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Upscaling the Solid-Phase Synthesis of a Tetrahydrocarbazole in Chemical Development

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Abstract:

A multigram solid-phase synthesis of tetrahydrocarbazole 1 was developed based a milligram-scale synthesis from Automated Medicinal Chemistry (Koppitz, M.; Reinhardt, G.; van Lingen, A. *Tetrahedron Lett.* 2005, *46*, 911−914). It was shown that a fast scale-up by a factor of 2000 of the solid-phase synthesis is possible in this case. Highly loaded Rink Amide resin was used, and the eight-step-synthesis was performed within 5 days, yielding 35 g (34%) of desired product 1.

Introduction

The application of solid-phase synthesis within research is widespread in combinatorial medicinal chemistry because this methodology allows the preparation of a broad range of different molecules in a very short time. Additional increase of speed and number of new chemical entities can be achieved by parallel synthesis employing lab robots.[2]

The fast delivery of multigram amounts of drug substance for first toxicological and formulation studies is an important issue in early-phase drug development. To accelerate this process solid-phase chemistry should be considered as an option. Solid-phase procedures are fast compared to liquid-phase chemistry because no purification of intermediate products is necessary. In general the resins are washed and used directly for the next reaction step. Until now, only a few examples of large-scale solid-phase syntheses of non-peptide molecules are reported.[3] Due to the limited number of publications in this research area we started a project to deepen knowledge and practical experiences on this field.

We wanted to investigate if a scale-up of a solid-phase synthesis of the new non-peptide potential drug-substance **1**, which originated from our own research, to a multigram scale is possible. By this strategy a time-consuming transfer to liquid-phase chemistry would be avoided at this early phase of development. A liquid-phase procedure was not available at that point and would have required additional development time. Therefore, solid-phase chemistry was chosen for a fast scale-up. Among the reactions performed on solid phase are not only peptide couplings but also a Fischer indole synthesis and a urea formation.

Research Synthesis

The synthesis of **1** in combinatorial chemistry was performed on a 0.1 mmol scale using commercially available Rink Amide resin **2**[4] with a loading of 0.7 mmol/g (Scheme 1). A solid-phase anchored dipeptide was synthesized by straightforward peptide coupling techniques with Fmoc-protected valine, deprotection with piperidine, and subsequent coupling with amino-cyclohexane-4-one carboxylic acid, **3**. Then, Fischer indole synthesis with phenylhydrazine/$ZnCl_2$, deprotection with piperidine, formation of the urea, and finally cleavage from the resin with TFA yielded the desired compound **1** as a mixture of diastereomers which could be separated by prep-HPLC. The acidic cleavage conditions gave rise to a byproduct (about 10%) of which the structure was resolved during the scale-up process (see below).

According to the research preparation the amide couplings were performed twice, each time with a 3-fold excess of amino acid and the highly reactive, but expensive, HATU ((2-(1-H-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate), **4** (2000 Euro/100 g), as a coupling reagent. Another significant drawback of the research synthesis was the high excess of quite expensive 2-phenylethyl-isocyanate (200 Euro/25 g; 10 equiv) in the urea formation.[1,5] Nevertheless, the yield of the medicinal research reaction sequence was 15 mg of tetrahydrocarbazole **1** (0.1 mmol scale, 31% o. th.).

Scale-Up Results

The main effort was put into rapid optimization of the reaction conditions of the research synthesis rather than the selection of different solid supports. Alternative resins such as the Sasrin resin[6] or the well-known Wang resin[7] were not investigated since these solid supports are less susceptible

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(1) Koppitz, M.; Reinhardt, G.; van Lingen, A. *Tetrahedron Lett.* **2005**, *46*, 911−914.

(2) (a) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555−601. (b) Früchtel, J. S.; Jung, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 17−42. (c) Hermkens, P. H. H.; Ottenheijm, H. C. J. *Tetrahedron* **1996**, *52*, 4527−4554.

(3) (a) Raillard, S.; Ji, G.; Mann, A. D.; Baer, T. A. *Org. Process Res. Dev.* **1999**, *3*, 177−183. (b) Meisenbach, M.; Allmendinger, T.; Mak, C.-P. *Org. Process Res. Dev.***2003**, *7*, 553−558.

(4) (a) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787−3790. (b) Bernatowicz, M. S.; Daniels, S. B.; Köster, H. *Tetrahedron Lett.* **1989**, *30*, 4645−4648. (c) Albericio, F.; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730−3743.

(5) (a) Kick, E. K.; Ellman, J. A. *J. Med. Chem.* **1995**, *38*, 1427−1430. (b) Kolodzicj, S. A.; Hamper, B. C. *Tetrahedron Lett.* **1996**, *37*, 5277−5280. (c) Kaldor, S. W.; Fritz, J. E.; Tang, J.; McKinney, E. R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 3041−3044. (d) Flynn, D. L.; Crich, J. Z.; Devraj, R. V.; Hockermann, S. L.; Parlow, J. J.; South, M. S.; Woodard, S. *J. Am. Chem. Soc.* **1997**, *119*, 4874−4881.

(6) (a) Albericio, F.; Barany, G. *Int. J. Peptide Prot. Res.* **1987**, *30*, 206−216. (b) Fivush, A. M.; Willson, T. M. *Tetrahedron Lett.* **1997**, *38*, 7151−7154. (c) Boojamra, C. G.; Burow, K. M.; Thompson, L. A.; Ellman, J. A. *J. Org. Chem.* **1997**, *62*, 1240−1256.

(7) Penke, B.; Rivier, J. *J. Org. Chem.* **1987**, *52*, 1197−1200.

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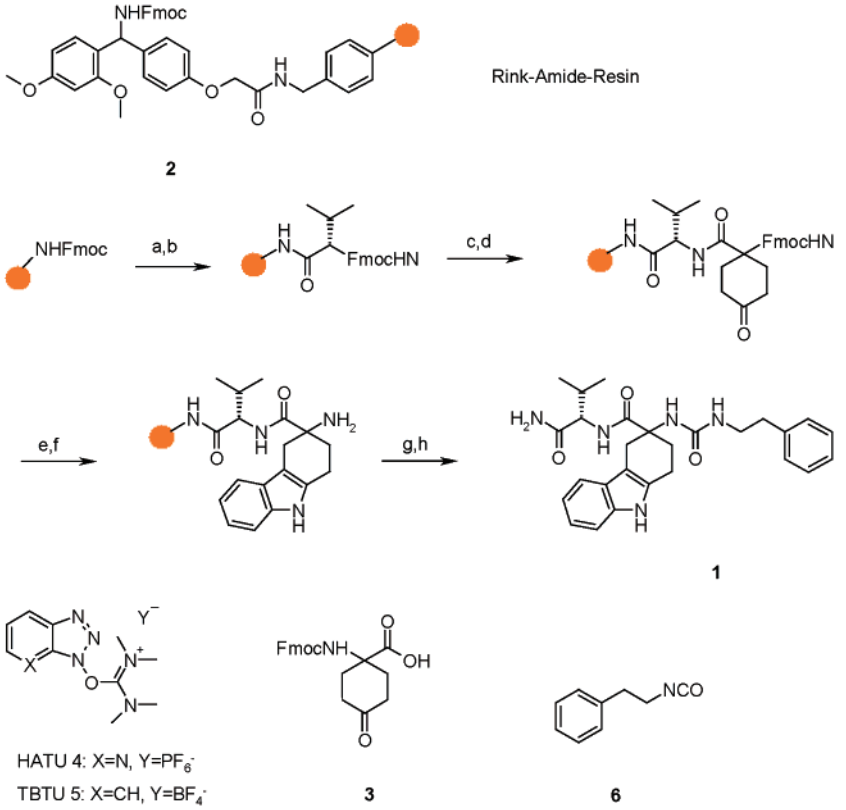
Scheme 1. **Solid-phase synthesis of 1**[a]

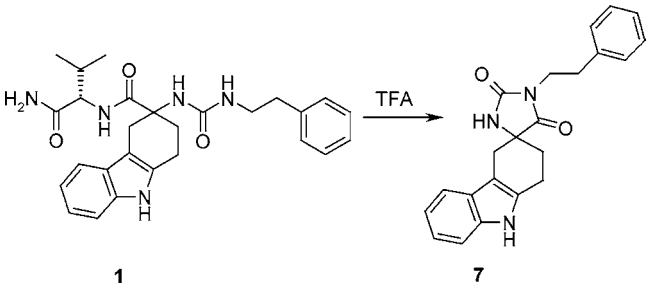


[a] Reagents and conditions: a) piperidine, DMF, RT, 15 min; b) Fmoc-L-Val-OH, TBTU **5**, NMM (*N*-methylmorpholine), DMF, 40 °C, 4 h; c) piperidine, DMF, RT, 15 min. d) **3**, TBTU **5**, NMM, DMF, 40 °C, 4 h; e) AcOH/NMP (*N*-methylpyrrolidone) 7:1, $PhNHNH_2$, $ZnCl_2$; f) piperidine, DMF, RT, 15 min; g) $PhCH_2CH_2NCO$ **6**, DCM (dichloromethane), RT, 18 h; h) 25% TFA in DCM, RT, 4 h.

to acidic cleavage than the Rink Amide resin. More acidic cleavage conditions and longer reaction times were expected, resulting in an increased formation of a side product known from the research synthesis. Analytics were performed at the end of the complete synthesis, and no byproducts due to incomplete reaction steps were found by HPLC after cleavage of the product from the solid support. HPLC analysis showed no products lacking an amino acid unit, and the reactions were shown to deliver reproducible results.

In a first series of experiments with commercially available Rink Amide (0.7 mmol/g loading), it turned out that a repetition of the amide couplings is not necessary, and it could be shown that expensive HATU can be replaced by cheaper TBTU (2-(1-H−benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate), **5**. Furthermore, the excesses of amino acids and coupling reagents were reduced to 1.3 equiv. Especially, the reduced demand for the noncommercially available amino-cyclohexane-4-one carboxylic acid, **3**, had a significant impact on the effectiveness of the synthesis because this building block had to be synthesized in a separate four-step procedure.[8] No further effort was put toward optimizing the coupling reagent at this early stage of drug development. The Fischer indole synthesis from Automated Med. Chem.[1,8,9] was scaled up without any problems. All reagents were soluble under the reaction conditions, preventing diffusion problems. After the reaction,

Scheme 2. **Side reaction of 1 upon exposure to TFA, yielding hydantoin 7**

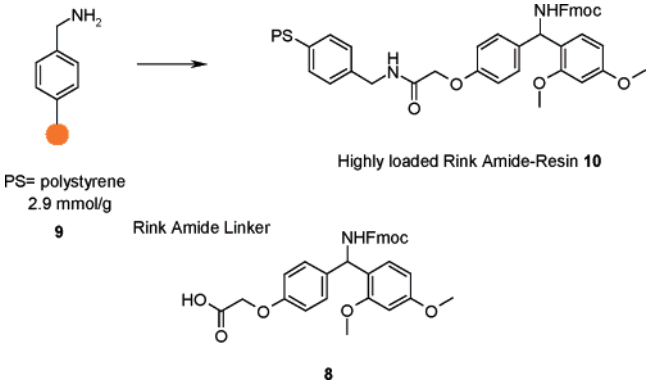


the resin was filtrated, and all reagents were removed by simply washing the resin. No byproducts due to incomplete conversions of the Fisher indole synthesis were observed by HPLC in the final product **1**. In the urea formation the amount of toxic and expensive 2-phenylethylisocyanate, **6**, was reduced to 1.5 equiv (10 equiv in research synthesis), still leading to full conversion even though the amino functionality suffers strong steric hindrance.

The cleavage of the final product from the resin with 95% TFA in water was challenging. Already on a 0.1 mmol scale in research a byproduct was formed (10%) resulting from cleavage of the valine-unit and formation of a hydantoin **7** (Scheme 2). Due to larger reaction volumes during the scale-up process the removal of TFA took significantly more time, resulting in larger amounts of the byproduct **7** (up to 50%). This issue was approached by exchanging the aqueous TFA mixture by a 25% TFA in DCM solution for cleavage. Evaporation of the solvent was much faster, and the amount

(8) Britten, A.; Lockwod, G. *J. Chem. Soc., Perkin Trans.* **1974**, *1*, 1824−1827.
(9) Hutchins, S. M.; Chapman, K. T. *Tetrahedron Lett.* **1996**, *37*, 4869−4872.

Scheme 3. **Synthesis of a highly loaded Rink Resin 10**[a]



[a] Reagents and conditions: Rink Amide linker **8**, TBTU (1.3 equiv each), NMM, DMAP (cat.), DMF, 3 h, 40 °C.

of side product was pushed below 20%. In-depth analysis and improvement of the cleavage step in a similar case are currently ongoing.

A neutralization of the large amounts of TFA with a base was not practical since it results in the generation of large amounts of waste.

During optimization, this synthesis was performed on a 2 g-scale (10 mmol) in good yields (>50%) several times with reproducible results even with reduced amounts of reagents. This is an improvement compared to the research synthesis still using the same Rink Amide resin as a solid support. However, due to the relatively low loading of this resin (0.7 mmol/g) large amounts of solvents and big flasks had to be used in a larger-scale synthesis. To address these issues there was need for a higher loaded resin. The synthesis of such highly loaded Rink Amide resin is already known.[3] Amino-functionalized polystyrene **9** (2.9 mmol/g) was coupled with commercially available Rink Amide linker **8**, yielding the highly loaded Rink Amide resin **10** (Scheme 3).

The eight-step synthesis of **1** was repeated on large scale with the highly loaded resin **10**. 35 g (34%, >95% purity by HPLC) of pure product were obtained from ca. 200 g of resin with only one chromatographic purification procedure after the last step.

Interestingly, the yield was lower (34%) than the ones obtained with commercially available resins (0.7 mmol/g) on a 10 mmol scale. A decrease in yield at higher loading was observed by Raillard[3a] as well. This topic was also addressed by Hudson[10] and Zikos[11] who proposed a decreased accessibility of reactive sites on higher loaded resins. However, Meisenbach et al.[3b] reported constant yields independent of the resin loading.

Comparison to Alternative Syntheses

Since all development syntheses start from the research procedures we did a comparison of both procedures to validate our improvements. Compared to the research synthesis the yield was slightly increased (31% research; 34% development); however, the most significant improvement was made as far as the costs are concerned and the time required for the whole synthesis. In chemical development the fast delivery of first amounts of drug substance is a critical issue as well as the costs. A comparison of the costs of the optimized synthesis with that of the original research synthesis is provided in the following table. Compared to the research synthesis we accomplished a scale-up by a factor of 2000 and significantly shortened the time for the whole procedure. In addition we were able to cut the costs for the whole synthesis by a factor of 4 (see Table 1).

Table 1. **Comparison of research and development synthesis**

	research synthesis	development synthesis
scale	0.1 mmol	208 mmol
yield	15 mg = 31%	35 g = 34%
time for the whole synthesis of **1**	2 weeks	5 days
upscale into gram scale possible?	not practical[12]	yes
costs[13] per 35 g of **1**	25.000 Euro	5.500 Euro

Compared to a hypothetical liquid-phase synthesis our solid-phase procedure is likely to be more expensive. Synthesizing these 35 g of **1** on solid phase required significant amounts of resin and linker. After all 4000 Euro were spent for the resin and the linker whereas reagents and building blocks cost only around 1000 Euro for a 30−40-g campaign which would have to be spent in a liquid-phase procedure as well. Interestingly, most of these additional costs were caused by the Rink Amide linker **8** (2500 Euro), and only 1500 Euro had to be paid for the resin **9** which indicates that the cost of a solid-phase synthesis depends on the sorts of resin and linker that are used. These costs have to be balanced to the amount of work and time which would be needed to develop a solution-phase synthesis. Here, workup procedures would have to be developed which might even lead to a change of reaction conditions (e.g., change of solvents). In solution this reaction sequence would include many purification procedures since equimolar amounts of byproducts (about 70 g) are formed, resulting from TBTU/HATU in the peptide couplings. The workup procedure on that scale would take at least one full day per peptide coupling step. Alternatively, the search for other coupling reagents would definitely afford some time.

After all, a very stable and reliable procedure was developed (the procedure was performed four times with similar results). Performing the whole reaction sequence on a 35-g scale took only 5 days of lab work under the optimized conditions. This amount of drug substance **1** is sufficient to start formulation development and early toxicological studies. Compared to the known large-scale solid-phase synthesis in the literature,[3] these results confirm and expand the findings of Raillard[3a] and Meisenbach[3b] due to the different types of chemical transformations on the solid support, thus making the solid-phase methodology an attractive alternative for fast scale-up of small molecules in early-phase drug development. However, it should be noted that a further scale-up of the synthesis of **1** is still challenging due to decomposition of the product by TFA. This is not necessarily a general problem

(10) Adams, J. H.; Cook, R. M.; Hudson, D.; Jammalamadaka, V.; Lyttle, M. H.; Songster, M. F. *J. Org. Chem.* **1998**, *63*, 3706−3716.
(11) Zikos, C. C.; Frederigos, N. G. *Tetrahedron Lett.* **1995**, *36*, 3741−3744.

of solid-phase chemistry but is likely to happen quite often since cleavage conditions often are relatively harsh.

Currently, this methodology is applied to more sophisticated molecules to broaden the scope of chemical transformations and to deepen the knowledge about solid-phase scale-up techniques and their limits. Publications on the results of that work are currently in preparation.

Conclusion

Solid-phase procedures from combinatorial chemistry can be scaled up to a 30−40-g scale (scale increase by a factor of 2000) without changes to the synthetic approach but with significant changes to the reaction conditions. Large excesses of reagents can be avoided, and thus the costs can be reduced by a factor of 4. This was demonstrated by the fast synthesis of 35 g (34% yield) of **1** on solid phase. These results show the opportunity to use solid-phase chemistry to rapidly provide first amounts of drug substance for early toxicological and formulation studies in drug development. This is of particular interest for development candidates of which solid-phase procedures already exist and a liquid-phase procedure has not been developed yet.

Experimental Procedures

All reagents and solvents were obtained from commercial suppliers and used without further purification. Highly loaded aminomethylated polystyrene (2.9 mmol/g) **9** was obtained from NovaBiochem (Läufelingen, Switzerland). Rink Amide linker **8** was purchased from Iris Biotech GmbH (Marktredwitz, Germany). All resins are 1% cross-linked with divinylbenzene. Amino-cyclohexan-4-one carboxylic acid was prepared according to Britten et al.[8] The introduction of the Fmoc-group was performed as described by Koppitz et al.[1,14]

Reactions were performed using standard laboratory glassware. Standard glass-frits were used for filtrations.

Loading of Aminomethylated Polystyrene. The aminomethylated polystyrene resin (72.4 g, 2.9 mmol/g) was suspended in 1600 mL of DMF. TBTU (87.6 g, 0.273 mol, 1.31 equiv), Rink Amide linker **8** (147.3 g, 0.273 mol, 1.31 equiv), DMAP (1.65 g, 0.0137 mol, 6.5 mol %), and *N*-methylmorpholine (69.0 g, 0.683 mol, 3.3 equiv) were added, and the mixture was stirred at 40 °C for 4 h. The resin was filtered off and washed four times with 1500 mL of DMF. The resin was used without any further manipulations.

Performing Peptide Couplings. The resin was suspended in 1300 mL of DMF, and 300 mL of piperidine were added. The mixture was stirred for 20 min, and the resin was filtered off and washed with four times with 1500 mL of DMF. Then the resin was suspended in 1600 mL of DMF. TBTU (87.6 g, 0.273 mol), Fmoc-protected amino acid (0.273 mol), DMAP (1.65 g, 0.0137 mol, 5 mol %), and *N*-methylmorpholine (69.0 g, 0.683 mol, 2.5 equiv) were added, and the mixture was stirred at 40 °C for 4 h. The resin was filtered off and washed four times with 1500 mL of DMF.

Fischer Indole Synthesis. A mixture of 1200 mL of AcOH, 200 mL of NMP, $ZnCl_2$ (286.2 g, 2.1 mol), and phenylhydrazine (226.8 g, 2.1 mol) was added to the resin and stirred at 70 °C for 18 h. The resin was filtered off and washed four times with 1500 mL of DMF.

Urea Formation. The resin was suspended in 1300 mL of DMF, and 300 mL of piperidine were added. The mixture was stirred for 20 min, and the resin was filtered off and washed four times with 1500 mL of DMF. Then the resin was suspended in 1600 mL of DCM, and phenylethylisocyanate **6** (46.3 g, 0.315 mol) was added. The mixture was stirred for 24 h at room temperature. The resin was filtered off and washed three times with 1500 mL of DCM.

Cleavage. The resin was suspended in 1200 mL of DCM, and 400 mL of TFA was added. The mixture was stirred at room temperature for 3 h. The resin was filtered off, and the procedure was repeated once.

The combined filtrates were evaporated to dryness, and 300 mL of 1 N NaOH and 500 mL of EtOAc were added. A white precipitate was formed which is filtered off to give a first crop of product (11 g). The aqueous phase was extracted with EtOAc (500 mL) and discarded afterwards. The white precipitate which had formed was filtered off and washed with EtOAc (5 g). Half of the solvent was evaporated, and another crop was collected after crystallization at −18 °C for 72 h (2 g). The solvent was removed completely, and the resulting red oil was purified by column chromatography (EtOAc, silica gel). Overall yield: 35.0 g (34%; >95% purity).

Analysis was performed by HPLC. (Agilent 1100 HPLC DAD (diode array detector) and MSD (mass selective detector) SL; column: Kromasil 100 C18 5 μm, 125 mm × 3.0 mm; Eluent A: H_2O + 0.1% HCOOH; Eluent B: ACN + 0.1% HCOOH (A:B 8:2→1:9); UV-detection at 214 nm.

A separation of the diastereomers for analysis was achieved by preparative HPLC using a Purosphere Star C18 5μm (125 mm × 25 mm) using 0.2% NH_3 in H_2O/acetonitrile as an eluent (25 mL/min).

1 (Diastereomer 1): ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.72 (s, 1H), 7.48 (s, 1H), 7.07−7.32 (m, 9H), 6.96 (td, J = 7.5 Hz, J = 1.2 Hz, 1H), 6.88 (td, J = 7.5 Hz, J = 1.0 Hz, 1H), 6.07 (t, J = 6.0 Hz, 1H), 6.12 (s, 1H), 4.13 (dd, J = 9.0 Hz, J = 4.8 Hz, 1H), 3.14 (m, 2H), 2.89 (q, J = 19.2 Hz, 2H), 2.79−2.55 (m, 3H), 2.58 (t, J = 6.3 Hz, 2H), 2.00 (m, 2H), 0.88 (d, J = 6.9 Hz, 3H), 0.79 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 174.5, 173.0, 157.4, 139.6, 136.1, 133.0, 128.6, 128.2, 127.4, 125.9, 120.2, 118.1, 117.0, 110.6, 104.8, 57.9, 56.9, 41.1, 35.9, 30.7, 30.4, 28.3, 19.4, 19.1, 17.2; $[\alpha]^{20}_{540}$ = 137.8 (c = 0.567 mg/mL DMF); mp 248 °C.

1 (Diastereomer 2): ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.73 (s, 1H), 7.45 (s, 1H), 7.07−7.32 (m, 9H), 6.96 (td, J = 7.5 Hz, J = 1.2 Hz, 1H), 6.89 (td, J = 7.5 Hz, J = 1.0 Hz, 1H), 6.18 (t, J = 6.0 Hz, 1H), 6.14 (s, 1H), 4.09 (dd, J = 8.4 Hz, J = 4.5 Hz, 1H), 3.15 (q, J = 6.8 Hz, 2H), 2.90 (s, 2H), 2.59 (t, J = 7.5 Hz, 2H), 2.45−2.73 (m, 3H), 1.98−

(12) A repetitive execution of the small-scale research synthesis is not practical due to large amounts of needed building bock **3**.

(13) Only material costs are considered for total costs of the product.

(14) Kortenaar, P. B. W.; Van Dijk, B. G.; Peeters, J. M.; Raaben, B. J.; Hans, P. J.; Adams, M.; Tesser, G. I. *Int. J. Peptide Prot. Res.* **1986**, *27*, 398−400.

2.18 (m, 2H), 0.87 (d, $J = 7.2$ Hz, 3H), 0.79 (d, $J = 7.2$ Hz, 3H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 174.8, 173.0, 157.8, 139.5, 136.1, 133.2, 128.6, 128.2, 127.3, 125.9, 120.2, 118.1, 117.0, 110.6, 104.5, 57.9, 57.1, 40.6, 35.9, 31.0, 29.8, 27.8, 19.4, 19.1, 17.2; $[\alpha]^{20}_{540} = -116.4$ ($c = 0.613$ mg/mL DMF); IR (KBr) 3370, 3061, 3027, 2967, 2929, 1650, 1635, 1555, 1510, 1265, 743 cm^{-1}; MS (ES) m/z (%) 498 (40, [M^+ + Na^+]), 476 (100, [M^+ + H]), 459 (28), 360 (22), 329 (31), 310 (18), 293 (21); HRMS (EI) calcd 475.2594 for $C_{27}H_{32}N_5O_3$, found 475.2590; mp 249 °C.

Hydantoin 7: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.49 (s, 1H), 7.30−7.15 (m, 7H), 6.98 (dt, $J = 8.0$ Hz, $J = 1$,2 Hz, 1H), 6.90 (dt, $J = 7.8$ Hz, $J = 1.2$ Hz, 1H), 3.62 (t, $J = 7.2$ Hz, 2H), 2.92−2.78 (m, 5H), 2.56 (m, 1H), 1.96 (m, 1H), 1.72 (m, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 176.9, 156.2, 138.6, 136.5, 133.3, 129.3, 128.8, 127.5, 126.9, 120.9, 118.7, 117.5, 111.1, 104.6, 60.3, 39.2, 34.5, 31.0, 30.4, 19.5; IR (KBr) 3370, 3135, 1762, 1702, 1454, 1423 cm^{-1}; MS (ES) m/z (%) 382 (30, [M^+ + Na]), 360 (100, [M^+ + H]); HRMS (EI) calcd 359.1634 for $C_{22}H_{21}N_3O_2$, found 359.1637; mp 213 °C.

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