Enantioselective Synthesis of N-Protected α-Amino Acid Hydrazides

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Abstract: A new, mild, general, and efficient synthesis of N-protected α-amino acid hydrazides is described. This two-step preparation uses *N*-aminophthalimide as protected hydrazine to prepare N-protected α-amino acid hydrazide precursors, and subsequent dephthaloylation with an aminomethyl polystyrene resin yields N-protected α-amino acid hydrazides. It has the advantages of avoiding the use of the toxic hydrazine reagent and being compatible with the most commonly used N-protecting groups. This strategy is particularly interesting in the case of *N*-(9-fluorenylmethoxycarbonyl)-protected amino acids. Within the limits of chiral HPLC detection, no epimerization is apparent.

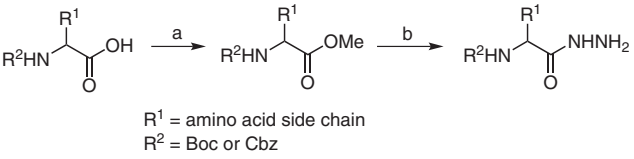
Key words: amino acids, hydrazides, epimerization, protecting groups, aminomethyl resins

Hydrazides have been studied for their biological[1] and chemical properties. This functional group can be used in peptide synthesis as a carboxy protecting group[2] or for hydrazide peptide preparations. Hydrazides can also be used as precursors in heterocyclic chemistry.[3,4] Our study focused on the synthesis of α-amino acid hydrazides that can be obtained from α-amino acids. The use of α-amino acids introduces a chiral center in the target molecules that can be conserved. Additionally, α-amino acids need orthogonal protection to potentially reactive groups.

Several strategies have been described for the synthesis of N-protected α-amino acid hydrazides. There are mainly two synthetic pathways, namely hydrazinolysis of esters and acylation of protected hydrazines.

The most commonly employed method is hydrazinolysis of alkyl esters, mainly methyl esters (Scheme 1). This reaction needs strong conditions and employs toxic reagents, including hydrazine monohydrate. In the case of N-protected α-amino acids, the yields range from low to medium. In our study, the major problem was the use of hydrazine which did not allow the 9-fluorenylmethoxycarbonyl (Fmoc) group to be used for amine protection.

Another example of this strategy has been described by Krysin[5] and concerns hydrazinolysis of trimethylsilyl esters of N-protected amino acids. The hydrazinolysis step was achieved with anhydrous hydrazine. The reported yields are good but the main problem is the use of anhydrous hydrazine, which is difficult to handle and incompatible with the presence of *N*-Fmoc protection.



Scheme 1 Hydrazinolysis of N-protected amino acid methyl esters. *Reagents and conditions*: (a) MeI (5 equiv), DBU, MeCN, reflux, 6 h; (b) $H_2NNH_2 \cdot H_2O$ (3 equiv), MeOH, r.t., overnight.

In our attempt to develop a general method for the synthesis of N-protected α-amino acid hydrazides, we looked at different strategies in which protected hydrazines were used as key reagents. Quibell[6] described the acylation of (benzyloxycarbonyl)-protected hydrazine with N-protected valine and lysine esters. In a second step, the benzyloxycarbonyl (Cbz) protecting group was removed by hydrogenolysis. In the case of *N*-Fmoc protection, standard hydrogenolysis conditions can lead to partial or total deprotection of the Fmoc group.[6,7] This last method has three major limitations: during (benzyloxycarbonyl)hydrazine acylation, a small amount of the Fmoc group was aminolyzed; the workup is very long and difficult; and it has been described, to our knowledge, only for Fmoc-valine and Fmoc-lysine(Boc).

Stravropoulos[8] reported the solid-phase synthesis of small hydrazide peptides. We applied this method to the synthesis of Fmoc-phenylalanine hydrazide. This reaction, for which trityl resin is used, proceeds in good yield and results in good purity, and workup is easy. The limitations are related to the cleavage conditions that are not compatible with the *tert*-butoxycarbonyl (Boc) protecting group, as well as the cost of this strategy.

The Stravropoulos and Quibell studies convinced us that the use of a protected hydrazide was a good way for developing a general multigram synthesis of N-protected α-amino acid hydrazides in solution (especially with the Fmoc protecting group). The choice of a protected hydrazine, easily deprotected under mild conditions with respect to Boc, Cbz, and Fmoc protecting groups, is very important for the synthesis of α-amino acid hydrazides.

Brosse[9] described a two-step synthesis of isoniazide in which *N*-aminophthalimide was used as a protecting group: the procedure consisted of activation of isonicotinic acid with 1,1′-carbonyldiimidazole (CDI) and coupling with *N*-aminophthalimide followed by dephthaloylation. The yields reported by Brosse are good, but, to apply this strategy for α-amino acid hydrazides, the use of 1,1′-car-

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bonyldiimidazole under strong conditions (for α-amino acids) can induce epimerization, and the use of amine or hydrazine during the dephthaloylation process is not compatible with the Fmoc protecting group. Nevertheless, we decided to explore this strategy to adapt it to our goal.

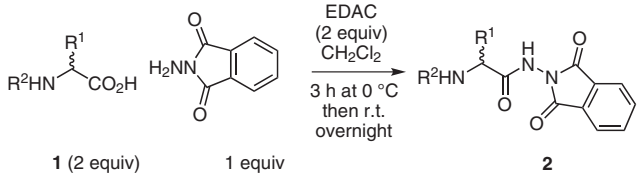
The first challenge was the activation of the carboxylic acid; Brosse commented on the difficulty of the coupling step (mainly due to the poor reactivity of the NH_2 group of *N*-aminophthalimide). For the coupling of N-protected amino acid **1** with *N*-aminophthalimide, we tried different activating reagents widely used in peptide synthesis and known to avoid epimerization (Table 1, see also Scheme 2).

Table 1 Conditions and Reagents Used for the Coupling Step[a]

Activating agent	Conditions
IBCF (1 equiv)	NMM, THF, –10 °C to r.t.
PyBOP (1 equiv)	DIEA, 0 °C to r.t.
HBTU (1 equiv)	DIEA, 0 °C to r.t.
DIC (1 equiv)	DMAP (0.01 equiv), 0 °C to r.t.
EDAC[b] (2 equiv)[c]	0 °C to r.t.

[a] Reaction conditions: **1** (1 equiv), $PhthNNH_2$ (1.1 equiv), activating agent.
[b] EDAC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride.
[c] EDAC (1.4 equiv) was generally used, but for some difficult reactions EDAC (2 equiv) was used, and is generally best used. DCC is similarly effective, but results in a difficult workup.

Symmetrical anhydrides obtained by carbodiimide reagents {e.g., 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDAC)} seemed to give the best results (Table 1, Scheme 2). It allowed us to get the corresponding N-protected hydrazides in good to excellent yields (Table 2).



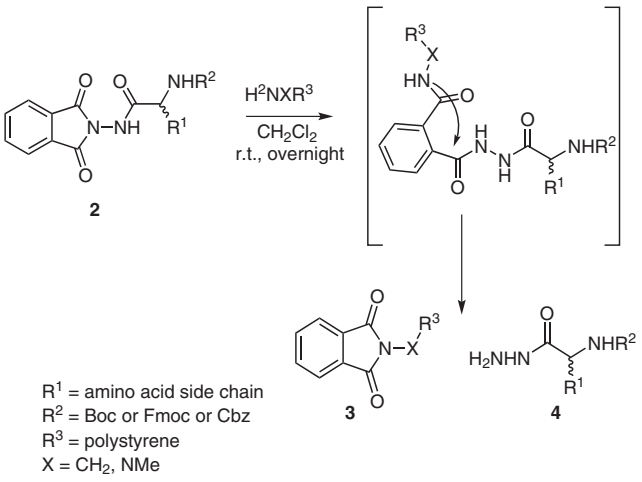
Scheme 2 Coupling of N-protected amino acids **1** with *N*-aminophthalimide in the presence of a carbodiimide activating reagent

The second step, that is, deprotection (or dephthaloylation) of **2** gave more difficulties. Known methods for the dephthaloylation include the use of primary amines (e.g., methylamine) or unsubstituted or monosubstituted hydrazines (e.g., phenylhydrazine, methylhydrazine).[9] These two procedures were not compatible with our attempt to synthesize *N*-Fmoc α-amino acid hydrazides. Therefore we decided to use a supported reagent to avoid partial Fmoc cleavage. Two kinds of supported reagents on polystyrene resin were tested, namely resin substituted with aminomethyl (X = CH_2) and *N*′-methylhydrazino (X = NMe; Scheme 3). The most efficient resin was found to be the aminomethyl resin. During the dephthaloylation, no Fmoc deprotection was observed by LCMS analysis of the crude product. The yields of the dephthaloylated α-amino acid hydrazides **4** are collected in Table 3.

Table 2 Isolated Yields for the Coupling Step[a]

N-Protected residue[b] C(O)CHR[1]NHR[2]	2	Yield[c] (%)
Cbz(L)Phe	**2a**	71[d]
Fmoc(L)Phe	**2b**	56[d]
Boc(L)Tyr(Dzb)	**2c**	39[d]
Boc(L)Trp	**2d**	81[d]
Boc(D)Trp	**2e**	83
Boc(L)Lys(Cbz)	**2f**	100
Fmoc(L)Asp(*t*-Bu)	**2g**	98
BocGly	**2h**	n.d.[e]
Boc(L)Orn(Cbz)	**2i**	29
Fmoc(L)Orn(Boc)	**2j**	98
Fmoc(L)Ile	**2k**	65[d]
Fmoc(L)Pro	**2l**	100

[a] See also footnote c of Table 1.
[b] The 'N-protected residue' corresponds to the C(O)CHR[1]NHR[2] moiety.
[c] All final compounds were identified by LCMS.
[d] Compounds were characterized by ^1H and ^{13}C NMR spectroscopy. See experimental section.
[e] n.d. = not determined.



Scheme 3 Dephthaloylation of **2** with supported reagent

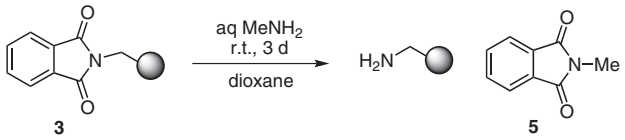
Table 3 Isolated Yields Obtained for the Dephthaloylation Step

Phthalimide **2**	Product **4**	Yield[a] (%)
2a	**4a**	88
2b	**4b**	83[b]
2c	**4c**	51[b]
2d	**4d**	70[b]
2e	**4e**	72
2f	**4f**	74
2g	**4g**	72[b]
2h	**4h**	89
2i	**4i**	68[b]
2j	**4j**	51[b]
2k	**4k**	90
2l	**4l**	82

[a] All final compounds were identified by LCMS.
[b] Compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy; see experimental section.

We then checked the optical purity of the obtained hydrazides. For this purpose, two Trp enantiomer derivatives were synthesized, and the optical purity was monitored by chiral HPLC (Scheme 4).[10] Under our experimental conditions, no epimerization could be observed within the limits of chiral HPLC detection. In addition, the specific rotation of **4d** and **4e** was also measured: $[\alpha]_D^{20}$ +11.7 and –11.5, respectively (*c* 1.00, MeOH).

In the synthesis described above, the aminomethyl resin can be regenerated upon treatment of **3** with aqueous methylamine to give the aminomethylated resin (Scheme 5), which can be used again in the dephthaloylation step. Mitchell[11] carried out the dephthaloylation with hydrazine monohydrate. More recently, Adams[12] reinvestigated this process using aqueous methylamine, a less toxic reagent. For resin regeneration, we chose to use



Scheme 5 Resin recycling

a dioxane/methylamine mixture. Under these conditions, the reaction proceeded at room temperature.

In conclusion, we reported a new method for the synthesis of α-amino acid hydrazides using nontoxic *N*-aminophthalimide. This strategy could be applied to the more currently used protecting groups in peptide chemistry. The use of mild conditions and very selective cleavage of the hydrazine protecting group brought us closer to the possibility of the synthesis of hydrazide peptides in solution. Moreover, no or little epimerization was detected under these conditions, and the resin used for the removal of the *N*-phthalimide protecting group could be regenerated and recycled for use in further deprotection cycles.

Solvents and other chemicals were obtained commercially and used as received. NMR spectra were recorded on a Bruker AMX-300 spectrometer. Column chromatography was performed on silica gel (230–400 mesh).

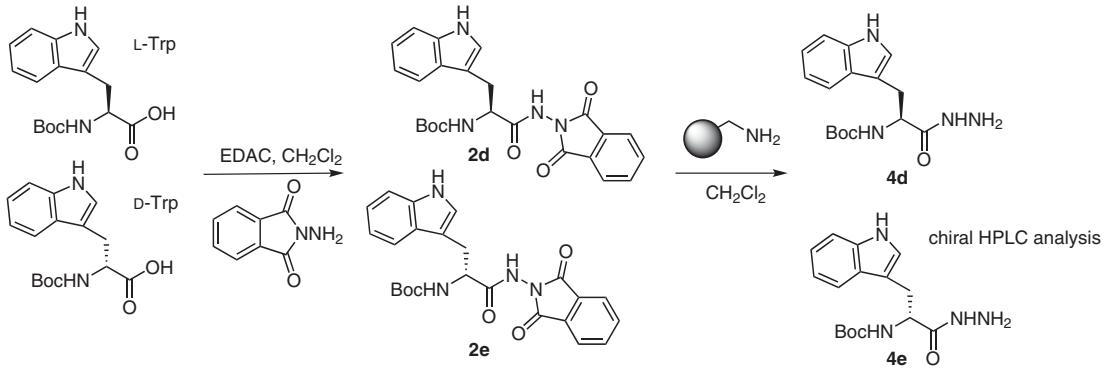
N-Protected Hydrazides 2a–l; General Procedure

The protected amino acid **1** (2 equiv) and EDAC (2 equiv) were dissolved in the minimum amount of CH_2Cl_2 at 0 °C. The mixture was stirred at 0 °C for 2 h. $PhthNNH_2$ (1 equiv) was added and the mixture was stirred at 0 °C for 1 h and then at r.t. overnight. Distillation of the solvent under reduced pressure gave a yellow-white solid which was dissolved in EtOAc and washed successively with 1 M $KHSO_4$, sat. aq $NaHCO_3$, and brine. The organic layer was then dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was precipitated with Et_2O to yield compound **2**. If necessary, the residue was purified by column chromatography (silica gel, hexane–EtOAc, 3:7 to 7:3).

Cbz(L)-Phenylalanine-phthalimide (2a)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.91 (dd, *J* = 11, 14 Hz, 1 H, CH_2β Phe), 3.19 (dd, *J* = 4, 14 Hz, CH_2β Phe), 4.57 (m, 1 H, CHα Phe), 4.98 (d, *J* = 4 Hz, 2 H, CH_2 Cbz), 7.23–7.39 (m, 10 H, CH aromatics Cbz, Phe), 7.76 (d, *J* = 9 Hz, 1 H, NH Cbz), 7.97 (m, 4 H, CH aromatics Phth), 10.99 (s, 1 H, NH hydrazide).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 37.6 (CH_2β Phe), 54.7 (CHα Phe), 65.3 (CH_2 Cbz), 123.7 (C_4C_7 Phth), 126.4 (C_4 Phe), 127.3



Scheme 4 Synthesis of Trp enantiomers for chiral HPLC analysis

(C_2C_6 Phe), 127.6 (C_4 Cbz), 128.1–128.2–129.2 ($C_2C_3C_5C_6$ Cbz, Phe), 129.5 (C_8C_9 Phth), 135.2 (C_5C_6 Phth), 136.9 (C_1 Cbz), 137.5 (C_1 Phe), 155.8 (CO Cbz), 164.9 (C_1C_3 Phth), 171.2 (CO hydrazide).

Fmoc(L)-Phenylalanine-phthalimide (2b)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.93 (dd, J = 11, 13 Hz, 1 H, CH_2β Phe), 3.19 (dd, 1 H, 3, 14 Hz, CH_2β Phe), 4.17 (m, 3 H, CH_2CH Fmoc), 4.56 (m, 1 H, CHα Phe), 7.19–7.34 (m, 6 H, $H_2H_3H_4H_5H_6$ Phe, NH Fmoc), 7.40 (m, 4 H, $H_3H_6H_2H_7$ Fmoc), 7.65 (m, 2 H, H_4H_5 Fmoc), 7.88 (d, J = 7 Hz, 2 H, H_1H_8 Fmoc), 7.97 (m, 4 H, $H_4H_5H_6H_7$ Phth), 11.00 (s, 1 H, NH hydrazide).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 38.9 (CH_2β Phe), 46.5 ($CHCH_2$ Fmoc), 54.6 (CHα Phe), 65.8 ($CHCH_2$ Fmoc), 120.0 (C_1C_8 Fmoc), 123.7 (C_4C_7 Phth), 125.3 (C_2C_7 Fmoc), 126.4 (C_4 Phe), 127.0 (C_4C_5 Fmoc), 127.6 (C_3C_6 Fmoc), 128.1, 129.2 ($C_2C_3C_5C_6$ Phe), 129.5 (C_8C_9 Phth), 135.2 (C_5C_6 Phth), 137.6 (C_1 Phe), 140.6–143.6 ($C_{4a/b}$, $C_{8a/b}$ Fmoc), 155.8 (CO Fmoc), 164.9 (C_1C_3 Phth), 171.2 (CO hydrazide).

Boc(L)-Tyrosine(DCB)-phthalimide (2c)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.31 (s, 9 H, CH_3 Boc), 2.82 (dd, J = 9, 14 Hz, 1 H, CH_2β Tyr), 3.09 (dd, J = 3, 14 Hz, 1 H, CH_2β Tyr), 4.42 (m, 1 H, CHα Tyr), 5.20 (s, 2 H, CH_2 DCB), 6.98–7.10 (dd, J = 8, 9 Hz, 2 H, H_3H_5 Tyr, NH Boc), 7.34 (d, J = 8 Hz, 2 H, H_2H_6 Tyr), 7.44–7.57 (m, 3 H, $H_3H_4H_5$ DCB), 7.92–8.00 (m, 4 H, $H_4H_5H_6H_7$ Phth), 10.85 (s, 1 H, NH hydrazide).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 28.1 (CH_3 Boc), 36.8 (CH_2β Tyr), 54.4 (CHα Tyr), 64.8 (CH_2 DCB), 78.2 (C quat Boc), 114.2 (C_3C_5 Tyr), 123.7 (C_4C_7 Phth), 128.7 (C_3C_5 DCB), 129.5 (C_8C_9 Phth), 130.2 (C_1 Tyr), 130.3 (C_2C_6 Tyr), 131.4 (C_4 DCB), 135.2 (C_5C_6 Phth), 136.0 (C_1 DCB), 155.2 (CO Boc), 157.1 (C_4 Tyr), 164.9 (C_1C_3 Phth), 171.3 (CO hydrazide).

Boc(L)-Tryptophan-phthalimide (2d)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.32 (s, 9 H, CH_3 Boc), 3.03 (dd, J = 14, 10 Hz, 1 H, CH_2 β Trp), 3.28 (m, 1 H, CH_2 β Trp), 4.50 (m, 1 H, CHα Trp), 6.98 (t, J = 8 Hz, 1 H, H_5 Trp), 7.02 (d, J = 7 Hz, 1 H, H_4 Trp), 7.08 (t, J = 7 Hz, 1 H, H_6 Trp), 7.23 (s, 1 H, H_2 Trp), 7.35 (d, J = 8 Hz, 1 H, H_7 Trp), 7.68 (d, J = 8 Hz, 1 H, NH Boc), 7.97 (m, 4 H, H_4 H_5 H_6 H_7 Phth), 10.86 (m, 2 H, NH indole Trp, NH hydrazide).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 27.9 (CH_2 β Trp), 28.1 (CH_3 Boc), 53.5 (CH α Trp), 78.2 (C quat Boc), 109.6 (C_3 Trp), 111.3 (C_7 Trp), 118.2 (C_4 Trp), 118.4 (C_5 Trp), 120.8 (C_6 Trp), 123.7 (C_4C_7 Phth), 123.9 (C_2 Trp), 127.3 (C_9 Trp), 129.5 (C_8C_9 Phth), 135.2 (C_5C_6 Phth), 136.0 (C_8 Trp), 155.1 (CO Boc), 165.0 (C_1C_3 Phth), 171.6 (CO hydrazide).

Boc-Glycine-phthalimide (2h)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.39 (s, 9 H, CH_3 Boc), 3.83 (d, J = 6 Hz, 2 H, CH_2α Gly), 7.13 (t, J = 6 Hz, 1 H, NH Boc), 7.95 (m, 4 H, $H_4H_5H_6H_7$ Phth), 10.65 (s, 1 H, NH hydrazide).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 27.8 (CH_3 Boc), 41.5 (CH_2 α Gly), 78.2 (C quat Boc), 123.7 (C_4C_7 Phth), 129.4 (C_8C_9 Phth), 135.2 (C_5C_6 Phth), 155.7 (CO Boc), 165.0 (C_1C_3 Phth), 169.0 (CO hydrazide).

Fmoc(L)-Isoleucine-phthalimide (2k)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.88 (t, J = 7 Hz, 3 H, CH_3δ Ile), 1.02 (d, J = 6 Hz, 3 H, CH_3γ Ile), 1.23, 1.56 (m, 2 H, CH_2γ Ile), 1.84 (m, 1 H, CHβ Ile), 4.17–4.33 (m, 4 H, $CHCH_2$ Fmoc, CHα Ile), 7.34 (m, 2 H, H_3H_6 Fmoc), 7.44 (m, 2 H, H_2H_7 Fmoc), 7.72 (d, J = 10 Hz, 1 H, NH Fmoc), 7.77 (m, 2 H, H_4H_5 Fmoc), 7.92 (m, 6 H, $H_4H_5H_6H_7$ Phth, H_1H_8 Fmoc), 10.88 (s, 1 H, NH hydrazide).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 10.7 (Cδ Ile), 15.1 (CH_3γ Ile), 24.3 (CH_2γ Ile), 36.5 (CHβ Ile), 46.6 (CH Fmoc), 57.3 (CHα Ile), 65.8 (CH_2 Fmoc), 120.0 (C_1C_8 Fmoc), 123.7 (C_4C_7 Phth), 125.4 (C_2C_7 Fmoc), 127.0 (C_4C_5 Fmoc), 127.6 (C_3C_6 Fmoc), 129.4 (C_8C_9 Phth), 135.2 (C_5C_6 Phth), 140.7, 143.6, 143.9 ($C_{4a/b}$, $C_{8a/b}$ Fmoc), 156.0 (CO Fmoc), 165.0 (C_1C_3 Phth), 170.0 (CO hydrazide).

Compounds 4a–l; General Procedure

Aminomethylated polystyrene resin (3 equiv; 1.1 mmol/g, 100–200 mesh) was conditioned for 10 min at r.t. in CH_2Cl_2. Then **2** (1 equiv) was added, and the mixture was slowly stirred for 24 h at r.t. The mixture was filtered and the solvent was evaporated in vacuo to give product **4** as a white solid.

Fmoc(L)-Phenylalanine-$NHNH_2$ (4a)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.85 (dd, J = 10 Hz, 1 H, CH_2β Phe), 2.93 (dd, J = 13 Hz, 1 H, CH_2β Phe), 4.20 (m, 6 H, CH_2CH Fmoc, $NHNH_2$, CHα Phe), 7.20 (d, J = 6 Hz, 1 H, NH Fmoc), 7.24–7.35 (m, 7 H, $H_2H_3H_4H_5H_6$ Phe, H_2H_7 Fmoc), 7.41 (t, J = 7 Hz, 2 H, H_3H_6 Fmoc), 7.64 (dd, J = 7, 12 Hz, 2 H, H_4H_5 Fmoc), 7.88 (d, J = 7 Hz, 2 H, H_1H_8 Fmoc), 9.21 (s, 1 H, $NHNH_2$).

^{13}C NMR (75 MHz, DMSO-d_6): δ =37.7 (CH_2β Phe), 46.5 ($CHCH_2$ Fmoc), 54.9 (CHα Phe), 65.6 ($CHCH_2$ Fmoc), 120.0 (C_3C_6 Fmoc), 125.2, 125.3 (C_2C_7 Fmoc), 126.2 (C_4 Phe), 127.0 (C_4C_5 Fmoc), 127.5 (C_3C_6 Fmoc), 128.0, 129.1 ($C_2C_3C_5C_6$ Phe), 138.0 (C_1 Phe), 140.6, 143.7 ($C_{4a/4b}$, $C_{8a/8b}$ Fmoc), 155.6 (CO Fmoc), 170.7 (CO hydrazide).

Boc(L)-Tyrosine(DCB)-$NHNH_2$ (4c)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.30 (s, 9 H, CH_3 Boc), 2.65–2.73 (dd, J = 10, 13 Hz, 1 H, CH_2β Tyr), 2.81–2.87 (1 H, dd, J = 4, 14 Hz, CH_2β Tyr), 4.08 (m, 1 H, CHα Tyr), 4.30 (sl, 2 H, NH_2 hydrazide), 5.18 (s, 2 H, CH_2 DCB), 6.83 (d, 1 H, 8 Hz, NH Boc), 6.95 (d, J = 8 Hz, 2 H, H_3H_5 Tyr), 7.19 (d, J = 8 Hz, 2 H, H_2H_6 Tyr), 7.43–7.57 (m, 3 H, $H_3H_4H_5$ DCB), 9.09 (s, 1 H, NH hydrazide).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 28.1 (CH_3 Boc), 37.0 (CH_2β Tyr), 54.6 (CHα Tyr), 64.8 (CH_2 DCB), 77.8 (C quat Boc), 114.1 (C_3C_5 Tyr), 128.7 (C_3C_5 DCB), 130.2 (C_1 Tyr), 130.6 (C_2C_6 Tyr), 131.4 (C_4 DCB), 131.7 (C_2C_6 Tyr), 136.0 (C_1 DCB), 155.0 (CO Boc), 156.9 (C_4 Tyr), 170.9 (CO hydrazide).

Boc(L)-Tryptophan-$NHNH_2$ (4d)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.31 (s, 9 H, CH_3 Boc), 2.89 (dd, J = 9, 14 Hz, 1 H, CH_2β Trp), 3.02 (dd, J = 5, 14 Hz, 1 H, CH_2 β Trp), 4.17 (dd, J = 8 Hz, 1 H, CHα Trp), 4.29 (s, 2 H, NH_2 hydrazide), 6.72 (d, J = 8 Hz, 1 H, NHBoc), 6.97 (t, J = 8 Hz, 1 H, H_5 Trp), 7.06 (t, J = 7 Hz, 1 H, H_6 Trp), 7.13 (s, 1 H, H_2 Trp), 7.32 (d, J = 8 Hz, 1 H, H_4 Trp), 7.59 (d, J = 8 Hz, 1 H, H_7 Trp), 9.12 (s, 1 H, NH hydrazide), 10,78 (s, 1 H, NH Trp).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 28.7 (CH_3 Boc), 39.2 (CH_2β Trp), 53.7 (CHα Trp), 77.8 (C quat Boc), 110.1 (C_3 Trp), 111.2 (C_7 Trp), 118.1 (C_4 Trp), 118.4 (C_5 Trp), 120.7 (C_6 Trp), 127.3 (C_9 Trp), 136.0 (C_8 Trp), 155.0 (CO Boc), 171.3 (CO hydrazide).

Fmoc(L)-Aspartic Acid(*t*-Bu)-$NHNH_2$ (4g)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.37 (s, 9 H, CH_3 *t*-Bu), 2.64 (m, 2 H, CH_2β Asp), 4.24 (m, 6 H, CH_2CH Fmoc, $NHNH_2$, CHα Asp), 7.33 (s, 2 H, H_2H_7 Fmoc), 7.42 (s, 2 H, H_3H_6 Fmoc), 7.57 (d, J = 7 Hz, 1 H, NH Fmoc), 7.71 (s, 2 H, H_4H_5 Fmoc), 7.89 (d, J = 6 Hz 2 H, H_1H_8 Fmoc), 9.11 (s, 1 H, $NHNH_2$).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 27.6 (CH_3 *t*-Bu), 37.5 (CH_2β Asp), 46.6 (CHα Asp), 50.3 (CH Fmoc), 65.7 (CH_2 Fmoc), 80.1 (C quat Boc), 120.0 (C_1C_8 Fmoc), 125.2 (C_2C_7 Fmoc), 127.0 (C_3C_6 Fmoc), 127.6 (C_4C_5 Fmoc), 140.6 ($C_{4a/b}$ Fmoc), 143.7 ($C_{8a/b}$ Fmoc), 155.6 (CO Fmoc), 169.2, 169.6 (CO *t*-Bu, hydrazide).

Boc(L)-Ornithine(Cbz)-NHNH$_2$ (4j)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.37–1.58 (m, 14 H, CH_3 Boc, $CH_2\gamma$, $CH_2\beta$ Orn), 2.97 (m, 2 H, $CH_2\delta$ Orn), 3.85 (m, J = 6, 8 Hz, 1 H, CHα Orn), 4.24 (s, 2 H, NH_2 hydrazide), 5.00 (s, 2 H, CH_2 Cbz), 6.74 (d, J = 8 Hz, 1 H, NH Boc), 7.20 (m, 1 H, NH Cbz), 7.33 (m, 5 H, $H_2H_3H_4H_5$ Cbz), 8.98 (s, 1 H, NH hydrazide).

^{13}C NMR (75 MHz, DMSO-d_6): δ = 26.0 (CH_2 γ Orn), 28.1 (CH_3 Boc), 29.5 ($CH_2\beta$ Orn), 39.9 ($CH_2\gamma$ Orn), 52.6 (CHα Orn), 65.1 (CH_2 Cbz), 77.9 (Cq Boc), 127.6 ($C_2C_6C_4$ Cbz), 128.3 (C_3C_5 Cbz), 137.2 (C1 Cbz), 155.1, 156.0 (CO Cbz, Boc), 171.3 (CO hydrazide).

Resin Recycling

The methylphthalimide polystyrene was suspended in 1,4-dioxane and stirred until fully swollen. A 40% aq soln of $MeNH_2$ was added, and the mixture was stirred at r.t. for 3 d. The suspension was filtered and the beads were washed with 1,4-dioxane–H_2O (4:1), 1,4-dioxane, DMF, CH_2Cl_2, and MeOH. After air-drying, the resin could be reused without any further treatment.

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