J. Chem. Sci., Vol. 120, No. 1, January 2008, pp. 163–173.

Propargyloxycarbonyl as a protecting group for the side chains of serine, threonine and tyrosine

RAMAPANICKER RAMESH, KAVITA DE, SHIPRA GUPTA and SRINIVASAN CHANDRASEKARAN[†,*]

Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012
[†]Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore 560 064
e-mail: scn@orgchem.iisc.ernet.in

Abstract. Propargyloxycarbonyl group is used as a protecting group for the hydroxyl groups of serine, threonine and tyrosine. The propargyloxycarbonyl derivatives of these hydroxy amino acids are stable to acidic and basic reagents commonly employed in peptide synthesis. The deprotection of the *O*-Poc derivatives using tetrathiomolybdate does not affect commonly used protecting groups such as *N*-Boc, *N*-Cbz, *N*-Fmoc, methyl and benzyl esters. The di- and tripeptides synthesized using *O*-Poc derivatives of serine, threonine and tyrosine are stable, isolable compounds and give the hydroxy peptides in good yields when treated with tetrathiomolybdate.

Keywords. Protecting groups; amino acids; peptide synthesis; hydroxy residues.

1. Introduction

The protection of the hydroxyl groups of serine, threonine and tyrosine is essential for the effective preparation of peptides bearing these residues.[1,2] The most commonly used protecting groups for the side chains of these amino acids include *tert*-butyl and trityl ethers and rarely benzyloxycarbonyl (Cbz) and related carbonates.[1,2] Although preparation of carbamates seems to be the method of choice for the protection of amines, protection of hydroxyl groups as the corresponding carbonates is not a commonly used strategy.[3] Among the various carbonates used for the protection of alcohols, benzyl carbonates (Cbz derivatives) are the most studied.[3,4] From our laboratory, we have reported the utility of propargyloxycarbonyl (Poc) as an efficient protecting group for the protection of hydroxyl groups in carbohydrates.[5] Poc group has been shown to be stable during glycosylation conditions. The efficient and fast deprotection of *O*-Poc group can be achieved under mild and neutral conditions using benzyltriethylammonium tetrathiomolybdate [$(PhCH_2NEt_3)_2MoS_4$, **1**].[5,6] The orthogonal stability of Poc group with a number of other *O*-protecting groups has also been established.[5] Here, we report the results of our efforts to protect the side chain hydroxyl groups of serine, threonine and tyrosine as Poc derivatives (figure 1) and the applications of these derivatives in solution phase peptide synthesis.

2. Experimental

2.1 *General experimental procedures*

All reactions were performed in oven dry apparatus and were stirred magnetically. Melting points and optical rotation (at 25°C) were recorded on digital instruments. Infrared spectra were recorded using an FT–IR instrument and the frequencies are reported in wave number (cm^{-1}) and intensities of the peaks are denoted as s (strong), w (weak), m (medium). ^1H and ^{13}C NMR spectra were recorded on a 300 MHz and 75 MHz spectrometer respectively. Chemical shifts are reported in parts per million downfield from the internal reference, tetramethylsilane. Multiplicity is indicated using the following abbreviations: *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), *qu* (quintet), *m* (multiplet), *bs* (broad singlet) and *bd* (broad doublet). Coupling constants are reported wherever it is necessary in Hertz (Hz). Mass spectra were recorded on a Q-TOF electrospray instrument.

2.2 *Preparation of benzyltriethylammonium tetrathiomolybdate*[9]

Ammonium molybdate (10 g) was dissolved in a mixture of ammonium hydroxide (60 mL) and water

*For correspondence

(20 mL), and the solution was filtered. Hydrogen sulfide was bubbled rapidly at room temperature (28°C) into the solution until it was saturated and the temperature was raised to 60°C while maintaining a slow stream of hydrogen sulfide. After 60 min, the mixture was cooled to 0°C and kept under refrigeration for 30 min. The granular product thus obtained was isolated by filtration. The crystalline solid was washed with isopropyl alcohol (25 mL × 2), ether (25 mL × 4) and dried under vacuum to get brick red crystals of ammonium tetrathiomolybdate (13·4–14·2 g, 92%).

A solution of benzyltriethylammonium chloride (23·31 g, 102·5 mmol) in distilled water (60 mL) was added to a well-stirred solution of ammonium tetrathiomolybdate (13 g, 50 mL) in distilled water (60 mL). Rapid stirring was continued for 2 h at room temperature and the solid that separated was filtered, washed with isopropyl alcohol (40 mL × 2) ether (40 mL × 4). The brick red powder of benzyltriethylammonium tetrathiomolybdate (**1**) was dried under vacuum and stored in a desiccator (24 g, 80%). Melting point: decomposes at 150°C.

2.3 *Preparation of propargyloxycarbonyl chloride*

To a stirred solution of triphosgene (2·23 g, 7·5 mmol) in anhydrous diethyl ether (30 mL), activated charcoal (0·05 g) was added and stirred for 1 h at room temperature (28°C). The solution was cooled to 0°C and propargyl alcohol (0·9 mL, 15 mmol) in anhydrous diethyl ether (10 mL) was added drop-wise. The resultant solution was stirred for 12 h and filtered. The diethyl ether layer was concentrated under reduced pressure (100 mbar) and the remaining pale green liquid was used for reactions without any further purification. Boiling point: 118–122°C; Density (28°C): 1·215 g/cm^3; FTIR (neat): 3303, 2982, 2131, 1777; δ_H (300 MHz, $CDCl_3$): 2·6 (*t*, *J* = 2·4 Hz, 1H), 4·7 (*d*, *J* = 2·4 Hz, 2H); δ_C (75 MHz, $CDCl_3$): 150·2, 77·6, 74·9, 58·3.

2.4 *General procedure for the synthesis of O-Poc derivatives of serine, threonine and tyrosine*

Serine, threonine or tyrosine, with the amino and carboxyl groups suitably protected, (**3a–i** or **8**, 5 mmol) is dissolved in anhydrous CH_2Cl_2 (25 mL). The solution is cooled to -78°C and 12·5 mmol (1 mL) of pyridine was added drop-wise. The mixture was allowed to stir for 5 min and PocCl (0·55 mL, 5·5 mmol) was added over a period of 30 min. The reaction mixture was allowed to attain room temperature and stirred for 2 h. The crude reaction mixture was diluted with CH_2Cl_2 (100 mL), washed twice with saturated citric acid solution (40 mL), once with water (40 mL) and then with brine (50 mL). The crude solution was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The *O*-Poc derivatives (**4a-i** or **9**) were isolated as pure compounds after column chromatography (silica gel, 100–200 mesh) using a solution of ethyl acetate (10–20%) in petroleum ether as eluent.

2.4a *Boc–Ser(Poc)–OMe,* ***4a****:* Colourless oil; Yield: 90%; $[\alpha]_D$: +34 (*c* = 1, MeOH); FTIR (Neat): 3289 (*br*), 2130 (*w*), 1754 (*s*), 1713 (*s*); δ_H (300 MHz, $CDCl_3$): 5·39 (*bd*, *J* = 7·8 Hz, 1H), 4·73 (*d*, *J* = 1·8 Hz, 2H), 4·52–4·60 (*m*, 2H), 4·44 (*dd*, J_1 = 11 Hz, J_2 = 3·6 Hz, 1H), 3·79 (*s*, 3H), 2·56 (*t*, *J* = 2·7 Hz, 1H), 1·45 (*s*, 9H); δ_C (75 MHz, $CDCl_3$): 169·7, 155·0, 154·0, 80·3, 75·9, 67·9, 55·6, 52·8, 28·2; *m/z* (HRMS): Calcd. for $C_{13}H_{19}NO_7$ + Na: 324·1060; Found: 324·1058.

2.4b *Boc–Ser(Poc)–OBn,* ***4b****:* Colourless oil; Yield: 83%; $[\alpha]_D$: +10 (*c* = 1, MeOH); FTIR (Neat): 3381 (*br*), 3291 (*m*), 2129 (*w*), 1762 (*s*), 1713 (*s*); δ_H (300 MHz, $CDCl_3$): 7·36 (*bs*, 5H), 5·39 (*bd*, *J* = 8 Hz, 1H), 5·23 (*d*, *J* = 12 Hz, 1H), 5·18 (*d*, *J* = 12 Hz, 1H), 4·68 (*d*, *J* = 2·7 Hz, 2H), 4·55–4·63 (*m*, 2H), 4·43 (*dd*, J_1 = 10·5 Hz, J_2 = 3·3 Hz, 1H), 2·54 (*t*, *J* = 2·7 Hz, 1H), 1·44 (*s*, 9H); δ_C (75 MHz, $CDCl_3$): 169·1, 155·0, 153·9, 134·9, 128·6, 128·5, 128·2, 80·4, 77·2, 75·9, 67·9, 67·6, 55·5, 52·9, 28·2; *m/z* (HRMS): Calcd. for $C_{19}H_{23}NO_7$ + Na: 400·1373; Found: 400·1375.

2.4c *Cbz–Ser(Poc)–OMe,* ***4c****:* Colourless oil; Yield: 88%; $[\alpha]_D$: +13 (*c* = 1, MeOH); FTIR (Neat): 3285 (*br*), 2129 (*w*), 1749 (*s*), 1705 (*s*); δ_H (300 MHz, $CDCl_3$): 7·35 (*bs*, 5H), 5·70 (*bd*, *J* = 8·1 Hz, 1H), 5·12 (*s*, 2H), 4·70 (*d*, *J* = 2·4 Hz, 2H), 4·62–4·67 (*m*, 1H), 4·55 (*dd*, J_1 = 11 Hz, J_2 = 3·3 Hz, 1H), 4·47 (*dd*, J_1 = 11 Hz, J_2 = 3·3 Hz, 1H), 3·77 (*s*, 3H), 2·53 (*t*, *J* = 2·4 Hz, 1H); δ_C (75 MHz, $CDCl_3$): 169·3, 155·7, 153·9, 135·9, 128·5, 128·2, 128·0, 76·5, 75·9, 67·6, 67·2, 55·6, 53·2, 52·9; *m/z* (HRMS): Calcd. for $C_{16}H_{17}NO_7$ + Na: 358·0903; Found: 358·0899.

2.4d *Fmoc–Ser(Poc)–OMe,* ***4d****:* Colourless oil; Yield: 85%; $[\alpha]_D$: +17 (*c* = 1, MeOH); FTIR (Neat): 3359

(*br*), 3292 (*br*), 2130 (*w*), 1755 (*s*), 1724 (*s*); δ_H (300 MHz, $CDCl_3$): 7·76 (*d*, *J* = 7·8 Hz, 2H); 7·60 (*d*, *J* = 7·2 Hz, 2H), 7·40 (*t*, *J* = 7·5 Hz, 2H), 7·31 (*t*, *J* = 7·5 Hz, 2H), 5·71 (*bd*, *J* = 8·4 Hz, 1H), 4·72 (*d*, *J* = 2·7 Hz, 2H), 4·64–4·67 (*m*, 1H), 4·58 (*dd*, J_1 = 11 Hz, J_2 = 3·3 Hz, 1H), 4·48 (*dd*, J_1 = 11 Hz, J_2 = 3·3 Hz, 1H), 4·41 (*d*, *J* = 6·9 Hz, 2H), 4·22 (*t*, *J* = 6·9 Hz, 1H), 3·79 (*s*, 3H), 2·53 (*t*, *J* = 2·4 Hz, 1H)); δ_C (75 MHz, $CDCl_3$): 169·4, 155·7, 153·9, 143·7, 143·6, 141·2, 127·7, 127·0, 125·0, 119·9, 76·0, 67·6, 67·3, 55·6, 53·2, 52·9; *m/z* (HRMS): Calcd. for $C_{23}H_{21}NO_7$ + Na: 446·1216; Found: 446·1211.

2.4e *Boc–Thr(Poc)–OMe,* ***4e****:* Colourless oil; Yield: 79%; $[\alpha]_D$: +22 (*c* = 1, MeOH); FTIR (Neat): 3292 (*br*), 2129 (*w*), 1753 (*s*), 1715 (*s*); δ_H (300 MHz, $CDCl_3$): 5·23–5·33 (*m*, 2H), 4·73 (*dd*, J_1 = 15 Hz, J_2 = 2·4 Hz, 1H), 4·67 (*dd*, J_1 = 15 Hz, J_2 = 2·4 Hz, 1H), 4·46 (*dd*, J_1 = 10 Hz, J_2 = 2·1 Hz, 1H), 3·75 (*s*, 3H), 2·53 (*t*, *J* = 2·4 Hz, 1H), 1·46 (*s*, 9H), 1·38 (*d*, *J* = 6·3 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 170·2, 155·8, 153·4, 80·3, 76·7, 75·9, 75·3, 56·9, 55·4, 52·7, 28·2, 16·8; *m/z* (HRMS): Calcd. for $C_{14}H_{21}NO_7$ + Na: 338·1216; Found: 338·1220.

2.4f *Boc–Thr(Poc)–OBn,* ***4f****:* Colourless oil; Yield: 73%; $[\alpha]_D$: +13 (*c* = 1, MeOH); FTIR (Neat): 3292 (*br*), 2129 (*w*), 1753 (*s*), 1715 (*s*); δ_H (300 MHz, $CDCl_3$): 7·32–7·40 (*m*, 5H), 5·30–5·36 (*m*, 1H), 5·26 (*bd*, *J* = 9·9 Hz, 1H), 5·20 (*d*, *J* = 12 Hz, 1H), 5·13 (*d*, *J* = 12 Hz, 1H), 4·67 (*dd*, J_1 = 15 Hz, J_2 = 2·7 Hz, 1H), 4·58 (*dd*, J_1 = 15 Hz, J_2 = 2·7 Hz, 1H), 4·50 (*dd*, J_1 = 10 Hz, J_2 = 2·4 Hz, 1H), 2·52 (*t*, *J* = 2·7 Hz, 1H), 1·45 (*s*, 9H), 1·36 (*d*, *J* = 6·3 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 169·6, 155·8, 153·3, 135·1, 128·6, 128·4, 80·3, 76·7, 75·9, 75·3, 67·6, 57·0, 55·4, 28·2, 16·8; *m/z* (HRMS): Calcd. for $C_{20}H_{25}NO_7$ + Na: 414·1529; Found: 414·1531.

2.4g *Cbz–Thr(Poc)–OMe,* ***4g****:* Colourless oil; Yield: 76%; $[\alpha]_D$: +34 (*c* = 1, MeOH); FTIR (Neat): 3403 (*br*), 3284 (*m*), 2129 (*w*), 1752 (*s*), 1718 (*s*); δ_H (300 MHz, $CDCl_3$): 7·27–7·36 (*m*, 5H), 5·60 (*bd*, *J* = 9·6 Hz, 1H), 5·31 (*dq*, J_1 = 6 Hz, J_2 = 2·4 Hz, 1H), 5·16 (*d*, *J* = 12 Hz, 1H), 5·11 (*d*, *J* = 12 Hz, 1H), 4·70 (*dd*, J_1 = 12 Hz, J_2 = 2·4 Hz, 1H), 4·64 (*dd*, J_1 = 12 Hz, J_2 = 2·4 Hz, 1H), 4·53 (*dd*, J_1 = 10 Hz, J_2 = 2·4 Hz, 1H), 3·74 (*s*, 3H), 2·53 (*t*, *J* = 2·4 Hz, 1H), 1·36 (*d*, *J* = 6 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 169·8, 156·4, 153·3, 135·9, 128·4, 128·1, 127·9, 75·9, 74·9, 67·2, 57·3, 55·4, 52·7, 16·7; *m/z* (HRMS): Calcd· for $C_{19}H_{18}NO_7$ + Na: 372·3250; Found: 372·3242.

2.4h *Boc–Tyr(Poc)–OMe,* ***4h****:* Colourless oil; Yield: 93%; $[\alpha]_D$: +26 (*c* = 1, MeOH); FTIR (Neat): 3397 (*br*); 3288 (*m*), 2129 (*w*), 1767 (*s*), 1749 (*s*), 1713 (*s*); δ_H (300 MHz, $CDCl_3$): 7·11–7·17 (*m*, 4H), 5·08 (*bd*, *J* = 8·1 Hz, 1H), 4·83 (*d*, *J* = 2·1 Hz, 2H), 4·54–4·61 (*m*, 1H), 3·70 (*s*, 3H), 3·13 (*dd*, J_1 = 14 Hz, J_2 = 6·6 Hz, 1H), 3·03 (*dd*, J_1 = 14 Hz, J_2 = 6·6 Hz, 1H), 2·61 (*t*, *J* = 2·4 Hz, 1H), 1·42 (*s*, 9H); δ_C (75 MHz, $CDCl_3$): 172·4, 155·4, 153·3, 150·3, 134·5, 130·7, 121·2, 80·3, 76·8, 76·6, 56·2, 54·6, 52·6, 37·9, 28·6; *m/z* (HRMS): Calcd· for $C_{19}H_{23}NO_7$ + Na: 400·1373; Found: 400·1369.

2.4i *Cbz–Tyr(Poc)–OMe,* ***4i****:* White solid; Yield: 89%; Melting point: 82°C; $[\alpha]_D$: +34 (*c* = 1, MeOH); FTIR (KBr): 3354 (*br*), 3291 (*m*), 2130 (*w*), 1765 (*s*), 1746 (*s*), 1725 (*s*); δ_H (300 MHz, $CDCl_3$): 7·26–7·36 (*m*, 5H), 7·07–7·10 (*m*, 4H), 5·33 (*bd*, *J* = 8·4 Hz, 1H), 5·09 (*s*, 2H), 4·82 (*d*, *J* = 2·1 Hz, 2H), 4·61–4·68 (*m*, 1H), 3·70 (*s*, 3H), 3·14 (*dd*, J_1 = 12 Hz, J_2 = 6 Hz, 1H), 3·05 (*dd*, J_1 = 12 Hz, J_2 = 6 Hz, 1H), 2·59 (*t*, *J* = 2·4 Hz, 1H); δ_C (75 MHz, $CDCl_3$): 172·1, 155·9, 153·3, 150·4, 136·5, 134·2, 130·7, 128·9, 128·5, 128·4, 121·3, 76·9, 76·6, 67·3, 56·2, 55·1, 52·7, 37·8; *m/z* (HRMS): Calcd· for $C_{22}H_{21}NO_7$ + Na: 434·1216; Observed: 434·1221.

2.4j *Boc–Thr(Poc)–OPfp,* ***9****:* Colourless oil; Yield: 70%; $[\alpha]_D$: –5 (*c* = 1, MeOH); FTIR (Neat): 3385 (*br*), 3311 (*m*), 2133 (*w*), 1797 (*s*), 1758 (*s*), 1721 (*s*); δ_H (300 MHz, $CDCl_3$): 5·48–5·52 (*m*, 1H), 5·41 (*bd*, *J* = 6·6 Hz, 1H), 4·89 (*dd*, J_1 = 6·6 Hz, J_2 = 2·7 Hz, 1H), 4·73–4·75 (*m*, 2H), 2·56 (*t*, *J* = 2·4 Hz, 1H), 1·47 (*bs*, 12H); δ_C (75 MHz, $CDCl_3$): 166·2, 155·6, 153·2, 80·9, 76·4, 75·9, 74·9, 57·0, 55·6, 28·1, 16·5; m/z (HRMS): Calcd· for $C_{19}H_{18}F_5NO_7$ + Na: 490·0901; Found: 490·0899.

2.5 *Procedure for the synthesis of Boc–Thr–OPfp (****8****)*

Boc–Thr–OH (5 mmol, 1·1 g) was dissolved in acetonitrile (15 mL) and pentafluorophenol (7·5 mmol, 1·40 g) was added to the solution with stirring. The solution was then cooled to 0°C and a solution of DCC (7·5 mmol, 1·6 g) in acetonitrile was added. Reaction mixture was allowed to come to room temperature and stirring was continued for 4 h. The solvent was removed under vacuum and the

residue was extracted with cold ethyl acetate (50 mL) and filtered over a celite pad. The ethyl acetate solution was washed with saturated Na_2CO_3 solution (40 mL) and then with brine (30 mL). The crude solution of the pentafluorophenyl was dried over anhydrous Na_2SO_4 and concentrated. The active ester **8** was then purified by column chromatography (silica gel, 100–200 mesh) eluting with a solution of ethyl acetate (10–25%) in petroleum ether. White solid; Melting point: 98°C; Yield: 82%; $[\alpha]_D$: +9 (*c* = 1, MeOH); FTIR (KBr): 3352 (*br*), 1831 (*s*), 1694 (*s*); δ_H (300 MHz, $CDCl_3$): 5·46 (*bs*,1H), 4·62 (*bd*, *J* = 9·3 Hz, 1H), 4·53 (*bs*, 1H), 1·48 (*s*, 9H), 1·37 (*d*, *J* = 8·7 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 167·8, 155·9, 80·7, 67·9, 58·8, 28·2, 20·1; *m/z* (HRMS): Calcd. for $C_{15}H_{16}F_5NO_5$ + Na: 408·0847; Found: 408·0850.

2.6 *General procedure for the synthesis of peptides* ***7a–g***

The *O*-Poc derivative of serine, threonine or tyrosine (2 mmol of **4a**, **4e** or **4h**) was dissolved in dichloromethane (5 mL) and TFA (5 mL) was added. The reaction mixture was stirred at r.t. (28°C) for 30 min. Dichloromethane and TFA were removed under vacuum and the crude trifluoroacetate salt was used directly for peptide coupling without purification.

The trifluoroacetate salt (2 mmol, obtained as above), an *N*-protected amino acid (2 mmol) and HOBt (0·270 g, 2 mmol) were dissolved in acetonitrile (15 mL). The solution was cooled to 0°C and *N*-methyl morpholine (0·24 mL, 2·2 mmol) was added drop-wise. A solution of DCC (0·62 g, 3 mmol) in acetonitrile (5 mL) was added to the reaction mixture. Reaction mixture was allowed to come to r.t. (28°C) and stirring was continued for 4 h. The solvent was removed under vacuum and the residue was extracted with cold ethyl acetate (50 mL) and filtered over a celite pad. The ethyl acetate solution was washed with saturated citric acid solution (40 mL), saturated Na_2CO_3 solution (40 mL) and finally with brine (40 mL). The crude solution of the peptide was dried over anhydrous Na_2SO_4 and concentrated. The peptides (**7a-g**) were purified by column chromatography (silica gel, 100–200 mesh) eluting with a solution of ethyl acetate (20–40%) in petroleum ether.

2.6a *Boc–Phe–Ser(Poc)–OMe,* ***7a****:* White solid; Melting point: 92°C; Yield: 82%; $[\alpha]_D$: +5 (*c* = 1, MeOH); FTIR (KBr): 3290 (*m*), 2130 (*w*), 1756 (*s*), 1666 (*s*); δ_H (300 MHz, $CDCl_3$): 7·19–7·31 (*m*, 5H), 7·01 (*bd*, *J* = 6 Hz, 1H), 5·14 (*bd*, *J* = 6·6 Hz, 1H), 4·81–4·86 (*m*, 1H), 4·71 (*d*, *J* = 2·4 Hz, 2H), 4·41–4·53 (*m*, 3H), 3·75 (*s*, 3H), 3·05–3·16 (*m*, 2H), 2·57 (*t*, *J* = 2·4 Hz, 1H), 1·40 (*s*, 9H); δ_C (75 MHz, $CDCl_3$): 171·4, 168·9, 155·2, 153·9, 136·3, 129·2, 128·5, 126·8, 80·1, 75·9, 67·3, 55·5, 55·3, 52·8, 51·4, 38·1, 28·1; *m/z* (HRMS): Calcd. for $C_{22}H_{28}N_2O_8$ + Na: 471·1744; Found: 471·1738.

2.6b *Fmoc–Lys(Boc)–Ser(Poc)–OMe,* ***7b****:* White wax; Yield: 74%; $[\alpha]_D$: +9 (*c* = 1, MeOH); FTIR (Neat): 3299 (*br*), 2129 (*w*), 1758 (*s*), 1685 (*s*), 1669 (*s*); δ_H (300 MHz, $CDCl_3$): 7·75 (*d*, *J* = 7·5 Hz, 2H), 7·60 (*d*, *J* = 6·9 Hz, 2H), 7·39 (*t*, *J* = 7·2 Hz, 2H), 7·30 (*t*, *J* = 7·2 Hz, 2H), 7·17 (*bd*, *J* = 7·2 Hz, 1H), 5·69 (*bd*, *J* = 6·9 Hz, 1H), 4·87–4·90 (*m*, 1H), 4·76 (*bs*, 1H), 4·66 (*d*, *J* = 2·7 Hz, 2H), 4·59 (*dd*, J_1 = 8 Hz, J_2 = 3·3 Hz, 1H), 4·45 (*dd*, J_1 = 11 Hz, J_2 = 3·3 Hz, 1H), 4·29–4·39 (*m*, 3H), 4·21 (*t*, *J* = 6·9 Hz, 2H), 3·76 (*s*, 3H), 3·05–3·15 (*m*, 2H), 2·50 (*t*, *J* = 2·4 Hz, 1H), 1·48–1·73 (*m*, 2H), 1·42 (*bs*, 13H); δ_C (75 MHz, $CDCl_3$): 171·9, 169·1, 156·1, 153·9, 143·9, 143·6, 141·2, 127·6, 127·0, 125·1, 119·9, 79·1, 76·1, 67·3, 67·1, 55·6, 54·5, 52·9, 51·5, 47·0, 39·8, 32·2, 29·5, 28·4, 22·2; *m/z* (HRMS): Calcd. for $C_{34}H_{41}N_3O_{10}$ + Na: 674·2690; Found: 674·2695.

2.6c *Boc–Ala–Ser(Poc)–OMe,* ***7c****:* Colourless oil; Yield: 86%; $[\alpha]_D$: +22 (*c* = 1, MeOH); FTIR (Neat): 3293 (*br*), 2129 (*w*), 1757 (*s*), 1696 (*s*), 1683 (*s*), 1670 (*s*); δ_H (300 MHz, $CDCl_3$): 6·97 (*bd*, *J* = 6·3 Hz, 1H), 5·02 (*bs*, 1H), 4·83-4·88 (*m*, 1H), 4·73 (*d*, *J* = 2·1 Hz, 2H), 4·56 (*dd*, J_1 = 11 Hz, J_2 = 3·3 Hz, 1H), 4·48 (*dd*, J_1 = 11 Hz, J_2 = 3·3 Hz, 1H), 4·18–4·23 (*m*, 1H), 3·79 (*s*, 3H), 2·56 (*t*, *J* = 2·1 Hz, 1H), 1·44 (*s*, 9H), 1·38 (*d*, *J* = 7·2 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 172·7, 169·1, 155·3, 154·1, 80·2, 76·0, 67·4, 55·6, 52·9, 51·6, 50·4, 28·2, 18·2; *m/z* (HRMS): Calcd. for $C_{16}H_{24}N_2O_8$ + Na: 395·1431; Found: 395·1427.

2.6d *Boc–Met–Ser(Poc)–OMe,* ***7d****:* Yellow oil; Yield: 78%; $[\alpha]_D$: +10 (*c* = 1, MeOH); FTIR (Neat): 3295 (*br*), 2129 (*w*), 1756 (*s*), 1710 (*s*), 1693 (*s*), 1679 (*s*); δ_H (300 MHz, $CDCl_3$): 7·17 (*bd*, *J* = 7·5 Hz, 1H), 5·13 (*bd*, *J* = 8·1 Hz, 1H), 4·84–4·89 (*m*, 1H), 4·73 (*d*, *J* = 2·4 Hz, 2H), 4·57 (*dd*, J_1 = 11 Hz, J_2 = 3·3 Hz, 1H), 4·48 (*dd*, J_1 = 11 Hz,

J_2 = 3·3 Hz, 1H), 4·24–4·37 (*m*, 1H), 3·79 (*s*, 3H), 2·58–2·63 (*m*, 3H), 1·89–2·14 (*m*, 5H), 1·45 (*s*, 9H); δ_C (75 MHz, $CDCl_3$): 171·6, 169·0, 155·3, 153·9, 80·1, 76·0, 67·2, 55·6, 53·3, 52·9, 51·5, 31·6, 29·9, 28·2, 15·1; *m/z* (HRMS): Calcd. for $C_{18}H_{28}N_2O_8S$ + Na: 455·1464; Found: 455·1452.

2.6e *Boc–Ala–Thr(Poc)–OMe, **7e**:* Colourless oil; Yield: 73%; $[\alpha]_D$: +26 (*c* = 1, MeOH); FTIR (Neat): 3295 (*br*), 2128 (*w*), 1752 (*s*), 1714 (*s*), 1697 (*s*), 1678 (*s*); δ_H (300 MHz, $CDCl_3$): 6·92 (*bd*, *J* = 7·2 Hz, 1H), 5·34 (*dq*, J_1 = 6·6 Hz, J_2 = 2·4 Hz, 1H), 5·19–5·22 (*m*, 1H), 4·70–4·72 (*m*, 2H), 4·25–4·29 (*m*, 1H), 3·75 (*s*, 3H), 2·57 (*t*, *J* = 2·4 Hz, 1H), 1·46 (*s*, 9H), 1·40 (*d*, *J* = 7·5 Hz, 3H), 1·33 (*d*, *J* = 6·3 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 173·3, 169·5, 155·3, 153·4, 80·1, 75·9, 75·0, 55·4, 55·0, 52·8, 50·1, 28·2, 18·0, 16·6; *m/z* (HRMS): Calcd. for $C_{17}H_{26}N_2O_8$ + Na: 409·1587; Found: 409·1590.

2.6f *Boc–Pro–Tyr(Poc)–OMe, **7f**:* White solid; Melting point: 72°C; Yield: 81%; $[\alpha]_D$: –35 (*c* = 1, MeOH); FTIR (KBr): 3311 (*br*), 2129 (*w*), 1746 (*s*), 1668 (*s*); δ_H (300 MHz, $CDCl_3$): 7·09–7·16 (*m*, 4H), 6·53 (*bs*, 1H), 4·77–4·90 (*m*, 3H), 4·26–4·31 (*bs*, 1H), 3·72 (*s*, 3H), 3·18–3·31 (*m*, 3H), 3·01 (*dd*, J_1 = 14 Hz, J_2 = 7·2 Hz, 1H), 2·62 (*t*, *J* = 2·4 Hz, 1H), 1·79–2·06 (*m*, 4H), 1·44 (*s*, 9H); δ_C (75 MHz, $CDCl_3$): 171·8, 153·2, 150·3, 134·5, 130·5, 121·2, 80·8, 76·8, 76·6, 60·2, 60·0, 56·2, 53·2, 52·6, 47·2, 37·7, 31·2, 28·5, 24·4, 23·8; *m/z* (HRMS): Calcd. for $C_{24}H_{30}N_2O_8$ + Na: 497·1900; Found: 497·1903.

2.6g *Boc–Ala–Tyr(Poc)–OMe, **7g**:* Colourless oil; Yield: 79%; $[\alpha]_D$: +30 (*c* = 1, MeOH); FTIR (Neat): 3295 (*br*), 2129 (*w*), 1766 (*s*), 1748 (*s*), 1697 (*s*), 1671 (*s*); δ_H (300 MHz, $CDCl_3$): 7·11–7·13 (*m*, 4H), 6·75 (*bd*, *J* = 6·6 Hz, 1H), 5·11 (*bd*, *J* = 7·5 Hz, 1H), 4·83 (*d*, *J* = 2·4 Hz, 3H), 4·13 (*bs*, 1H), 3·70 (*s*, 3H), 3·17 (*dd*, J_1 = 14 Hz, J_2 = 6 Hz, 1H), 3·07 (*dd*, J_1 = 14 Hz, J_2 = 6 Hz, 1H), 2·61 (*t*, *J* = 2·4 Hz, 1H), 1·44 (*s*, 9H), 1·30 (*d*, *J* = 7·2 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 172·8, 171·9, 155·7, 153·3, 150·4, 134·3, 130·7, 121·3, 80·4, 76·9, 76·6, 56·2, 53·4, 52·7, 50·4, 37·5, 28·6, 18·5; *m/z* (HRMS): Calcd. for $C_{22}H_{28}N_2O_8$ + Na: 471·1744; Found: 471·1738.

The tripeptide **15** was synthesized from the dipeptide **7c** and Boc–Leu–OH (**14**) using a procedure similar to that described for the preparation of peptides **7a–g**.

2.6h *Boc–Leu–Ala–Ser(Poc)–OMe, **14**:* Colourless oil; Yield: 78%; $[\alpha]_D$: –27 (*c* = 1, MeOH); FTIR (Neat): 3302 (*br*), 3063 (*m*), 2130 (*w*), 1741 (*s*), 1681 (*s*); δ_H (300 MHz, CDCl3): 7·52 (*bd*, *J* = 7·8 Hz, 1H), 7·16 (*bs*, 1H), 5·36 (*bs*, 1H), 4·87–4·92 (*m*, 1H), 4·62–4·73 (*m*, 3H), 4·56 (*dd*, J_1 = 11 Hz, J_2 = 3·6 Hz, 1H), 4·43 (dd, J_1 = 11 Hz, J_2 = 3·6 Hz, 1H), 4·19–4·22 (*m*, 1H), 3·79 (*s*, 3H), 2·58 (*t*, *J* = 2·7 Hz, 1H), 1·37-1·71 (*m*, 15H), 0·92–0·94 (*m*, 6H); δ_C (75 MHz, $CDCl_3$): 172·7, 172·4, 169·2, 155·7, 153·9, 79·7, 75·9, 67·3, 60·3, 55·5, 52·8, 51·4, 48·6, 41·3, 28·2, 24·6, 22·9, 21·7, 18·3; *m/z* (HRMS): Calcd. for $C_{22}H_{35}N_3O_9$ + Na: 508·2271; Found: 508·2267.

2.7 *Procedure for the preparation of peptides **7h** and **7i***

To a solution of Boc–Thr(Poc)–OPfp (**10**, 1 mmol) in acetonitrile (5 mL), the *C*-protected amino acid (H–Pro–OMe or H–Phe–OMe, 1 mmol) was added and the reaction mixture was stirred for 4 h. The solvent was removed under vacuum and the residue was extracted with ethyl acetate (30 mL). The ethyl acetate solution was washed with saturated citric acid solution (40 mL), saturated Na_2CO_3 solution (40 mL) and finally with brine (40 mL). The crude solution of the peptide was dried over anhydrous Na_2SO_4 and concentrated. The peptides **7h** and **7i** were purified by silica gel (100–200 mesh) column chromatography eluting with a solution of ethyl acetate (20–40%) in petroleum ether.

2.7a *Boc–Thr(Poc)–Pro–OMe, **7h**:* Colourless oil; Yield: 86%; $[\alpha]_D$: –44 (*c* = 1, MeOH); FTIR (Neat): 3427 (*br*), 2132 (*w*), 1822 (*s*), 1746 (*s*), 1711 (*s*); δ_H (300 MHz, $CDCl_3$): 5·45 (*bd*, *J* = 9·3 Hz, 1H), 5·07–5·14 (*m*, 1H), 4·66–4·74 (*m*, 2H), 4·45–4·63 (*m*, 2H), 3·72–3·84 (*m*, 5H), 2·53–2·55 (*m*, 1H), 2·21–2·30 (*m*, 1H), 1·95–2·14 (*m*, 3H), 1·35–1·43 (*m*, 12H); δ_C (75 MHz, $CDCl_3$): 171·9, 167·8, 155·4, 153·8, 79·9, 76·9, 75·6, 74·8, 58·9, 55·3, 55·2, 52·1, 47·2, 28·8, 28·2, 24·9, 16·3; *m/z* (HRMS): Calcd. for $C_{19}H_{28}N_2O_8$ + Na: 435·1744; Found: 435·1739.

2.7b *Boc–Thr(Poc)–Phe–OMe, **7i**:* Colourless oil; Yield: 87%; $[\alpha]_D$: + 38 (*c* = 1, MeOH); FTIR (Neat): 3294 (*br*), 2124 (*w*), 1746 (*s*), 1713 (*s*), 1699 (*s*); δ_H (300 MHz, $CDCl_3$): 7·25–7·34 (*m*, 3H), 7·08–7·11 (*m*, 2H), 6·74 (*bd*, *J* = 7·2 Hz, 1H), 5·32–5·39 (*m*, 1H), 5·23 (*bd*, *J* = 9 Hz, 1H), 4·78–4·85 (*m*, 1H),

4·72–4·74 (*m*, 2H), 4·30 (*bd*, J = 6 Hz, 1H), 3·74 (*s*, 3H), 3·16 (*dd*, J_1 = 14 Hz, J_2 = 6 Hz, 1H), 3·09 (*dd*, J_1 = 14 Hz, J_2 = 6 Hz, 1H), 2·54 (*t*, J = 2·7 Hz, 1H), 1·45 (*s*, 9H), 1·33 (*d*, J = 6·3 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 171·3, 168·4, 155·5, 153·1, 135·5, 129·2, 128·6, 127·1, 80·7, 75·9, 74·5, 57·5, 55·4, 53·4, 52·3, 37·6, 28·1, 15·9; *m/z* (HRMS): Calcd. for $C_{23}H_{30}N_2O_8$ + Na: 485·1900; Found: 485·1899.

2.8 *General procedure for the removal of O-Poc group using benzyltriethylammonium tetrathiomolybdate (**1**)*

To a solution of the *O*-Poc derivative (1 mmol of **4a–i**, **7a–i** or **15**) in acetonitrile, benzyltriethylammonium tetrathiomolybdate (**1**, 1·1 mmol, 0·67 g) was added at r.t. (28°C) and the reaction mixture was stirred for 1 h. Acetonitrile was removed under vacuum and the residue was extracted with a mixture of ethyl acetate and chloroform (9 : 1). The crude products were purified by column chromatography (silica gel, 100–200 mesh) eluting with a solution of ethyl acetate in petroleum ether.

2.8a *Boc–Phe–Ser–OMe, **11a**:* White solid; melting point: 60°C, Yield: 90%; $[\alpha]_D$: +16 (c = 1, MeOH); FTIR (KBr): 3320 (*br*), 1745 (*s*), 1691 (*s*), 1678 (*s*); δ_H (300 MHz, $CDCl_3$): 7·20–7·31 (*m*, 6H), 5·38 (*bd*, J = 7·2 Hz, 1H), 4·60–4·64 (*m*, 1H), 4·41–4·53 (*m*, 1H), 3·90 (*bs*, 2H), 3·74 (*s*, 3H), 3·14 (*dd*, J_1 = 14 Hz, J_2 = 8 Hz, 1H), 2·98 (*dd*, J_1 = 14 Hz, J_2 = 8 Hz, 1H), 1·37 (*s*, 9H); δ_C (75 MHz, $CDCl_3$): 171·8, 170·6, 155·7, 136·5, 129·3, 128·5, 126·8, 80·3, 62·6, 55·8, 54·7, 52·6, 38·2, 28·1; *m/z* (HRMS): Calcd. for $C_{18}H_{26}N_2O_6$ + Na: 389·1689; Found: 389·1693.

2.8b *Fmoc–Lys(Boc)–Ser–OMe, **11b**:* White solid; Melting point: 144°C; Yield: 83%; $[\alpha]_D$: –11 (c = 1, MeOH); FTIR (KBr): 3323 (*br*), 1689 (*s*); δ_H (300 MHz, $CDCl_3$): 7·74 (*d*, J = 6·9 Hz, 2H), 7·57 (*d*, J = 6·9 Hz, 2H), 7·25–7·40 (*m*, 5H), 5·95 (*bd*, J = 8·7 Hz, 1H), 4·83 (*bs*, 1H), 4·67 (*bs*, 1H), 4·08–4·36 (*m*, 4H), 3·90–4·01 (*m*, 2H), 3·73 (*s*, 3H), 3·10 (*bs*, 2H), 1·85 (*bs*, 1H), 1·72 (*bs*, 1H), 1·42 (*bs*, 13H); δ_C (75 MHz, $CDCl_3$): 172·3, 170·8, 156·5, 156·3, 143·8, 143·6, 141·2, 127·7, 127·0, 125·0, 119·9, 79·2, 67·2, 62·5, 54·7, 52·6, 46·9, 39·8, 32·1, 29·4, 28·4, 22·3; *m/z* (HRMS): Calcd. for $C_{30}H_{39}N_3O_8$ + Na: 592·2635; Found: 592·2639.

2.8c *Boc–Ala–Ser–OMe, **11c**:* Colourless oil; Yield: 92%; $[\alpha]_D$: –18 (c = 1, MeOH); FTIR (Neat): 3332 (*br*), 1746 (*s*), 1693 (*s*), 1681 (*s*); δ_H (300 MHz, $CDCl_3$): 7·10 (*bd*, J = 8 Hz, 1H), 5·24 (*bd*, J = 5 Hz, 1H), 4·63–4·67 (*m*, 1H), 4·15–4·20 (*m*, 1H), 3·96 (*bs*, 2H), 3·79 (*s*, 3H), 1·44 (*s*, 9H), 1·39 (*d*, J = 6·9 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 173·0, 170·8, 155·7, 80·5, 62·7, 54·8, 52·7, 50·4, 28·3, 18·0; *m/z* (HRMS): Calcd. for $C_{12}H_{22}N_2O_6$ + Na: 313·1376; Found: 313·1380.

2.8d *Boc–Met–Ser–OMe, **11d***: Yellow oil; Yield: 81%; $[\alpha]_D$: +10 (c = 1, MeOH); FTIR (Neat): 3319 (*br*), 1747 (*s*), 1669 (*s*); δ_H (300 MHz, $CDCl_3$): 7·57 (*bs*, 1H), 5·84 (*bs*, 1H), 4·63–4·69 (*m*, 1H), 4·34–4·41 (*m*, 1H), 3·98 (*dd*, J_1 = 11 Hz, J_2 = 3·3 Hz, 1H), 3·88 (*dd*, J_1 = 11 Hz, J_2 = 3·3 Hz, 1H), 3·77 (*s*, 3H), 2·60 (*t*, J = 7·5 Hz, 2H), 2·03–2·11 (*m*, 4H), 1·92–2·01 (*m*, 1H), 1·43 (*s*, 9H); δ_C (75 MHz, $CDCl_3$): 172·2, 170·5, 155·8, 80·0, 62·2, 54·5, 53·4, 52·4, 31·9, 29·8, 28·1, 15·0; *m/z* (HRMS): Calcd. for $C_{14}H_{26}N_2O_6S$ + Na: 373·1410; Found: 373·1408.

2.8e *Boc–Ala–Thr–OMe, **11e**:* White solid; Melting point: 95°C; Yield: 83%; $[\alpha]_D$: –14 (c = 1, MeOH); FTIR (KBr): 3344 (*br*), 1745 (*s*), 1690 (*s*), 1669 (*s*); δ_H (300 MHz, $CDCl_3$): 7·11 (*bd*, J = 8·1 Hz, 1H), 5·37 (*bs*, 1H), 4·62 (*dd*, J_1 = 9 Hz, J_2 = 2·7 Hz, 1H), 4·32–4·36 (*m*, 1H), 4·25–4·27 (*m*, 1H), 3·77 (*s*, 3H), 3·24 (*bs*, 1H), 1·44 (*s*, 9H), 1·39 (*d*, J = 6·9 Hz, 3H), 1·20 (*d*, J = 6·6 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 173·4, 171·3, 155·6, 80·2, 68·2, 57·3, 52·5, 50·2, 28·2, 19·8, 18·2; *m/z* (HRMS): Calcd. for $C_{13}H_{24}N_2O_6$ + Na: 327·1532; Found: 327·1529.

2.8f *Boc–Pro–Tyr–OMe, **11f***: Colourless oil; Yield: 94%; $[\alpha]_D$: –29 (c = 1, MeOH); FTIR (Neat): 3330 (*br*), 1744 (*s*), 1690 (*s*), 1681 (*s*); δ_H (300 MHz, $CDCl_3$): 7·90 (*bs*, 1H), 6·94 (*d*, J = 8·4 Hz, 2H), 6·75 (*d*, J = 6·9 Hz, 2H), 6·58 (*bs*, 1H), 4·84 (*bs*, 1H), 3·73 (*s*, 3H), 3·35 (*bs*, 2H), 3·10–3·14 (*m*, 1H), 2·88–2·95 (*m*, 1H), 2·53 (*bs*, 1H), 1·65–2·10 (*m*, 3H), 1·44 (*s*, 9H); δ_C (75 MHz, $CDCl_3$): 172·6, 171·7, 155·8, 154·7, 130·1, 126·5, 115·5, 81·2, 80·6, 60·7, 60·2, 54·2, 53·3, 52·7, 52·3, 46·9, 37·2, 30·5, 28·1, 24·5, 23·7; *m/z* (HRMS): Calcd. for $C_{20}H_{28}N_2O_6$ + Na: 415·1845; Found: 415·1839.

2.8g *Boc–Ala–Thr–OMe, **11g**:* Colourless oil; Yield: 96%; $[\alpha]_D$: –8 (c = 1, MeOH); FTIR (Neat): 3331

(*br*), 1743 (*s*), 1692 (*s*), 1680 (*s*); δ_H (300 MHz, $CDCl_3$): 7·52 (*bs*, 1H), 6·92 (*d*, *J* = 8·4 Hz, 1H), 6·84 (*bs*, 1H), 6·71 (*d*, *J* = 8·1 Hz, 2H), 5·24 (*bd*, *J* = 6·6 Hz, 1H), 4·78–4·85 (*m*, 1H), 4·16 (*bs*, 1H), 3·72 (*s*, 3H), 3·07 (*dd*, J_1 = 14 Hz, J_2 = 5 Hz, 1H), 2·98 (*dd*, J_1 = 14 Hz, J_2 = 5 Hz, 1H), 1·44 (*s*, 9H), 1·30 (*d*, *J* = 6·9 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 172·7, 171·8, 155·6, 130·3, 126·7, 115·5, 80·4, 60·5, 53·4, 52·3, 50·0, 37·0, 28·2, 18·2; *m/z* (HRMS): Calcd. for $C_{18}H_{26}N_2O_8$ + Na: 389·1689; Found: 389·1692.

2.8h *Boc–Thr–Pro–OMe,* ***11h****:* Colourless oil; Yield: 80%; $[\alpha]_D$: –62 (*c* = 1, MeOH), FTIR (Neat): 3440 (*br*), 1823 (*s*), 1746 (*s*), 1714 (*s*); δ_H (300 MHz, $CDCl_3$): 5·56 (*bd*, *J* = 9·3 Hz, 1H), 4·51–4·55 (*m*, 1H), 4·41 (*dd*, J_1 = 9 Hz, J_2 = 2·7 Hz, 1H), 4·11–4·20 (*m*, 1H), 3·74–3·87 (*m*, 5H), 3·58 (*bs*, 1H), 2·18–2·29 (*m*, 1H), 1·94–2·11 (*m*, 3H), 1·44 (*s*, 9H), 1·23 (*d*, *J* = 6·6 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 172·4, 170·7, 155·9, 79·7, 67·3, 58·7, 55·6, 52·3, 47·1, 28·8, 28·1, 24·7, 18·4; *m/z* (HRMS): Calcd. for $C_{15}H_{26}N_2O_6$ + Na: 353·1689; Found: 353·1691.

2.8i *Boc–Thr–Phe–OMe,* ***11i****:* Colourless oil; Yield: 82%; $[\alpha]_D$: +1 (*c* = 1, MeOH); FTIR (Neat): 3405 (*br*), 3336 (*br*), 1744 (*s*), 1712 (*s*), 1668 (*s*); δ_H (300 MHz, $CDCl_3$): 7·12–7·29 (*m*, 6H), 5·57 (*bd*, *J* = 7·8 Hz, 1H), 4·81–4·87 (*m*, 1H), 4·27 (*bd*, *J* = 5·1 Hz, 1H), 4·11 (*bd*, *J* = 7·2 Hz, 1H), 3·70 (*s*, 3H), 3·59 (*bs*, 1H), 3·15 (*dd*, J_1 = 14 Hz, J_2 = 6 Hz, 1H), 3·04 (*dd*, J_1 = 14 Hz, J_2 = 6 Hz, 1H), 1·46 (*s*, 9H), 1·15 (*d*, *J* = 6·3 Hz, 3H); δ_C (75 MHz, $CDCl_3$): 171·6, 170·8, 156·1, 135·7, 129·1, 128·4, 126·9, 80·1, 66·8, 58·2, 53·2, 52·2, 37·7, 28·1, 17·9; *m/z* (HRMS): $C_{19}H_{28}N_2O_6$ + Na: 403·1845; Found: 403·1851.

2.8j *Boc–Leu–Ala–Ser–OMe,* ***15****:* Colourless oil; Yield: 88%; $[\alpha]_D$: –35 (*c* = 1, MeOH); FTIR (Neat): 3304 (*br*), 1745 (*s*), 1688 (*s*), 1679 (*s*), 1659 (*s*); δ_H (300 MHz, $CDCl_3$): 7·67 (*d*, *J* = 8·1 Hz, 1H), 7·30 (*bd*, *J* = 4·5 Hz, 1H), 5·40 (*bd*, *J* = 7·5 Hz, 1H), 4·60–4·67 (*m*, 2H), 4·11–4·16 (*m*, 1H), 3·80–3·98 (*m*, 2H), 3·77 (*s*, 3H), 1·36–1·69 (*m*, 15H), 0·91–0·95 (two doublets, 6H); δ_C (75 MHz, $CDCl_3$): 173·4, 172·4, 170·7, 155·8, 80·2, 62·5, 54·8, 53·3, 52·5, 48·9, 41·1, 28·2, 24·6, 22·9, 21·6, 20·6, 18·1; *m/z* (HRMS): Calcd· for $C_{18}H_{33}N_3O_7$ + Na: 426·2217; Found: 426·2222.

3. Results and discussion

The propargyloxycarbonyl derivatives were synthesized by treating the corresponding derivatives of the amino acids with propargyloxycarbonyl chloride (PocCl, **2**) at low temperatures. For example, treatment of Boc–Ser–OMe (**3a**) with **2** (CH_2Cl_2, –78°C, 2 h) gave Boc–Ser(Poc)–OMe (**4a**) in 90% yield (scheme 1). The Poc derivatives (**4a–i**) were obtained in high yields from the corresponding derivatives (**3a–i**) of serine, threonine and tyrosine (table 1). Treatment of Boc–Ser(Poc)–OMe (**4a**) with **1** (CH_3CN, 28°C, 1 h) resulted in efficient deprotection of the Poc group to give **3a** in excellent yield (scheme 1). Similarly, the *O*-Poc derivatives (**4b–i**) were deprotected to the corresponding hydroxy compounds (**3b–i**) in good yields using **1** (table 1). A comparison of the specific rotation of the compounds **3a** obtained after the deprotection of the *O*-Poc derivatives **4a**, with that known in the literature revealed that there is no racemization of the amino acid residues during the conditions used for the protection and the deprotection of Poc group.[7]

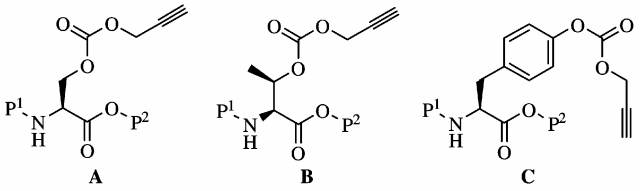
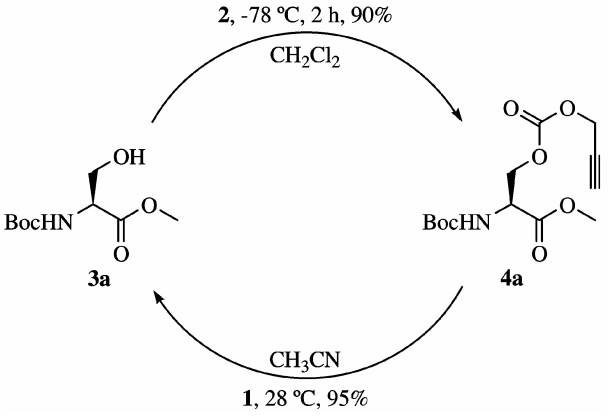
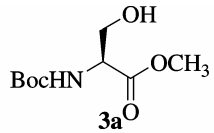
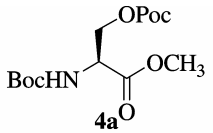
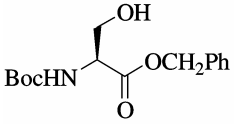
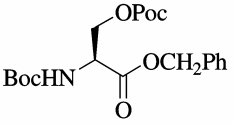
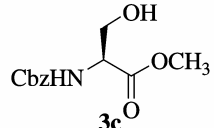
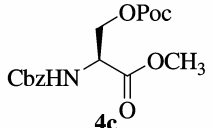
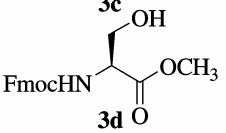
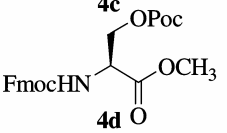
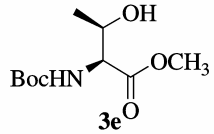
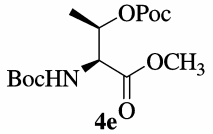
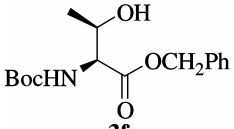
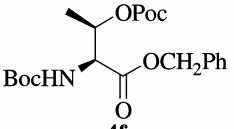
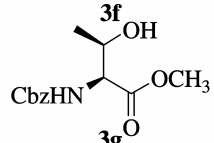
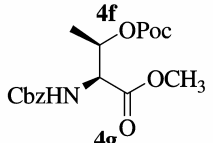
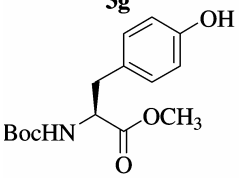
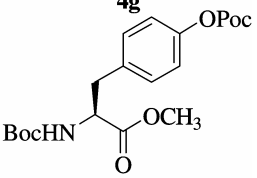
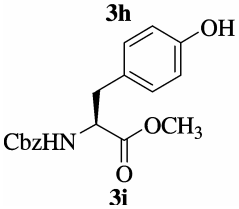
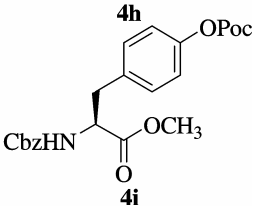


Figure 1. Poc derivatives of serine (**A**), threonine (**B**) and tyrosine (**C**).



Scheme 1. Protection of the serine derivative (**3a**) as the Poc derivative (**4a**) and the deprotection of the Poc group in 4a using tetrathiomolybdate (**1**).

Table 1. Preparation of *O*-Poc derivatives of serine, threonine and tyrosine and their deprotection using tetrathiomolybdate (**1**).

No.	Amino acid derivatives	*O*-Poc derivatives	Yield (%)[a]	Yield (%) after removal of Poc[a]
1	 **3a**	 **4a**	90	95
2	 **3b**	 **4b**	83	92
3	 **3c**	 **4c**	88	94
4	 **3d**	 **4d**	85	89
5	 **3e**	 **4e**	79	87
6	 **3f**	 **4f**	73	90
7	 **3g**	 **4g**	76	96
8	 **3h**	 **4h**	93	97
9	 **3i**	 **4i**	89	96

[a]Yields reported are of pure compounds isolated after column chromatography

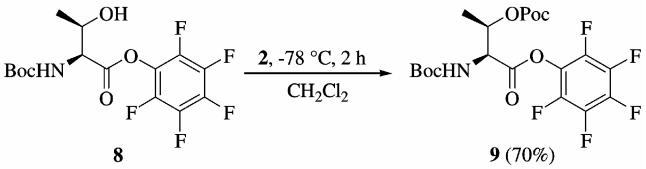
The Poc derivatives **4a–i** are stable compounds and can be stored at room temperature for more than six months. Treating Cbz–Ser(Poc)–OMe (**4c**) with neat TFA did not result in the deprotection of *O*-Poc group and **4c** could be isolated quantitatively after 24 h. The stability of *O*-Poc groups to neat TFA would allow the deprotection of Boc and related *tert*-butyl protecting groups without affecting the Poc group.[3] Treating a solution of **4c** in dichloromethane with various organic bases (piperidine, DBU, triethylamine, NMM) did not result in any reaction or deprotection of the Poc group. However,

the Poc derivatives of serine and threonine were not stable to treatment with DBU in DMF, which resulted in the formation of corresponding dehydroamino acid derivatives.[8] The elimination of Poc group was observed only when the reaction was performed in DMF as the solvent.[8]

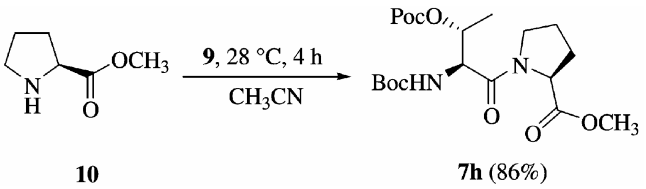
We were interested in examining the possibilities of using the *O*-Poc derivatives (**4a–i**) in solution phase peptide synthesis. Since the Boc group can easily be deprotected with 50% TFA in CH_2Cl_2,[3] the Poc derivatives **4a**, **4b**, **4e**, **4f** and **4h** could be used for solution phase peptide synthesis after removal of the Boc group. Accordingly, **4a** was treated with 50% TFA (CH_2Cl_2, 28°C, 30 min) to get the triflouroacetate salt TFA·H–Ser(Poc)–OMe (**5**). The trifluoroacetate salt (**5**) was then coupled with Boc–Phe–OH (**6**) using DCC (CH_3CN, HOBt, NMM, 28°C) and the dipeptide Boc–Phe–Ser–(Poc)–OMe (**7a**) was obtained in excellent yield (scheme 2).

Similarly a few other dipeptides were synthesized using the *N*-Boc derivatives **4a**, **4e** and **4h** and the corresponding dipeptides (**7b–g**) were obtained in good yields (table 2). However, our initial attempts to synthesize peptides using serine, threonine or tyrosine residues at the *N*-terminus were unsuccessful. Efforts to hydrolyse the ester groups in 4**a–i** resulted in the deblocking of the Poc group or in the formation of dehydroamino acids.[8] We overcame this problem by preparing the Poc derivative, Boc-Thr–(Poc)–OPfp (**9**) from the pentafluorophenyl ester Boc–Thr–OPfp (**8**) using **2** (scheme 3). Treating the active ester **9** with H-Pro-OMe (**10**) yielded the dipeptide Boc–Thr(Poc)–Pro–OMe (**7h**) in good yield (scheme 4, table 2: entry 8). Similarly, the peptide, Boc–Thr(Poc)–Phe–OMe (**7i**) was made in 83% yield from **9** and H–Phe–OMe (table 2: entry 9). Thus we have accomplished the synthesis of peptides bearing the *O*-Poc residues both at the *N*-terminus and at the *C*-terminus.

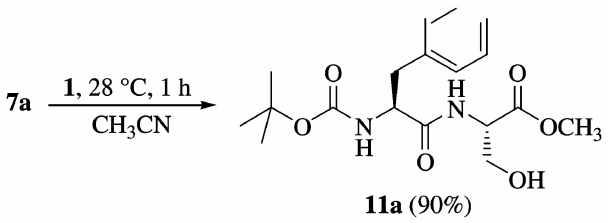
We anticipated that the deprotection of the *O*-Poc groups from the dipeptides (**7a–i**) could be achieved by treating them with tetrathiomolybdate (**1**). The dipeptide, Boc–Phe–Ser(Poc)–OMe (**7a**) when treated



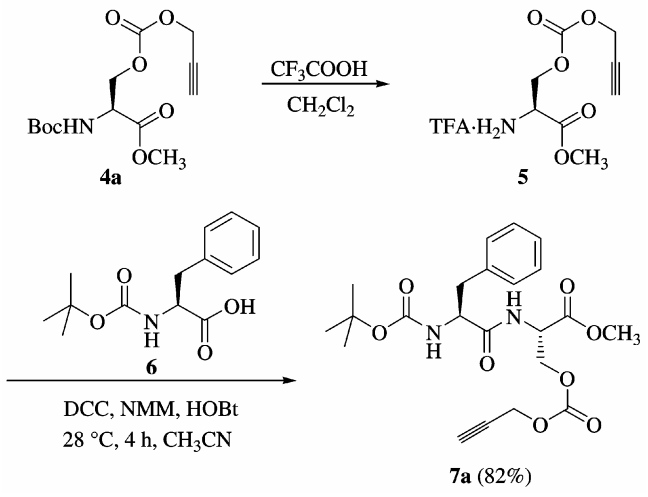
Scheme 3. Preparation of Boc–Thr(Poc)–OPfp (**9**).



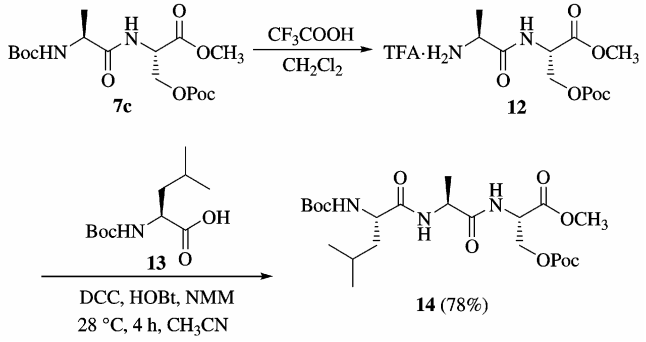
Scheme 4. Preparation of a dipeptide with the *O*-Poc residue on the *N*-terminus.



Scheme 5. Deprotection of *O*-Poc group to get the hydroxy peptide **11a**.



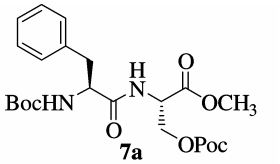
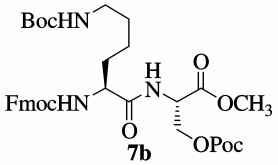
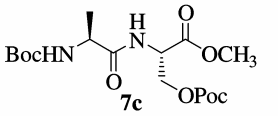
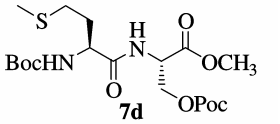
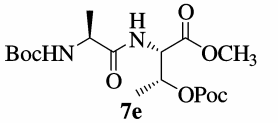
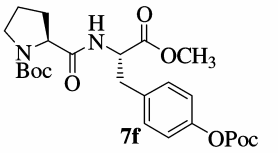
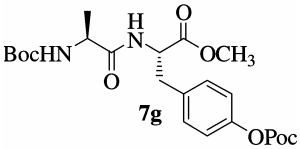
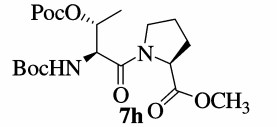
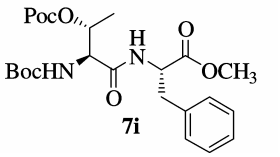
Scheme 2. Preparation of dipeptides using an *O*-Poc derivative of serine (**4a**).



Scheme 6. Synthesis of a tripeptide bearing an *O*-Poc residue.

with **1** (CH_3CN, 28°C, 1 h) yielded the corresponding hydroxy peptide Boc–Phe–Ser–OMe (**11a**) in excellent yield (scheme 5). All the other dipeptides (**7b–i**) could be similarly deprotected to the corresponding hydroxy peptides (**11b–i**) by treating with **1** (table 3).

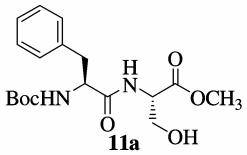
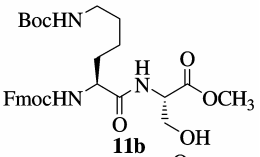
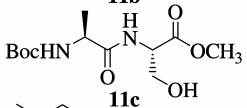
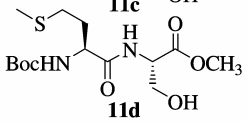
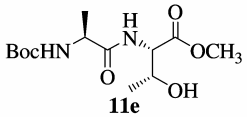
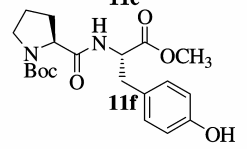
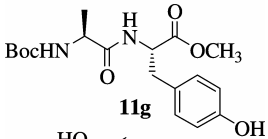
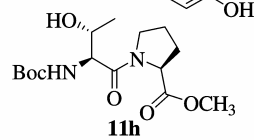
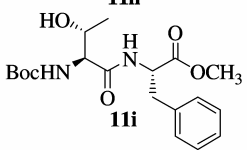
Table 2. Preparation of dipeptides with *O*-Poc derivatives of serine, threonine and tyrosine.

Entry	*O*-Poc derivatives	Dipeptides	Yield (%)[a]
1	**4a**	 **7a**	82
2	**4a**	 **7b**	74
3	**4a**	 **7c**	86
4	**4a**	 **7d**	78
5	**4e**	 **7e**	73
6	**4h**	 **7f**	81
7	**4h**	 **7g**	79
8	**9**	 **7h**	86
9	**9**	 **7i**	87

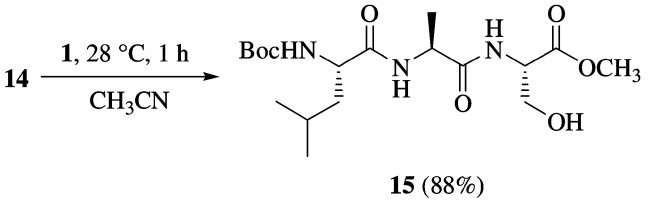
[a]Yields reported are of pure compounds isolated after column chromatography

We attempted the synthesis of a tripeptide from the dipeptide **4c** to show that the *O*-Poc groups are stable to multiple protection and deprotection steps used in the synthesis of oligopeptides. Boc–Ala–Ser(Poc)–OMe (**7c**) was treated with TFA (50% in CH_2Cl_2, 28°C, 30 min) to deblock the *N*-Boc group to get the trifluoroacetate salt **12**. The salt **12** was coupled with Boc–Leu–OH (**13**) to get the tripeptide

Table 3. Deprotection of *O*-Poc groups to get hydroxy peptides.

Entry	Dipeptides bearing *O*-Poc group	Hydroxy peptides	Yield (%)[a]
1	**7a**	 **11a**	90
2	**7b**	 **11b**	83
3	**7c**	 **11c**	92
4	**7d**	 **11d**	81
5	**7e**	 **11e**	83
6	**7f**	 **11f**	94
7	**7g**	 **11g**	96
8	**7h**	 **11h**	80
9	**7i**	 **11i**	82

[a]Yields reported are of pure compounds isolated after column chromatography



Scheme 7. Deprotection of *O*-Poc group to get the hydroxy tripeptide **15**.

Boc–Leu–Ala–Ser(Poc)–OMe (**14**) in good yield (scheme 6). The tripeptide **14** was treated with **1** (CH_3CN, 28°C, 1 h) to get the hydroxy tripeptide **15** in excellent yield (scheme 7).

Thus, we have clearly established the application of propargyloxycarbonyl group as an efficient protecting group for the hydroxy amino acids–serine, threonine and tyrosine. The protection of hydroxyl group as a Poc derivative is complimentary to strategies using *tert*-butyl protecting groups for the protection of amino group. Thus the methodology is quite useful in the synthesis of hydroxy peptides. It is quite evident that other hydroxy protecting groups used in peptide synthesis such as trityl, *tert*-butyl ether, etc. are orthogonal to Poc group making the methodology useful in the synthesis of peptides bearing diversely protected hydroxyl residues.

4. Conclusion

In conclusion, we have described an efficient protocol for the protection of the hydroxyl side chains of serine, threonine and tyrosine as their propargyloxycarbonyl derivatives. The *O*-Poc derivatives are stable to treatment with neat TFA, which make them orthogonal to *tert*-butyl protecting groups; but are efficiently deprotected to the corresponding hydroxyl residues using tetrathiomolybdate (**1**). The *O*-Poc derivatives of these hydroxyamino acids are used in the solution phase synthesis of peptides. The peptides bearing *O*-Poc residues are deprotected to synthesize hydroxy peptides in high yields.

References

1. Bodanszky M 1993 *Principles of peptide synthesis* (Berlin: Springer-Verlag) 2nd edn
2. Williams P L, Albericio F and Giralt E 1997 *Chemical approaches to the synthesis of peptides and proteins* (Boca Raton: Florida CRC Press)
3. Greene T W and Wuts P G M 1999 *Protective groups in organic synthesis* (New York: John Wiley & Sons Inc.) Chapter 2, 3rd edn, p 186
4. Sampson P 1995 *Encyclopedia of reagents in organic synthesis* (ed.) L A Pacquette (Chichester: West Sussex, John Wiley), vol 1, pp 323–326
5. Sridhar P R and Chandrasekaran S 2002 *Org. Lett.* **4** 4731
6. Ramesh R, Bhat R G and Chandrasekaran S 2005 *J. Org. Chem.* **70** 837
7. $[\alpha]_D^{25}$ +9·0 (c 1.0, $CHCl_3$); lit. $[\alpha]_D^{26}$ +9.1 (c 1.3, $CHCl_3$): Saito Y, Ouchi H and Takahata H 2006 *Tetrahedron* **62** 11599
8. Ramesh R, Kavita De and Chandrasekaran S 2007 *Tetrahedron* **63** 10534
9. Ramesha A R and Chandrasekaran S 1992 *Synth. Commun.* **22** 3277