Synthesis of N^{α}-Tetrachlorophthaloyl (TCP)-Protected Amino Acids under Microwave Irradiation (MWI)

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Abstract: A range of N^{α}-tetrachlorophthaloyl protected amino acids have been synthesized by an easy and efficient condensation procedure of the corresponding amino acid and tetrachlorophthaloyl anhydride under irradiation in an unmodified commercial microwave oven.

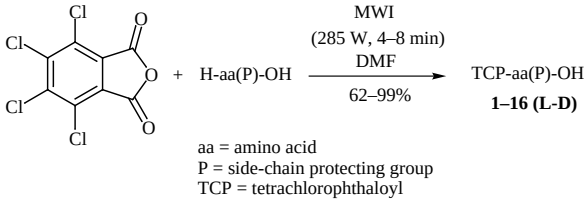
Key words: amino acids, microwave irradiation, protecting groups, phthalimide, tetrachlorophthaloyl

The use of the phthaloyl moiety as primary amine protecting group is extensively documented in the chemical literature.[1] However, the use of this protective group has not been practical in some sensitive substrates due to the quite harsh conditions required for its removal. Addition of electron-withdrawing groups to the phthalimide's aromatic ring greatly enhances deprotection under generally milder conditions than those previously encountered with the phthalimido group. In connection with their work in amino sugars, Fraser-Reid's and Schmidt's groups have described the use of the tetrachlorophthalimido function as a masked amino group. Nevertheless, the common application of the tetrachlorophthalimido protecting group has been limited to sugar chemistry.[2–4] To date, no efforts have been made towards the synthesis of a wide range of *N*-TCP protected amino acids suitable for SPPS (solid-phase synthesis of peptides).[5]

In general, *N*-substituted phthalimides are formed by condensation of phthalic anhydride and an amine by reflux[2–4] (Dean–Stark water separator[6]) or by direct fusion procedures.[7] The phthaloyl group has also been introduced by reacting the amine with 2-(ethoxycarbonyl)benzoic acid activated by PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluoro phosphate), followed by the thermally induced cyclization of the resulting phthalamic ester.[8]

On the other hand, the use of microwave irradiation in organic synthesis has been extensively reported.[9] Microwave assisted reactions can be carried out safely using conventional glassware in domestic microwave ovens, resulting on savings in reaction time and waste disposal. Bose et al. described the synthesis of tetrachlorophthalimidoacetic acid as an intermediate to α-amino-β-lactams from tetrachlorophthalic anhydride and glycine using microwave irradiation in high yields.[10]

We report in this paper an easy and efficient microwave-induced synthesis of *N*-TCP protected amino acids suitable for SPPS by condensing the corresponding amino acid with tetrachlorophthaloyl anhydride. This protocol includes mixing of the amino acid with tetrachlorophthaloyl anhydride in DMF (Scheme 1). After 4–8 minutes of irradiation (285 W) the reactions are complete. A range of L- and D-amino acids, including tri-functional with side-chain protection, have been protected and were obtained with high yields (80–99%) after straightforward workup (Table 1). Most *N*-TCP protected amino acids gave satisfactory elemental analyses without further purification (Table 2). In some syntheses the protected amino acid was purified by simple organic extraction.



Scheme 1

Whereas most of the amino acids were easily protected starting with 1 mmol of both the amino acid and the anhydride, same protections gave substantially better results using 2.1 mmol of the amino acid and 2.0 mmol of the anhydride (entries 2, 10 and 14, Table 1). This approach can be applied to the protection of a larger scale of amino acid obtaining similar to better yields. Thus, we have synthesized TCP-Leu-OH (**4L**), TCP-Ile-OH (**3L**) and TCP-Met-OH (**7L**) starting from 4.2 mmol, 6.3 mmol, and 8.4 mmol of the corresponding amino acid, respectively (entries 4, 6 and 12, Table 1). Moreover, we have shown that using MWI methodology the addition of a tertiary amine can be avoided, in contrast with previous reports that suggested the need of such bases.[10]

Under microwave irradiation *tert*-butyl ethers, esters, carbamates and the trityl function of cysteine were stable. Disappointingly, we observed some detritylation (5–25%) during the synthesis of TCP-His(Trt)-OH (**16L**) and TCP-D-His(Trt)-OH (**16D**); in these cases further purification by flash chromatography was required. TCP-His-OH formation was confirmed by treatment of **16L** with

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TFA-CH_2Cl_2 (1:1) and characterization of the product thus obtained.

All compounds showed optical rotations in good agreement with their stereochemical integrity, except for TCP-Cys(Trt)-OH (**15L-D**) and TCP-His(Trt)-OH (**16L-D**) where the large differences in $[\alpha]_D$ values indicate that some racemization had occurred (Table 2).

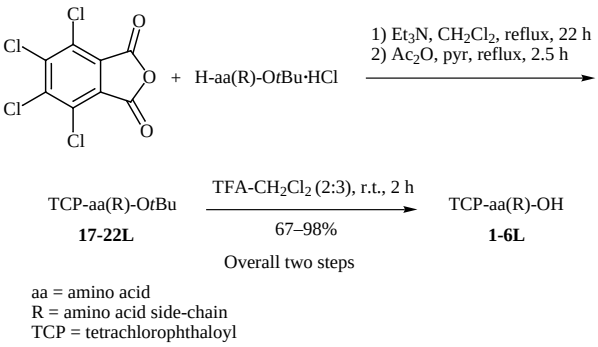
Interestingly, all *N*-TCP protected amino acids showed λ_{max} UV absorption at 334–336 nm which allows selective detection of *N*-TCP protected species and may be useful for monitoring the reactivity of *N*-TCP protected compounds. Furthermore, all are crystalline solids, so their handling and purification are facilitated.

Table 1 Synthesis of *N*-TCP Protected Amino Acids **1**–**14 (L**–**D)** under MWI and Reflux

Entry	Product	MWI[a] Yield (%)[b]	Reflux Yield (%)[c]
1	TCP-Gly-OH (**1**)	85	98
2	TCP-Ala-OH (**2L**)	62 (87)[d]	90
3	TCP-D-Ala-OH (**2D**)	80	
4	TCP-Ile-OH (**3L**)	82 (97)[e]	67
5	TCP-D-Ile-OH (**3D**)	98	
6	TCP-Leu-OH (**4L**)	92 (89)[f]	87
7	TCP-D-Leu-OH (**4D**)	97	
8	TCP-Val-OH (**5L**)	80	72
9	TCP-D-Val-OH (**5D**)	88	
10	TCP-Phe-OH (**6L**)	90 (97)[d]	91
11	TCP-D-Phe-OH (**6D**)	83	
12	TCP-Met-OH (**7L**)	90 (87)[g]	
13	TCP-D-Met-OH (**7D**)	86	
14	TCP-β-Ala-OH (**8**)	64 (89)[d]	
15	TCP-Tyr(Bu-*t*)-OH (**9L**)	99	
16	TCP-D-Tyr(Bu-*t*)-OH (**9D**)	88	
17	TCP-Ser(Bu-*t*)-OH (**10L**)	90	
18	TCP-D-Ser(Bu-*t*)-OH (**10D**)	96	
19	TCP-Thr(Bu-*t*)-OH (**11L**)	96	
20	TCP-D-Thr(Bu-*t*)-OH (**11D**)	96	
21	TCP-Asp(OBu-*t*)-OH (**12L**)	94	
22	TCP-D-Asp(OBu-*t*)-OH (**12D**) **12D**	98	
23	TCP-Glu(OBu-*t*)-OH (**13L**)	99	
24	TCP-D-Glu(OBu-*t*)-OH (**13D**)	89	
25	TCP-Lys(Boc)-OH (**14L**)	88	
26	TCP-D-Lys(Boc)-OH (**14D**)	95	
27	TCP-Cys(Trt)-OH (**15L**)	86	
28	TCP-D-Cys(Trt)-OH (**15D**)	95	
29	TCP-His(Trt)-OH (**16L**)	70	
30	TCP-D-His(Trt)-OH (**16D**)	69	

[a] Reactions carried out using 1 mmol of both the amino acid and tetrachlorophthalic anhydride.
[b] Isolated yield.
[c] Isolated yields determined via the synthesis of TCP-aa-OBu-*t* **17**–**22L** (Table 3). Hydrolysis of the *t*-Bu esters was quantitative.
[d] Results obtained from synthesis carried out using 2.1 mmol of the amino acid and 2.0 of tetrachlorophthalic anhydride.
[e] Results obtained from synthesis carried out using 6.3 mmol of the amino acid and 6.0 of tetrachlorophthalic anhydride.
[f] Results obtained from synthesis carried out using 4.2 mmol of the amino acid and 4.0 of tetrachlorophthalic anhydride.
[g] Results obtained from synthesis carried out using 8.4 mmol of the amino acid and 8.0 of tetrachlorophthalic anhydride.

To demonstrate the simplicity and efficiency of the microwave irradiation technique compared to the reflux methodology we have also synthesized *N*-TCP protected amino acids **1**–**6L** using the latter protocol (Scheme 2). This procedure consists of refluxing the amino acid *tert*-butyl ester hydrochloride and tetrachlorophthalic anhydride in the presence of triethylamine in CH_2Cl_2 for 22 hours, followed by treatment with acetic anhydride and pyridine (1:1) in CH_2Cl_2 for 2.5 hours. Finally, hydrolysis of the *tert*-butyl ester with TFA–CH_2Cl_2 (2:3) provided the *N*-TCP protected amino acids. As shown in Table 1, best yields and purities are generally achieved by the microwave-assisted reactions, which are also much faster, with easier workup and consumption of less quantity of solvent.



Scheme 2

We checked the stability of the TCP function towards standard SPPS treatments. Different portions of a TCP-Gly-Val-PAL-PEG-PS resin[11] were treated at room tem-

perature with DMF (24 h), piperidine–DMF (1:4, 30 min) and $(i\text{-Pr})_2$NEt–DMF (1:19, 30 min) followed by coupling with Boc-Phe-OH. Then, the resin was subjected to cleavage with TFA–H_2O (19:1, 2 h) and the crude product mixture was analyzed by HPLC and mass spectrometry. In all conditions tested, TCP-Gly-Val-NH_2 was obtained exclusively. Thus, the TCP protecting group is stable to standard Fmoc and Boc removal conditions, as well as, to TFA cleavage treatments. These observations agree with the results previously reported in the literature that TCP-sugar derivatives are stable to mildy basic and to very acidic conditions.[2c,d]

Preliminary studies on solid-phase TCP removal were carried out on a TCP-Gly-Val-PAL-PEG-PS resin[11] and we observed that after treatment with hydrazine–DMF (3:17, 1 h, 40 °C) or with ethylenediamine–DMF (1:199, 30 min, 50 °C) the TCP group was removed quantitatively.

In summary, we have described the synthesis of a range of N^{α}-tetrachlorophthaloyl protected amino acids suitable for solid phase synthesis of peptides in high yields and purities by a convenient procedure using domestic microwave ovens.

Commercially available reagents were used throughout without prior purification, except solvents, which were distilled. Mps were determined with a Mel-Temp device. Spectrophotometric analyses were performed on a Shimadzu 160 spectrophotometer. Optical rotations were measured on a Perkin Elmer 243B polarimeter at 20 °C. Aluminum-backed plates coated with silica gel (Scharlau or Macherey-Nagel UV_{254}) were used for analytical TLC; spots were visualized by UV. Analytical reversed phase HPLC analyses were carried out with a Thermo Separation Products P2000 binary pump, a Perkin Elmer LC290 UV/VIS detector, and a Spherisorb ODS-2 column (0.46 × 25 cm; 5 m particle size). Low-resolution fast atom bombardment mass spectroscopy (FABMS) was carried out in a 3-nitrobenzyl alcohol (3-NBA) matrix on a VG Quattro spectrometer at Servicios Xerais de Apoio á Investigación, Universidade da Coruña, Spain. Elemental analyses were determined with a Thermo Instruments elemental analyzer EA 1110 CHNS/O (Universitat de Girona) or with a Carlo Erba Instruments elemental analyzer EA 1108 CHNS/O (Universidade da Coruña). ^{1}H NMR spectra were recorded at 200 MHz on a Bruker OPX200 Avance instrument. Chemical shifts were expressed in ppm relative to TMS as internal reference, *J* values are given in Hz. ^{13}C NMR spectra were recorded on a Bruker OPX200 Avance instrument operating at 50.33 MHz. Amino acid abbreviations denote L-configuration unless otherwise noted. Microwave irradiations were conducted in a commercial Moulinex Optimo microwave oven. Peptide-resins (5–10 mg) were hydrolized in 12 N HCl–propionic acid (1:1, v/v) for 2 h at 160 °C. Amino acid analyses were performed on a Beckman 6300 Analyzer with a sulfated polystyrene cation-exchange column (0.4 cm × 21 cm).

Microwave-Induced Synthesis of *N*-TCP Protected Amino Acids; General Procedure

A mixture of tetrachlorophthalic anhydride (286 mg, 1 mmol) and the appropriate amino acid (conveniently protected if necessary) (1 mmol) was thoroughly grounded and transferred to a 250 mL beaker. DMF (1.7 mL) was added, the beaker was covered with a watch glass, placed in the microwave oven, and subjected to irradiation at 285 W at 20 s intervals for 4 to 8 min.[12] Reaction progress was followed by TLC. Then, the beaker was removed from the oven and allowed to cool to r.t. before the addition of cold H_2O (30 mL). The resultant precipitate was filtered, washed with cold H_2O and dried in vacuo over P_2O_5 to afford the *N*-TCP protected amino acids **1**–**16(L-D)** with 91% average yield (Tables 1 and 2). When purification was necessary the precipitate was taken up in EtOAc (20 mL) and washed with aq sat. NaCl solution (20 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure.

TCP-His(Trt)-OH (**16L**)

The general procedure described above was used with the following reagents and amounts: H-His(Trt)-OH (397 mg, 1 mmol, 1 equiv), tetrachlorophthalic anhydride (0.286 g, 1 mmol, 1 equiv) in DMF (1.7 mL). Purification of the crude product by column chromatography on silica gel ($CHCl_3$–MeOH, 2:1) provided 0.466 mg of **16L** (70%) as a white solid.

TCP-D-His(Trt)-OH (**16D**)

The general procedure described above was used with the following reagents and amounts: H-D-His(Trt)-OH (397 mg, 1 mmol, 1 equiv), tetrachlorophthalic anhydride (0.286 g, 1 mmol, 1 equiv) in DMF (1.7 mL). Purification of the crude product by column chromatography on silica gel ($CHCl_3$–MeOH, 2:1) provided 0.459 mg of **16D** (69%) as an off-white solid.

TCP-His-OH:

TCP-His(Trt)-OH (**16L**) (78 mg) was dissolved in TFA–CH_2Cl_2 (1:1, 10 mL). After 15 min stirring at r.t. the clear solution was concentrated to dryness under reduced pressure followed by addition of Et_2O. The resulting solid was collected by filtration and washed with Et_2O to yield pure TCP-His-OH.

^{1}H NMR (200 MHz, DMSO-d_6): δ = 3.23–3.46 (m, 2 H, β-CH_2), 4.85–4.87 (m, 1 H, α-CH), 6.82 (s, 1 H, CH_{imid}), 7.61 (s, 1 H, CH_{imid}).

Table 2 Characterization of Compounds **1**–**16(L-D)**

Product[a]	R_f[b]	t_R[c]	Mp (°C)	$[\alpha]_D$ (*c*)[d]	^{1}H NMR δ, *J* (Hz)	^{13}C NMR, δ	FAB-MS *m/z* (%)
1	0.55	18.1	288–292 (dec.)	-	4.42 (s, 2 H, α-CH_2)[e]	40.01 (α-CH_2), 127.98, 128.56, 138.85 (C_{arom}), 162.86 (CON), 168.36 (CO_2H)[e]	$[M + H]^+$ 341.98 (17), 344.0 (16), 346.0 (10), $[M - CO_2H]^+$ 295.9 (57), 297.9 (70), 299.9 (42)
2L	0.68	19.4	280–286 (dec.)	–4.10 (1.162)	1.70 (d, 3 H, *J* = 7.4, β-CH_3), 5.00 (q, 1 H, *J* = 7.4, α-CH)[f]	15.08 (β-CH_3), 49.09 (α-CH), 129.11, 129.91, 140.07 (C_{arom}), 163.58 (CON), 171.39 (CO_2H)[f]	$[M + H]^+$ 355.9 (46), 357.9 (55), 359.9 (28), $[M - CO_2H]^+$ 309.8 (89), 311.9 (100), 313.9 (53)

Table 2 Characterization of Compounds **1**–**16(L-D)** (continued)

Product[a]	R_f[b]	t_R[c]	Mp (°C)	$[\alpha]_D$ (*c*)[d]	^{1}H NMR δ, *J* (Hz)	^{13}C NMR, δ	FAB-MS *m*/*z* (%)
2D	0.67	19.7	283–286 (dec)	+4.72 (1.121)	Identical to **2L**	Identical to **2L**	$[M + H]^+$ 355.9 (36), 357.9 (46), 359.9 (23), $[M – CO_2H]^+$ 309.9 (46), 311.9 (57), 313.9 (30)
3L	0.73	21.6	174–176	–24.80 (0.138)	1.02 (t, 3 H, *J* = 7.4, δ-CH_3), 1.14–1.38 (m, 1 H, γ-CH_2), 1.27 (d, 3 H, *J* = 5.0, γ-CH_3), 1.68–1.82 (m, 1 H, γ-CH_2), 2.57–2.71 (m, 1 H, β-CH), 4.83 (d, 1 H, *J* = 8.0, α-CH)[f]	11.70 (δ-CH_3), 17.48 (γ-CH_3), 26.79 (γ-CH_2), 35.72 (β-CH), 58.45 (α-CH), 129.26, 130.49, 140.62 (C_{arom}), 164.29 (CON), 169.94 (CO_2H)[f]	$[M + H]^+$ 397.9 (85), 399.9 (100), 401.9 (47), $[M – CO_2H]^+$ 351.9 (78), 353.9 (95), 355.9 (47)
3D	0.69	22.0	173–175	+24.20 (0.124)	Identical to **3L**	Identical to **3L**	$[M + H]^+$ 398.0 (56), 400.0 (66), 401.9 (32), $[M – CO_2H]^+$ 352.0 (82), 354.0 (100), 356.0 (49)
4L	0.70	21.8	226–228	–6.75 (0.126)	0.97 (d, 3 H, *J* = 6.4, δ-CH_3), 1.01 (d, 3 H, *J* = 6.4, δ-CH_3), 1.51–1.60 (m, 1 H, γ-CH), 2.02 (ddd, 1 H, *J* = 4.5, 10.0, 14.2, β-CH_2), 2.34 (ddd, 1 H, *J* = 4.2, 11.3, 14.2, β-CH_2), 5.01 (dd, 1 H, *J* = 4.5, 11.3, α-CH)[f]	21.23 (δ-CH_3), 23.43 (δ-CH_3), 25.55 (γ-CH), 37.80 (β-CH_2), 51.84 (α-CH), 128.94, 130.05, 140.21 (C_{arom}), 163.83 (CON), 170.52 (CO_2H)[f]	$[M + H]^+$ 397.9 (17), 400.0 (19), 402.0 (9), $[M – O_2H]^+$ 352.0 (26), 354.0 (31), 356.0 (17)
4D	0.66	21.9	229–231	+4.66 (0.118)	Identical to **4L**	Identical to **4L**	$[M + H]^+$ 397.9 (56), 399.9 (67), 401.9 (32), $[M – CO_2H]^+$ 352.0 (60), 354.0 (72), 355.9 (37)
5L	0.70	20.8	210–212	–36.29 (1.017)	0.98 (d, 3 H, *J* = 6.7, γ-CH_3), 1.22 (d, 3 H, *J* = 6.7, γ-CH_3), 2.75 (dq, 1 H, *J* = 6.7, 8.0, β-CH), 4.65 (d, 1 H, *J* = 8.0, α-CH)[f]	19.68 (γ-CH), 21.19 (γ-CH_3), 28.65 (β-CH), 58.68 (α-CH), 128.87, 130.12, 140.24 (C_{arom}), 163.91 (CON), 169.50 (CO_2H)[f]	$[M + H]^+$ 383.9 (58), 386.0 (71), 387.9 (35), $[M – CO_2H]^+$ 338.0 (81), 340.0 (100), 341.9 (50)
5D	0.69	21.0	212–214	+37.25 (1.023)	Identical to **5L**	Identical to **5L**	$[M + H]^+$ 384.0 (44), 385.9 (51), 387.9 (25), $[M – CO_2H]^+$ 338.0 (81), 340.0 (100), 342.0 (48)
6L	0.67	21.6	250–254	–150.40 (0.124)	3.62 (dd, 1 H, *J* = 11.0, 14.3, β-CH_2), 3.78 (dd, 1 H, *J* = 5.1, 14.3, β-CH_2), 5.40 (dd, 1 H, *J* = 5.1, 11.0, α-CH), 7.29–7.40 (m, 5 H, CH_{arom})[f]	34.95 (β-CH_2), 54.71 (α-CH), 127.61, 128.49 (C_{arom}), 129.37, 129.68 (CH_{arom}), 130.12, 138.05, 140.48 (C_{arom}), 163.52 (CON), 169.70 (CO_2H)[f]	$[M + H]^+$ 431.9 (12), 433.9 (15), 436.1 (10), $[M – CO_2H]^+$ 385.9 (9), 387.9 (11), 390.0 (7)
6L	0.67	21.8	253–256	+144.30 (0.096)	Identical to **6L**	Identical to **6L**	$[M + H]^+$ 431.9 (14), 433.9 (18), 435.9 (9), $[M – CO_2H]^+$ 385.9 (14), 387.9 (18), 389.9 (10)
7L	0.65	20.3	171–173	–24.77 (0.107)	2.03 (s, 3 H, SCH_3), 2.27–2.46 (m, 2 H, β-CH_2), 2.48–2.90 (m, 2 H, γ-CH_2), 5.18 (dd, 1 H, *J* = 5.6, 8.8, α-CH)[e]	14.47 (SCH_3), 27.56 (β-CH_2), 29.86 (γ-CH_2), 51.31 (α-CH), 127.93, 128.53, 138.75 (C_{arom}), 162.99 (CON), 169.90 (CO_2H)[e]	$[M + H]^+$ 415.9 (62), 417.9 (79), 419.9 (40)
7D	0.71	20.9	172–174	+22.85 (0.116)	Identical to **7L**	Identical to **7L**	$[M + H]^+$ 415.9 (74), 417.9 (96), 419.9 (49)

Table 2 Characterization of Compounds **1**–**16(L-D)** (continued)

Product[a]	R_f[b]	t_R[c]	Mp (°C)	$[\alpha]_D$ (*c*)[d]	^{1}H NMR δ, *J* (Hz)	^{13}C NMR, δ	FAB-MS *m*/*z* (%)
8	0.73	18.4	230–240	-	2.70 (t, 2 H, *J* = 10.1, α-CH_2), 3.90 (t, 2 H, *J* = 10.1, β-CH_2)[e]	32.03 (β-CH_2), 34.36 (α-CH_2), 128.15,128.40, 138.31 (C_{arom}), 163.23 (CON), 172.03 (CO_2H)[e]	$[M + H]^+$ 355.8 (17), 357.9 (18), 359.9 (9)
9L	0.72	22.9	69–72	–147.38 (0.122)	1.27 (s, 9 H, 3 × CH_3), 3.50 (dd, 1 H, *J* = 6.1, 14.4, β-CH_2), 3.60 (dd, 1 H, *J* = 10.7, 14.4, β-CH_2), 5.19 (dd, 1 H, *J* = 6.1, 10.7, α-CH), 6.85 (d, 2 H, *J* = 8.5, CH_{arom}), 7.06 (d, 2 H, *J* = 8.5, CH_{arom})[g]	28.70 (3 × CH_3), 33.57 (β-CH_2), 53.81 (α-CH), 78.53 (*C*Bu-*t*), 124.48 (CH_{arom}), 127.02 (C_{arom}), 129.19 (CH_{arom}), 129.76, 131.03, 140.28 (C_{arom}), 154.22 ($C_{arom\text{-}OBu\text{-}t}$), 162.60 (CON), 172.04 (CO_2H)[g]	$[M + H]^+$ 504.0 (2), 506.0 (2), 508.1 (1), [M – *t*-Bu + H]$^+$ 447.0 (3), 449.0 (5), 451.0 (3)
9D	0.71	22.9	66–69	+150.97 (0.164)	Identical to **9L**	Identical to **9L**	$[M + H]^+$ 504.0 (1), 506.0 (1), 508.0 (0.6), [M – *t*-Bu + H]$^+$ 447.9 (3), 449.9 (4), 451.9 (2)
10L	0.73	21.8	173–175	–27.24 (0.112)	1.21 (s, 9 H, 3 × CH_3), 4.00 (dd, 1 H, *J* = 9.2, 9.4, β-CH_2), 4.17 (dd, 1 H, *J* = 6.7, 9.4, β-CH_2), 5.09 (dd, 1 H, *J* = 6.7 Hz, 9.2, α-CH)[g]	27.31 (3 × CH_3), 52.64 (α-CH), 58.31 (β-CH_2), 75.09 (*C*Bu-*t*), 127.34, 129.98, 140.43 (C_{arom}), 162.72 (CON), 169.87 (CO_2H)[g]	$[M + H]^+$ 428.0 (10), 430.0 (12), 432.0 (6), [M – *t*-Bu + H]$^+$ 372.0 (79), 373.9 (100), 375.9 (49)
10D	0.70	21.7	176–178	+20.20 (0.098)	Identical to **10L**	Identical to **10L**	$[M + H]^+$ 428.0 (11), 430.0 (13), 432.0 (7), [M – *t* Bu + H]$^+$ 371.9 (79), 373.9 (100), 375.9 (49)
11L	0.74	22.3	92–94	–72.27 (0.627)	1.21 (s, 9 H, 3 × CH_3), 1.45 (d, 3 H, *J* = 6.2, γ-CH_3), 4.22–4.35 (m, 1 H, β-CH), 4.87 (d, 1 H, *J* = 7.0, α-CH)[g]	21.46 (γ-CH_3), 28.26 (3 × CH_3), 56.71 (α-CH), 66.15 (β-CH), 76.18 (*C*Bu-*t*), 127.24, 129.96, 140.43 (C_{arom}), 162.57 (CON), 168.75 (CO_2H)[g]	$[M + H]^+$ 442.0 (6), 444.0 (8), 446.0 (4), [M – *t*-Bu + H]$^+$ 385.9 (77), 387.9 (100), 389.9 (49)
11D	0.70	22.8	88–91	+77.53 (0.688)	Identical to **11L**	Identical to **11L**	$[M + H]^+$ 442.0 (5), 444.0 (6), 446.0 (3), [M – *t*-Bu + H]$^+$ 385.9 (78), 387.9 (100), 389.9 (50)
12L	0.74	21.6	256–258	–15.84 (0.627)	1.43 (s, 9 H, 3 × CH_3), 3.11 (dd, 1 H, *J* = 9.3, 16.7, β-CH_2), 3.29 (dd, 1 H, *J* = 5.6, 16.7, β-CH_2), 5.39 (dd, 1 H, *J* = 5.6, 9.3, α-CH)[g]	27.90 (3 × CH_3), 34.66 (β-CH_2), 48.79 (α-CH), 82.03 (*C*Bu-*t*), 127.30, 130.04, 140.48 (C*arom*), 162.51 (CON), 168.92 (CO_2H), 171.85 (*C*O_2Bu-*t*)[g]	$[M + H]^+$ 455.9 (10), 458.0 (11), 460.0 (6), [M – *t*-Bu + H]$^+$ 399.9 (77), 401.9 (100), 403.9 (49)
12D	0.75	21.3	252–255	+19.71 (0.614)	Identical to **12L**	Identical to **12L**	$[M + H]^+$ 455.9 (11), 457.9 (13), 460.0 (7), [M – *t* Bu + H]$^+$ 399.9 (78), 401.9 (100), 403.9 (49)
13L	0.76	22.2	122–126	–22.90 (0.677)	1.44 (s, 9 H, 3 × CH_3), 2.38–2.30 (m, 2 H, β-CH_2), 2.66–2.45 (m, 2 H, γ-CH_2), 5.00 (dd, 1 H, *J* = 4.8, 9.4, α-CH)[g]	23.72 (β-CH_2), 27.97 (3 × CH_3), 32.00 (γ-CH_2), 51.85 (α-CH), 81.22 (*C*Bu-t), 127.33, 130.00, 140.43 (C_{arom}), 162.77 (CON), 171.40 (CO_2H), 172.83 (*C*O_2Bu-*t*)[g]	$[M + H]^+$ 470.0 (9), 472.0 (10), 474.0 (5), [M – *t*-Bu + H]$^+$ 413.9 (77), 415.9 (100), 417.9 (50)

Table 2 Characterization of Compounds **1**–**16(L-D)** (continued)

Product[a]	R_f[b]	t_R[c]	Mp (°C)	$[\alpha]_D$ (*c*)[d]	^{1}H NMR δ, *J* (Hz)	^{13}C NMR, δ	FAB-MS *m*/*z* (%)
13D	0.74	22.3	118–120	+20.37 (0.707)	Identical to **13L**	Identical to **13L**	$[M + H]^+$ 470.0 (10), 472.0 (12), 474.0 (6), $[M - t\text{-Bu} + H]^+$ 413.9 (78), 415.9 (100), 417.9 (50)
14L	0.79	21.2	125–128	–15.80 (1.045)	1.38 (s, 9 H, 3 × CH_3), 1.38–1.56 (m, 4 H, γ,δ-CH_2), 2.64 (dt, 2 H, *J* = 7.5, 8.0, β-CH_2), 3.08 (t, 2 H, *J* = 6.8, ∈-CH_2), 4.95 (t, 1 H, *J* = 7.5, α-CH)[g]	24.20 (γ-CH_2), 28.55 (3 × CH_3), 28.77 (β-CH_2), 30.07 (δ-CH_2), 40.46 (∈-CH_2), 53.53 (α-CH), 78.27 (*C*But), 128.99, 130.06, 140.19 (C_{arom}), 156.60 (CO-Boc), 163.80 (CON), 170.25 (CO_2H)[g]	$[M + H]^+$ 513.0 (2), 515.0 (2), 517.0 (1), $[M - C_5H_8O_2 + H]^+$ 413.0 (80), 415.0 (100), 417.0 (49)
14D	0.72	21.3	121–124	+13.49 (1.052)	Identical to **14L**	Identical to **14L**	$[M + H]^+$ 513.0 (1), 515.0 (1), 517.0 (0.7), $[M - C_5H_8O_2 + H]^+$ 413.0 (79), 415.0 (100), 417.0 (49)
15L	0.72	26.1[h]	82–84 (dec.)[e]	–29.75 (0.079)	3.04 (dd, 1 H, *J* = 4.4, 13.8, β-CH_2), 3.43 (dd, 1 H, *J* = 11.5, 13.8, β-CH_2), 4.35 (dd, 1 H, *J* = 4.4, 11.5, α-CH), 7.20–7.44 (m, 15 H, CH_{arom})[g]	30.22 (β-CH_2), 52.27 (α-CH), 67.65 (*C*Trt), 126.90 (CH_{arom}), 127.28 (C_{arom}), 128.23, 129.41 (CH_{arom}), 129.84, 140.29, 144.09 (C_{arom}), 162.45 (CON), 170.91 (CO_2H)[g]	$[M + H]^+$ 632.0 (0.04), $[Trt]^+$ 243.2 (100), $[Trt + H]^+$ 244.2 (23)
15D	0.70	25.0[h]	83–85 (dec.)	+11.84 (0.076)	Identical to **15L**	Identical to **15L**	$[M + H]^+$ 632.0 (0.07), $[Trt]^+$ 243.2 (100), $[Trt + H]^+$ 244.2 (72)
16L	0.72	24.6[h]	142–158 (dec.)	–70.20 (0.095)	3.46 (dd, 1 H, *J* = 9.60, 15.3, β-CH_2), 3.82 (dd, 1 H, *J* = 6.0, 15.3, β-CH_2), 5.16 (dd, 1 H, J = 6.0, 9.6, α-CH), 6.59 (s, 1 H, CH_{imid}), 7.00–7.39 (m, 15 H, CH_{arom}), 7.68 (s, 1 H, CH_{imid})[g]	26.43 (β-CH_2), 53.70 (α-CH), 77.20 (*C*Trt), 119.42 (CH_{imid}), 128.18 (C_{arom}), 128.18, 129.48 (CH_{arom}), 135.10 (C_{arom}), 137.28 (CH_{imid}), 139.79, 141.18, 146.85 (C_{arom}), 162.85 (CON), 170.21 (CO_2H)[g]	$[M + H]^+$ 664.0 (0.99), 666.0 (1.24), 668.0 (0.65), $[M - C_{19}H_{14} + H]^+$ 421.9 (4), 423.9 (5), 425.9 (2), $[M - Trt\text{-}CO_2H + H]^+$ 376.0 (2), 378.0 (3), 380.0 (1), $[Trt]^+$ 243.2 (100), $[Trt + H]^+$ 244.2 (78)
16D	0.66	24.2[h]	138–151 (dec.)	+59.01 (0.142)	Identical to **16L**	Identical to **16L**	$[M + H]^+$ 664.0 (0.45), 666.0 (0.55), 668.0 (0.28), $[M - C_{19}H_{14} + H]^+$ 421.9 (3), 423.9 (4), 425.9 (2), $[M - Trt - CO_2H + H]^+$ 376.0 (2), 378.0 (3), 380.0 (2), $[Trt]^+$ 243.2 (100), $[Trt + H]^+$ 244.2 (77)

[a] Satisfactory microanalyses obtained: C ±0.40, H ±0.31, N ±0.40, S ±0.26 (Exceptions: **9L**, C –1.24, N +0.92; **9D**, C –1.33, N +1.51; **11L**, H +0.48; **12D**, C –0.52, N +1.36; **13D**, N +0.65; **14L**, N +0.54; **14D**, C –1.06, N +1.06; **15L**, C –2.56, N +0.68, S –0.7; **15D**, H +0.49, N +1.27; **16L**, C +0.45, H –0.52; **16D**, C –0.75, N +0.43). Compounds **10L**, **11L**, and **13D** contained occluded water to an extent of 0.5 H_2O (**10L**, **11L**), 1 H_2O (**13D**, **16D**) and 2 H_2O (**16L**).
[b] EtOAc–MeOH–AcOH (2:3:0.3).
[c] Analytical HPLC was developed at 1.0 mL/min with 0.1% aq TFA–MeCN (2 min at 1:0, 1:0 to 0:1 over 23 min, then 5 min at 0:1); detection at 220 nm.
[d] EtOH.
[e] DMSO-d_6.
[f] Acetone-d_6.
[g] $CDCl_3$.
[h] Analytical HPLC was carried out at 1.0 mL/min with H_2O–MeCN (2 min at 2.3:1, 2.3:1 to 1:9 over 23 min, 1:9 to 0:1 over 10 min, then 10 min at 0:1); detection at 220 nm.

N-TCP Protected Amino Acids by Reflux; General Procedure

Tetrachlorophthalic anhydride (1 equiv) was added to a solution of the amino acid *tert*-butyl ester hydrochloride (1 equiv) and Et_3N (2 equiv) in CH_2Cl_2 (100 mL). The resultant solution was heated to reflux and stirred for 22 h. After cooling to r.t., the mixture was concentrated under reduced pressure. To the off white residue a solution of Ac_2O–pyridine (1:1, 40 mL) and CH_2Cl_2 (40 mL) was added, and the mixture was refluxed for 2.5 h. Then, it was allowed to cool to r.t. and concentrated in vacuo. 0.1 M HCl (50 mL) was added to the residue and the suspension was left 15 min at 4 °C. The resultant precipitate was collected by filtration and washed with cold 0.05 M HCl and water. The solid was dissolved in EtOH and the solution concentrated to dryness; this process was repeated twice. The *N*-TCP amino acid *tert*-butyl esters **17**–**22L** (Table 3) obtained were dried in vacuo over P_2O_5.

Compounds **17**–**22L** were dissolved in TFA–CH_2Cl_2 (2:3, 30 mL). After stirring for 2 h at r.t. the clear solution was concentrated to dryness under reduced pressure. The resultant solid was dissolved in CH_2Cl_2, following by evaporation of the solvent; this process was repeated twice, yielding the corresponding *N*-TCP protected amino acids **1**–**6L** (Tables 1, 2) which were dried in vacuo over P_2O_5.

Studies of the Stability of the TCP Function Towards Standard SPPS Treatments

Fmoc-PAL-PEG-PS resin (150 mg, 0.024 mmol, 0.16 mmol/g) was washed with DMF (3 × 2 min) and CH_2Cl_2 (3 × 2 min), treated with piperidine–DMF (3:7, 2 + 10 min), and finally washed with DMF (3 × 1 min) and CH_2Cl_2 (3 × 1 min). Fmoc-Val-OH (24 mg, 0.072 mmol, 3 equiv) was coupled to the resin with DIPCI (11 μl, 0.072 mmol, 3 equiv) and HOAt (10 mg, 0.072 mmol, 3 equiv) in DMF (0.8 mL) for 3 h, followed by washing with DMF (5 × 2 min) and CH_2Cl_2 (5 × 2 min). After Fmoc removal and the washings described above, TCP-Gly-OH (25 mg, 0.072 mmol, 3 equiv) was incorporated onto the resin with DIPCI (11 μl, 0.072 mmol, 3 equiv) and HOAt (10 mg, 0.072 mmol, 3 equiv) in DMF at 25 °C for 5.5 h (Kaiser ninhydrin test[13] negative after this time). The resin was washed with DMF (3 × 1 min) and CH_2Cl_2 (3 × 1 min) and dried in vacuo. An aliquot of this TCP-Gly-Val-PAL-PEG-PS resin was hydrolyzed for amino acid analysis: Val 1.0, Gly 0.89.

TCP-Gly-Val-NH_2 was characterized prior to the stability studies. A portion of the TCP-Gly-Val-PAL-PEG-PS resin (10 mg) was subjected to cleavage with TFA–H_2O (19:1) for 2 h, the filtrates were drawn off from the vessel with positive nitrogen pressure, and the cleaved resin was washed (2 × 0.5 mL) with further TFA–H_2O (19:1). The combined filtrates were evaporated to dryness, the residue dissolved in MeCN and analyzed by analytical HPLC (gradient in Table 1) and mass spectrometry: HPLC: t_R 18.9 min.

FAB-MS: $m/z = [M + H]^+$ 441.8, $[M + H - CONH_2]^+$ 396.9, $[M.]^-$ 440.8.

Portions of this resin (10 mg per experiment) were subjected to various TCP stability experiments, as follows: TCP-Gly-Val-PAL-

Table 3 Characterization of Compounds **17**–**22L**

Product	R_f[a]	^{1}H NMR δ, *J* (Hz)	^{13}C NMR δ
TCP-Gly-OBu-*t* (**17**)	0.79	1.51 (s, 9 H, 3 × CH_3), 4.37 (s, 2 H, α-CH_2)[b]	27.98 (3 × CH_3), 40.20 (α-CH_2), 83.35 (*C*Bu-t), 127.65, 129.92, 140.32 (C_{arom}), 162.86 (CON), 165.49 (*C*O_2Bu-*t*)[b]
TCP-Ala-OBu-*t* (**18L**)	0.85	1.49 (s, 9H, 3 × CH_3), 1.70 (d, 3 H, *J* = 7.4, β-CH_3), 4.91 (q, 1 H, *J* = 7.4, α-CH)[c]	14.99 (β-CH_3), 27.83 (3 × CH_3), 49.10 (α-CH), 82.85 (CBu-*t*), 127.46, 129.76, 140.17 (C_{arom}), 162.74 (CON), 167.79 (*C*O_2Bu-*t*)[d]
TCP-Ile-OBu-*t* (**19L**)	0.72	0.91 (t, 3 H, *J* = 7.2, δ-CH_3), 1.00–1.18 (m, 1 H, γ-CH_2), 1.14 (d, 3 H, *J* = 6.8, γ-CH_3), 1.47 (s, 9 H, 3 × CH_3), 1.47–1.61 (m, 1 H, γ-CH_2), 2.45–2.62 (m, 1 H, β-CH), 4.63 (d, 1 H, *J* = 7.8, α-CH)[b]	11.12 (δ-CH_3), 16.93 (γ-CH_3), 26.26 (γ-CH_2), 27.93 (3 × CH_3), 37.78 (β-CH), 58.84 (α-CH), 82.75 (*C*Bu-*t*), 127.32, 129.83, 140.28 (C_{arom}), 163.20 (CON), 167.12 (*C*O_2Bu-*t*)[b]
TCP-Leu-OBu-*t* (**20L**)	0.80	0.97 (d, 3 H, *J* = 6.6, δ-CH_3), 0.98 (d, 3 H, *J* = 6.4, δ-CH_3), 1.47 (s, 9 H, 3 × CH_3), 1.43–1.54 (m, 1 H, γ-CH), 1.95 (ddd, 1 H, *J* = 4.6, 10.0, 14.3, β-CH_2), 2.31 (ddd, 1 H, *J* = 4.4, 11.4, 14.3, β-CH_2), 4.88 (dd, 1 H, *J* = 4.6, 11.4, α-CH)[b]	21.01(δ-CH_3), 23.11 (δ-CH_3), 25.27 (γ-CH), 27.89 (3 × CH_3), 37.11 (β-CH_2), 52.41 (α-CH), 82.91 (CBu-*t*), 127.42, 129.81, 140.25 (C_{arom}), 163.14 (CON), 167.99 (*C*O_2Bu-*t*)[b]
TCP-Val-OBu-*t* (**21L**)	0.82	0.95 (d, 3 H, *J* = 6.8, γ-CH_3), 1.17 (d, 3 H, *J* = 6.8, γ-CH_3), 1.47 (s, 9 H, 3 × CH_3), 2.76 (dq, 1 H, *J* = 6.8, 8.0, β-CH), 4.54 (d, 1 H, *J* = 8.0, α-CH)[b]	19.70 (γ-CH_3), 21.01 (γ-CH_3), 27.91 (3 × CH_3), 28.56 (β-CH), 59.43 (α-CH), 82.76 (*C*Bu-*t*), 127.30, 129.84, 140.29 (C_{arom}), 163.17 (CON), 166.98 (*C*O_2Bu-*t*)[b]
TCP-Phe-OBu-*t* (**22L**)	0.84	1.52 (s, 3 H, 3 × CH_3), 3.51 (dd, 1 H, *J* = 10.6, 14.3, β-CH_2), 3.75 (dd, 1 H, *J* = 6.6, 14.3, β-CH_2), 5.14 (dd, 1 H, *J* = 6.6, 10.6, α-CH), 7.17–7.30 (m, 5 H_{arom})[b]	27.91 (3 × CH_3), 34.42 (β-CH_2), 54.83 (α-CH), 83.33 (*C*Bu-*t*), 126.91, 127.16, 128.62, 128.65, 129.70, 136.58, 140.19 (C_{arom}), 162.74 (CON), 166.98 (*C*O_2Bu-*t*)[b]

[a] EtOAc–MeOH–AcOH (2:3:0.3).
[b] $CDCl_3$.
[c] DMSO-d_6.
[d] Acetone-d_6.

PEG-PS resin was swollen in DMF (2 × 5 min), treated with DMF (24 h), piperidine–DMF (1:4, 30 min) or (*i*-Pr)$_2$NEt–DMF (1:19, 30 min), and washed with DMF (5 × 2 min). Then, Boc-Phe-OH (2 mg, 0.008 mmol, 5 equiv) was coupled to each of the resins with DIPCI (1.2 μl, 0.008 mmol, 5 equiv) and HOAt (1 mg, 0.008 mmol, 5 equiv) in DMF (0.2 mL) for 4.5 h. The resins were washed with DMF (3 × 1 min) and CH_2Cl_2 (3 × 1 min) and dried in vacuo. Final release of the peptide from the resins was carried out with TFA–H_2O (19:1) for 2 h as described above. The resulting residue was dissolved in MeCN and analyzed by analytical HPLC (gradient in Table 2). TCP-Gly-Val-NH_2 (t_R 18.9 min) was detected exclusively for all the conditions tested.

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