 cm^{-1} (NO_2, C—NO_2). Purity after cleavage (HPLC, 254 nm), 92% **4**.

Solid Support 5a (Route A), PS-5. A suspension of PS-**4** (1.50 g, 1.23 mmol, 1 equiv) in THF (5 mL) was treated with lithium acetanilide (prepared from acetanilide (4.15 g, 30.7 mmol) and *n*-BuLi (15.4 mL, 1.6 M solution in hexane)) and gently stirred for 67 h. Acetic acid (7.0 mL, 123 mmol, 100 equiv) was added to the deep-red reaction mixture, and the mixture was stirred for an additional hour. The obtained pale-colored resin was isolated by filtration and washed with DMF/25% AcOH (3×), DMF (4×), DCM (7×), and MeOH (3×). On-bead IR spectrum: 1697 cm^{-1} (CONHAr, C═O), 1644 cm^{-1} (ArCONH, C═O), 1336 cm^{-1} (NO_2, C—NO_2). A small portion of the resin was treated with the cleavage cocktail providing **5a** in 92% purity (HPLC, 254 nm). Theoretical loading, 0.84 mmol/g; found loading, 0.46 mmol/g. Prep-HPLC provided **5a** ((*S*)-3-cyclohexylmethyl-7-nitro-3,4-dihydro-1*H*-benzo-[*e*][1,4]diazepine-2,5-dione) as pale yellow crystals, mp 245−249 °C; $C_{16}H_{19}N_3O_4$ (317.35) calcd: C, 60.22%; H, 6.06%; N, 13.17%. Found: C, 60.21%; H, 5.96%; N, 13.07%. TLC: R_f = 0.55 (EtOAc). $[\alpha]^{20}_D$ = +132.2° (c = 1.02, MeOH). ^1H NMR (DMSO-d_6): δ (ppm) = 0.68−1.74 (m, 13H,), 3.80 (dt, J = 5.7 Hz, J = 8.5 Hz, 1H), 7.30 (d, J = 9.0 Hz, 1H), 8.35 (dd, J = 9.0 Hz, J = 2.8 Hz, 1H), 8.52 (d, 2.8 Hz, 1H), 8.71 (bd, J = 5.7 Hz, 1H), 10.96 (s, 1H).

TG-**5a** was prepared analogously, yielding a deep-yellow-colored resin. Theoretical loading, 0.251 mmol/g; found loading, 0.11 mmol/g. On-bead IR spectrum: 1698 cm^{-1} (CONHAr, C═O), 1637 cm^{-1} (ArCONH, C═O), 1330 cm^{-1} (NO_2, C—NO_2); purity (HPLC, 254 nm) 94% **5a**.

Solid Support 5b (Route A), PS-5b. Under argon, a solution of acetanilide (10.65 g, 78.8 mmol, 24 equiv) in THF (135 mL) was cooled to −78 °C and treated with *n*-BuLi (41.0 mL, 1.6 M solution in hexane, 65.7 mmol, 20 equiv). After 30 min at −78 °C, the white precipitate was dissolved by the addition of DMF (135 mL) while warming up to room temperature. Via a syringe, the lithium acetanilide solution was transferred to a suspension of PS-**4** (1.50 g, 1.23 mmol, 1 equiv) in THF (5 mL) and gently stirred for 25 h. Iodomethane (8.2 mL, 131.2 mmol, 40 equiv) was added to the deep-red reaction mixture, and the mixture was stirred for 22 h. The pale-colored resin was isolated by filtration and washed with DMF/25% AcOH (3×), DMF (4×), DCM (7×), and MeOH (3×) and dried to constant weight. A small portion of the resin was treated with the cleavage cocktail, yielding **5b** with 79% purity (HPLC, 254 nm). Theoretical loading, 0.84 mmol/g; found loading, 0.40 mmol/g. Prep-HPLC provided **5b** ((*S*)-3-cyclohexylmethyl-1-methyl-7-nitro-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione) as pale yellow crystals. mp 234−237 °C. $C_{17}H_{21}N_3O_4$ (331.37). ESI (MH^+) 332.4. ^1H NMR ($CDCl_3$): δ (ppm) = 0.82−1.97 (m, 13H), 3.48 (s, 3H), 3.80 (dt, J = 6 Hz, 9 Hz, 1H), 6.74 (m, 1H), 7.41 (d, J = 9 Hz, 1H), 8.41 (dd, J = 3 Hz, 1 Hz, 1H), 8.79 (d; J = 3 Hz, 1H).

Solid Support PS-6a (Route A). PS-**5a** (1.0 g, 0.84 mmol, 1 equiv) was added to a solution of $SnCl_2$ (4.77 g, 25.2 mmol, 30 equiv, 1.7 M) in DMF (15 mL), and the mixture was stirred for 20 h at 50 °C. After cooling to room temperature, the resin was collected by filtration and washed with DMF/25%, AcOH (3×), DMF/10% Et_3N (3×), DMF (2×), DCM (6×), and MeOH (2×). Drying in a vacuum to constant weight provided PS-**6a** as a brownish-red resin. On-bead IR: 1686 cm^{-1} (CONHAr, C═O), 1637 cm^{-1} (ArCONH, C═O). A small portion of the resin was treated with the

cleavage cocktail, yielding **6a** in 78% purity (HPLC, 254 nm). Theoretical loading, 0.86 mmol/g; found loading, 0.47 mmol/g. Prep-HPLC provided **6a** ((*S*)-7-amino-3-cyclohexylmethyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione) as brownish crystals. mp 176−178 °C; purity (HPLC, 254 nm), 98.7%. $[\alpha]_D^{20} = +223.6°$ ($c = 0.98$, MeOH), enantiomeric excess 97.0%. $C_{16}H_{21}N_3O_2$ (287.36) calcd: C, 66.88%; H, 7.37%; N, 14.62%. Found: C, 66.89%; H, 7.38%; N, 14.30%. TLC: $R_f = 0.26$ (DCM/MeOH = 10:1). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.68−1.74 (m, 13H), 3.57 (dt, J = 6.0 Hz, J = 8.2 Hz, 1H), 5.18 (bs, 2H, NH_2), 6.71 (dd, J = 8.5 Hz, J = 2.6 Hz, 1H), 6,78 (d, J = 8.5 Hz, 1H), 6,90 (d, 2.6 Hz, 1H), 8.21 (d, J = 6.0 Hz, 1H), 9.86 (s, 1H).

TG-**6a** was prepared analogously.

Solid Support PS-6b (Route A), PS-6b. PS-**5b** (3.9 g, 3.28 mmol, 1 equiv) was added to a solution of $SnCl_2$ (12.4 g, 65.6 mmol, 20 equiv, 1.7 M) in DMF (35 mL), and the mixture was stirred for 42 h at 50 °C. After cooling to room temperature, the resin was collected by filtration and washed with DMF/25% AcOH (3×), DMF/10% Et_3N (3×), DMF (2×), DCM (6×), and MeOH (2×). Drying in a vacuum to constant weight provided PS-**6b** as a brownish-red resin. A small portion of the resin was treated with the cleavage cocktail, yielding **6b** in 78% purity (HPLC, 210 nm). Theoretical loading, 0.86 mmol/g; found loading, 0.35 mmol/g. Prep-HPLC provided **6b** ((*S*)-7-amino-3-cyclohexylmethyl-1-methyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione) as colorless crystals. mp 138−141 °C. $[\alpha]_D^{20} = +208.1°$ ($c = 0.1$, MeOH). $C_{17}H_{23}N_3O_2$ (301.39) ESI MH^+ 302.3. ^{1}H NMR ($CDCl_3$) δ (ppm) = 0.70−1.80 (m; 12H), 1.87 (m; 1H), 3.34 (s; 3H), 3.84 (dt, J = 6 Hz, 9 Hz, 1H), 3.86 (br s; 2H), 6.09 (d, J = 6 Hz, 1H), 6.86 (dd, J = 3 Hz, 10 Hz, 1H), 7.04 (d, J = 10 Hz, 1H), 7.13 (d, J = 6 Hz, 1H).

Solid Support 6a (Route B), PS-6a. PS-**4** (1.00 g, 0.82 mmol, 1 equiv) was added to a solution of $SnCl_2$ (3.11 g, 16.4 mmol, 20 equiv) in DMF/water (15:1), and the mixture was gently stirred at 80 °C for 70 h. After cooling to room temperature, the resin was collected by filtration and washed with DMF/25% AcOH (3×), DMF/10% NMM (3×), DMF (4×), DCM (7×), und MeOH (3×). Drying in a vacuum at 50 °C yielded PS-**6a** as a golden-brownish-colored resin. On-bead IR spectrum: 1686 cm^{-1} (CONHAr, C═O), 1637 cm^{-1} (ArCONH). A small portion of the resin was treated with the cleavage cocktail, yielding **6a** in 73% purity (HPLC, 254 nm). Theoretical loading, 0.86 mmol/g; found loading, 0.38 mmol/g; 73% **6a**.

TG-**6a** was prepared analogously. On-bead IR spectrum: 1683 cm^{-1} (CONHAr, C═O), 1637 cm^{-1} (ArCONH, C═O). Purity after cleavage: 87% (HPLC, 254 nm). Theoretical loading, 0.251 mmol/g; found loading, 0.08 mmol.

General Acylation Protocol. Resins **6a** (100 mg) were suspended in *N*-methylpyrrolidone (2 mL), treated with acyl halogenides (1 mmol), and shaken for 16 h at room temperature. After washing with NMP (3×), DMF (3×), MeOH (3×) and dichloromethane (3×), the resins were dried in a vacuum to constant weight. Treating the resins with the cleavage cocktail followed by prep-HPLC provided the following final products.

***N*-((*S*)-3-Cyclohexylmethyl-2,5-dioxo-2,3,4,5-tetrahydro-1*H*-benzo[*e*][1,4]diazepin-7-yl)-acetamide (8a).** Colorless crystals, mp 171−175 °C; $C_{18}H_{23}N_3O_3 \cdot 0.65\ H_2O$ (341.11) calcd: C, 63.38%; H, 7.18%; N, 12.32%. Found: C, 63.48%; H, 7.21%; N, 11.96%. TLC: $R_f = 0.22$ (DCM/MeOH = 10:1). $[\alpha]_D^{20} = +227.8°$ ($c = 0.98$, THF). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.65−1.74 (m, 13H), 2.03 (s, 3H, $COCH_3$), 3.63 (dt, J = 5.9 Hz, J = 8.4 Hz), 7.02 (d, J = 8.7 Hz, 1H), 7.69 (dd, J = 8.7 Hz, J = 2.5 Hz, 1H), 7.98 (d, 2.5 Hz, 1H), 8.40 (bd, J = 5.9 Hz, 1H), 10.07 (s, 1H), 10.24 (s, 1H).

***N*-((*S*)-3-Cyclohexylmethyl-2,5-dioxo-2,3,4,5-tetrahydro-1*H*-benzo[*e*][1,4]diazepin-7-yl)-benzamide (8b).** Colorless crystals, mp 309−314 °C. $C_{23}H_{25}N_3O_3 \cdot 0.50\ H_2O$ (395.98) calcd: C, 69.77%; H, 6.49%; N, 10.61%. Found: C, 69.74%; H, 6.42%; N, 10.45%. TLC: $R_f = 0.33$ (DCM/MeOH = 10:1). $[\alpha]^{20}_D = +227.8°$ ($c = 0.98$, THF). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.68−1.75 (m, 13H), 3.67 (dt, J = 5.8 Hz, J = 8.0 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 7.48−7.70 (m, 3H), 7.93 (dd, J = 8.8 Hz, J = 2.5 Hz, 1H), 7.95−8,04 (m, 2H), 8.18 (d, 2.5 Hz, 1H), 8.43 (bd, J = 5.8 Hz, 1H), 10.31 (s, 1H), 10.40 (s, 1H).

Cyclohexanecarboxylic Acid ((*S*)-3-Cyclohexylmethyl-2,5-dioxo-2,3,4,5-tetrahydro-1*H*-benzo[*e*][1,4]diazepin-7-yl)-amide (8c). Colorless crystals, mp 298−302 °C; $C_{23}H_{31}N_3O_3 \cdot 0.50\ H_2O$ (406.53) calcd: C, 67.95%; H, 7.93%; N, 10.34%. Found: C, 67.92%; H, 7.83%; N, 10.23%. TLC: $R_f = 0.36$ (DCM/MeOH = 10:1). $[\alpha]_D^{20} = +236.0°$ ($c = 0.973$, THF). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.63−1.94 (m, 23H), 2.19−2.38 (m, 1H), 3.62 (dt, J = 5.9 Hz, J = 8.2 Hz, 1H), 7.01 (d, J = 8.7 Hz, 1H), 7.71 (dd, J = 8.7 Hz, J = 2.5 Hz, 1H), 8.00 (d, 2.5 Hz, 1H), 8.38 (bd, J = 5.9 Hz, 1H), 9.93 (s, 1H), 10.12 (s, 1H).

Fmoc-tyrosine ((*S*)-3-Cyclohexylmethyl-2,5-dioxo-2,3,4,5-tetrahydro-1*H*-benzo[*e*][1,4]diazepin-7-yl)amide (8d). Colorless crystals, mp 167−171 °C. $C_{40}H_{40}N_4O_6 \cdot 0.60\ H_2O$ (683.60) calcd: C, 70.28%; H, 6.07%; N, 8.20%. Found: C, 70.33%; H, 6.17%; N, 8.07%. TLC $R_f = 0.30$ (DCM/MeOH = 10:1). $[\alpha]^{20}_D = +131.9°$ ($c = 0.98$, THF). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.72−1.70 (m, 13H), 2.78 (dd, J = 13.5, J = 10.1, 1H), 2.93 (dd, J = 13.5 Hz, J = 5.0 Hz, 1H), 3.64 (dt, J = 5.5 Hz, J = 8.3 Hz, 1H), 4.07−4.24 (m, 3H), 4.29 (dt, J = 5.0 Hz, J = 9 Hz, 1H), 6.67 (d, J = 8.4 Hz, 2H), 7.04 (d, J = 8.8 Hz, 1H), 7.14 (d, J = 8.4 Hz, 2H), 7.25−7.36 (m, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.61−7.73 (m, 3H), 7.76 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 7.5 Hz, 2H), 8.05 (d, 2.5 Hz, 1H), 8.41 (d, J = 5.5 Hz, 1H), 9.19 (s, 1H, OH), 10.22 (s, 1H), 10.27 (s, 1H).

Side Products. When PS-**6a** was treated with benzoyl chloride and DIEA in DMF, significant amounts of **9** were formed, as determined after treating the resin with the cleavage cocktail and product isolation by prep HPLC.

***N*′-((*S*)-3-Cyclohexylmethyl-2,5-dioxo-2,3,4,5-tetrahydro-1*H*-benzo[*e*][1,4]diazepin-7-yl)-*N*,*N*-dimethylformamidine (9).** ($C_{19}H_{26}N_4O_2 + C_2HF_3O_2$) (342.21 + 113.99). ESI MH^+ 343.3. ^{1}H NMR ($CDCl_3$): δ (ppm) = 0.74−1.87 (m, 13H), 3.37/3.45 (2s, 6H), 3.73 (m, 1H), 7.02/7.43 (2d, J = 9 Hz, 2H), 7.75 (s, 1H), 8.35 (s, 1H), 9.34 (br d, 1H).

When PS/TG-**6a** was acylated with an excess of cyclohexylcarbonyl chloride and DIEA and treating the washed

and dried resin with the acidic cleavage cocktail for 16 h at room temperature, the following byproducts dominated the reaction mixture:

(*S*)-2-(2,5-Bis-benzoylamino-benzoylamino)-3-cyclohexyl-propionic Acid (10). ($C_{30}H_{31}N_3O_5$) 513.60. [MH^-] = 512.3, [$2MH^-$] = 1025.5. TLC: R_f = 0.15 (DCM/MeOH = 10:1). 1H NMR (250 MHz, CDCl3/DMSO-d_6 = 4:1): δ (ppm) = 0.83−1.92 (m, 13H, cyclohexylmethyl), 4.71 (dt, J = 5.7 Hz, J = 8.6 Hz, 1H, C2−H), 7.48−7.59 (m, 6H, phenyl−H), 7.93−8.03 (m, 5H, phenyl−H + Ar−C4−H), 8.21 (bd, J = 8.6 Hz, 1H, C2−NH), 8.24 (d, J = 2.4 Hz, 1H, Ar−C6−H), 8.72 (d, 9.0 Hz, 1H, Ar−C3−H), 10.13 (s, 1H, Ar−NH), 12.02 (s, 1H, Ar−NH).

2,5-Bis-(benzoylamino)-benzoic Acid (11). ($C_{21}H_{16}N_2O_4$) 360.37, [MH^-] = 359.1; [$2MH]^-$ = 719.3. TLC R_f = 0.07 (DCM/MeOH = 10:1), ^{1}H NMR (250 MHz, DMSO-d_6): δ (ppm) = 7.46−7.62 (m, 6H, phenyl−H), 7.97−8.05 (m, 4H, phenyl−H), 8.08 (dd, J = 9 Hz, J = 2 Hz, 1H, C_4−H), 8.60 (d, J = 2 Hz, 1H, C_6−H), 8.85 (d, 9 Hz, 1H, C_3−H), 10.12 (s, 1H, Ar−NH), 10.31 (s, 1H, Ar−NH).

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