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Synthesis of methyl (±)-3,5-bis(substitutedmethyl)pyrrolidine-2-carboxylates: a convenient approach to proline-mimetics

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ABSTRACT

Starting from readily available *N*-benzyl protected methyl 3,5-bis(hydroxymethyl)pirrolidinecarboxylate a number of racemic methyl *t*-3,*t*-5-disubstitutedprolinates have been synthesised, thus opening a practical way towards the preparation of a variety of putative proline-mimetics. In this context, *N*-Boc protected derivatives proved to be better intermediates than their *N*-benzyl counterparts in terms of cleanness and overall yield of the synthetic procedure.

1. Introduction

The key to understanding and modulating protein function as well as developing new therapies and diagnosis are Small Molecules.[1] In this context, the cyclic α-amino acids are synthetically interesting targets because they can be used as building blocks for the peptidomimetic structures synthesis with biological activity.[2] In recent years[3] small heterocyclic molecules have attracted considerable attention and between these, proline-derived structures constitute a particularly interesting class of molecules.[4]

For our interest the most bioactive peptides have proline in their sequence, the important roles of the neuropeptides GPE (glycyl-L-prolyl-L-glutamate)[5] and PLG (L-prolyl-L-leucyl-glycinamide)[6] (Fig. 1) in the central nervous system have prompted exploration of the possibility of using these species and/or their analogues to treat neurodegenerative pathologies such as Alzheimer's disease, Parkinson's disease and Huntington's chorea.

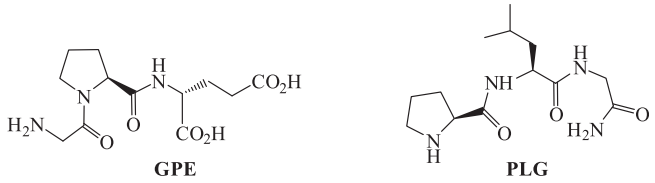

Figure 1.

The GPE (Glypromate®) tripeptide is a peptide that naturally occurring and has been studied extensively. It was reported that GPE stimulates acetylcholine release from cortical slices of the rat[7,8] and has considerable neuroprotective efficacy in multiple models. In vitro, Glypromate® has been shown to protect hippocampal neurons from glutamate-mediated excitotoxicity[9,10] and protects cerebellar and striate cells from excitotoxicity and oxidative stress. These effects have been shown efficacy in various animal models of acute brain injury in juvenile and adult rats, hypoxia-ischemia,[11–13] NMDA challenge,[8] and in animal models of Parkinson's,[14,15] the experimental autoimmune encephalomyelitis model of multiple sclerosis[16] and β-amyloid-induced depletion of somastatin for relevance to Alzheimer's disease.[17] This efficacy has been underpinned in the development of this drug as a neuroprotective agent and it is currently in Phase III clinical trials for the treatment of cognitive decline following on-pump cardiac surgery.[18]

The tripeptide PLG acts as a modulator of the dopamine receptor.[6] Experiments have shown that PLG enhances the conversion of dopamine receptors from their low-affinity state to a high-affinity state for enhanced agonist binding.[19] The precise mechanism of action behind PLG's ability to modulate the dopamine receptor is unclear. Preliminary evidence suggests the existence of a putative PLG binding site.[20,21]

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One subclass of PLG and GPE analogues is defined by replacement of the prolyl moiety by molecules that mimic proline and this is currently a very active area of investigation aimed at the development of new therapeutic agents.[21–26]

From a structural point of view, the most commonly used proline-mimetics are derivatives of proline itself with a wide range of substituents. In terms of the substitution patterns on the proline ring, derivatives with 2-, 3-, 4- and 5-monosubstitution,[24,26–29a,29b] 2,3,2,4- 3,4- and 3,5-disubstitution,[24,27c,29–31] and some 2,3-, 4-, 2,4,5- and 3,4,5-trisubstitution[24,27c,29a] patterns have been synthesised.

All these facts have contributed to the growing interest in finding short and efficient synthetic routes to substituted cyclic amino acids. This work describes here concerns 3,5-disubstituted proline derivatives and their potential as scaffolds in the synthesis of peptides with pharmacological activity. In this respect we have prepared 3,5-disubstituted proline analogues of type **I**. These derivatives have been obtained through synthetic routes associated with the synthesis of 2-azabicyclo[2.2.1]cyclohept-5-eno-3-carboxylic acid derivatives of type **II**, the synthesis of which has been pioneered by Stglich[32] and was extended by other investigators,[33–42] and subsequent synthetic exploitation of their stereoisomers[39,40] has been developed by our group (Fig. 2).

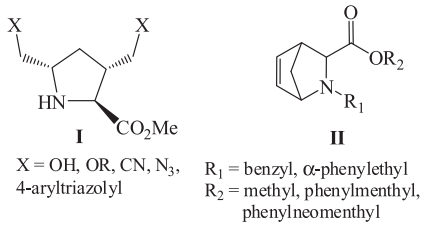


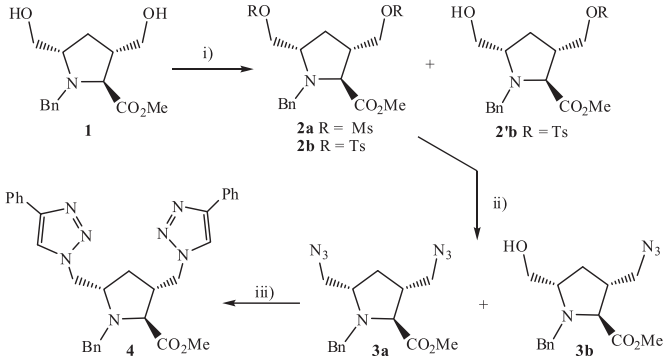
Figure 2.

2. Results and discussion

The synthesis of intermediate **1** (Scheme 1) was accomplished by an imino-Diels–Alder reaction[40] followed by a twofold hydroxylation and oxidative cleavage, as described previously.[41] Treatment of **1** with excess mesyl chloride (3 equiv) using a standard procedure gave **2a** (64% yield). The use of tosyl chloride in a similar proportion gave worse results, with **2b** obtained in only 17% yield. With the aim of preparing mono(tosyloxy)derivatives another experiment was carried out with a smaller amount of tosyl chloride (1.6 equiv). In this case **2b** was isolated in 22% yield after column chromatography along with a monotosyl derivative of alcohol **1**, which was identified as **2′b** [the 3-(tosyloxy)derivative, yield 9%]. This compound was formed because the hydroxymethyl group in position 3 of the pyrrolidine ring of diol **1** is sterically less crowded. Furthermore, previous investigations carried out by our group support this result[42] as attempts to selectively protect one of the hydroxy groups in **1** with *tert*-butyldiphenylsilyl chloride gave the derivative with the monosilyl ether on the hydroxymethyl group at position 3 as the major product.

Attempts to obtain diazide **3a** through nucleophilic substitution, by treatment with excess sodium azide, gave only poor-to-moderate yields (54%) of the desired product even when the most favourable reaction conditions were used (precursor **2a**). The use of **2b** as the starting material gave the 3-azidoderivative **3b** (Scheme 1). Subsequent Huisgen 1,3-dipolar cycloaddition[43] between **3a** and phenylacetylene in the presence CuI[44] gave the ditriazole derivative **4** in 54% yield.

Efforts were made to improve the low yields obtained [mesylate **2a** (64%), tosylate **2b** (17%), diazide **3a** (54%) and ditriazole derivative **4** (54%)] and minimise the difficult and laborious nature of the procedures described above, which can be attributed to the low reactivity of the functional groups on the methyl radicals in structures of type **I**. In this respect, it was decided to change the protecting group for the amino group of the proline, with *tert*-butoxycarbonyl (Boc) used instead of benzyl. The rationale for this change was that the presence of an electron-withdrawing group on the nitrogen of the pyrrolidine ring would increase the reactivity of the hydroxymethyl group, though other effects brought about by the introduction of a Boc group on the pyrrolidine ring such as changes of the geometry of the ring and in the orientation of the substituents at the 2 and 5 positions, due to both the minimisation of pseudoallylic 1,3-strain and to different intra-molecular H-bonding properties, may also be influential.



Scheme 1. Reagents, conditions and yields: (i) (a) MsCl, Et_3N, CH_2Cl_2, DMAP, rt, 1 h, **2a** (64%); (b) TsCl, pyridine, CH_2Cl_2, rt, 4 h, **2b** (22%)+**2′b** (9%); (ii) NaN_3, DMF, 90 °C, 14 h, **3a** (41%)+**3b** (13%) from **2b**, and **3a** (54%) from **2a**; (iii) phenylacetylene, CuI, DIPEA, toluene, *t*-BuOH, 70 °C, 13 h, 54%.

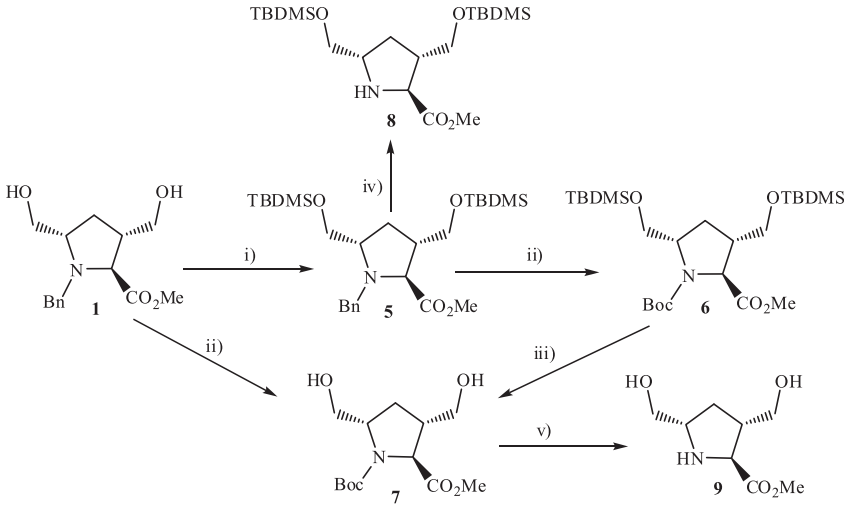
With this aim in mind, diol **7** was prepared by the route shown in Scheme 2. The hydroxy groups in diol **1** were first protected as silyl ethers by reaction with 3,5-bis(*tert*-butyltrimethylsilyl) chloride. The resulting disilyl ether **5** was hydrogenated in the presence of di-*tert*-butyl dicarbonate with $Pd(OH)_2$ as a catalyst. The resulting pyrrolidine derivative was reacted in situ to give the *N*-Boc derivative **6**[45] and subsequent cleavage of the silyl ethers in **6** with 1 M TBAF in THF gave **7** with an overall yield of 62%. Alternatively, a similar procedure carried out on compound **1** gave **7** directly in high yield (81%). Finally the treatment of **5** and **7** with trifluoroacetic acid gave proline-mimetics **8** and **9**, respectively.

The conversion of **7** to 3,5-bis(mesyloxymethyl)pyrrolidine derivative **10a** (Scheme 3) was practically quantitative on using a 1:3 ratio of **7**/mesyl chloride. However, the use of a lower ratio (1:2) also gave 3-(mesyloxymethyl)-5-(hydroxymethyl)pyrrolidine derivative **10b** (25%). Treatment of **10a** with NaN_3 under the same conditions used for the synthesis of **3a** led to diazide **11**, which underwent a 1,3-dipolar cycloaddition with ethynylbenzene, or (4-ethynylphenyl)methanol[46] to give, respectively **12a** or **12b** in good yields.

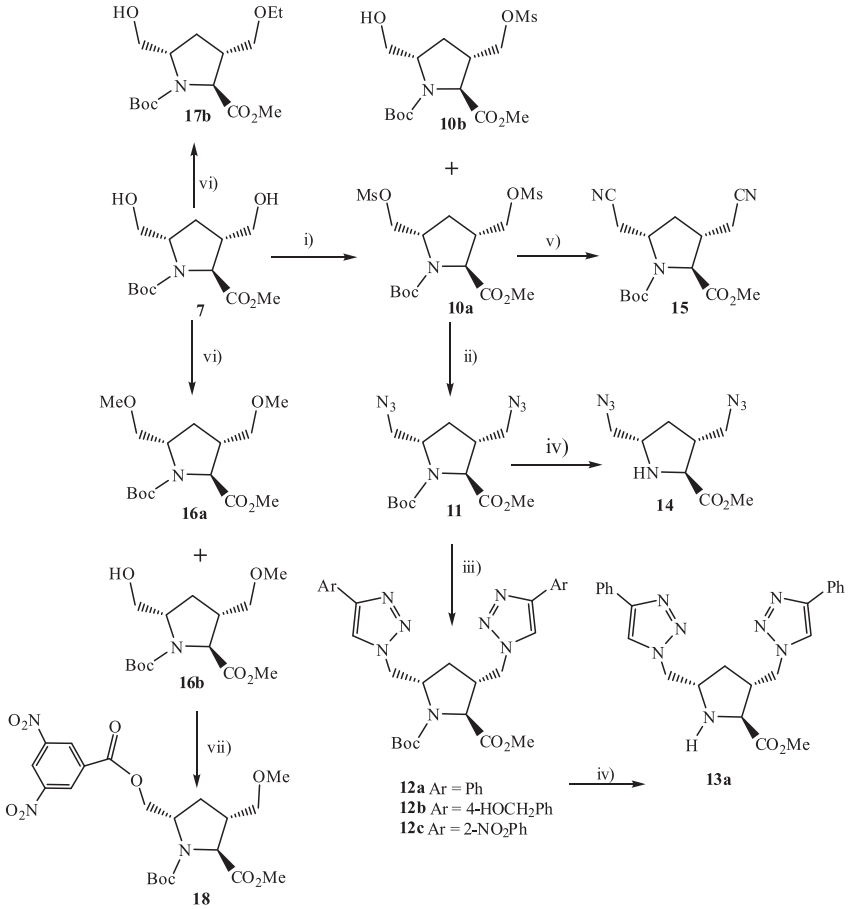
Finally, diazide **11** and 3,5-bis-[(4-arylltriazol)-1-ylmethyl]pyrrolidine derivatives **12a** were converted into the corresponding proline-mimetics **13a** and **14** by treatment with trifluoroacetic acid in dichloromethane at room temperature.

The reaction sequence (**1**→**7**→**10a**→**11**→**12a**), in which the pyrrolidine nitrogen of the substrates was protected as the *tert*-butylcarbamate, gave an overall yield of 51%, which compares favourably with the 19% yield obtained when the benzyl protecting group was used (**1**→**2a**→**3a**→**4**).

Other examples of utility of **7** or your (mesyloxymethyl)pyrrolidine derivative **10a** for the preparation of 3,5-disubstituted proline-mimetics was exemplified by the synthesis of the compounds **15**–**18**.



Scheme 2. Reagents and conditions: (i) TBDMSCl, imidazole, CH_2Cl_2, rt, 2 h, 92%; (ii) 20% $Pd(OH)_2/C$, H_2 1 atm, Boc_2O, EtOAc, rt, 18 h, 6 (77%); 7 (81%); (iii) 1 M TBAF in THF, rt, 1 h, 7 (88%); (iv) 20% $Pd(OH)_2/C$, H_2 1 atm, EtOAc, rt, 144 h, 8 (42%); (v) TFA, CH_2Cl_2, rt, 4 h, 9 (76%).



Scheme 3. Reagents, conditions and yields: (i) (a) MsCl, Et_3N, CH_2Cl_2, DMAP, rt, 1 h; ratio **7**/MsCl:1/3, **10a** (96%); ratio **7**/MsCl:1/2, **10a** (72%)+**10b** (25%); (ii) NaN_3, DMF, 90 °C, 2 h, 76%; (iii) arylacetylene, CuI, DIPEA, toluene, *t*-BuOH, rt, 24 h, **12a** (87%), **12b** (60%), **12c** ( ); (iv) CF_3CO_2H, CH_2Cl_2, rt 3 h, **13a** (98%); (v) NaCN, 18-crown-6, CH_3CN, 60 °C, 23 h, 61%; (vi) RI, Ag_2O, CH_3CN, 40 °C, 60 h, **16a** (36%)+**16b** (34%); **17b** (37%); (viii) 3,5-dinitrobenzyl chloride, THF, rt, 45 h, 46%.

Reaction of **10a** with NaCN in acetonitrile and 18-crown-6 at 60 °C[46] gave dicarbonitrile **15**. The structure of **15** was unequivocally determined by means of X-ray analysis of a single crystal (Fig. 3).[47] This confirmed the relative configuration of the **10a** intermediate key, and consequently that, of the complete series of derivatives of the same.

The preparation of **16a** proved problematical by the reaction of mesylate **10a** and MeOH in the presence of KOH, but the desired compound was finally obtained in 46% yield along with monoether **16b** (38%) by reaction of **7**, MeI and Ag_2O in acetonitrile[48] (Scheme 3). The product ratio of **16a**/**16b** was 46:38 and this remained unchanged even on using a large excess of methylating agent (molar ratio **10a**/CH_3I of 1:140) and prolonged reaction times (up to 120 h). The treatment of **7** with EtI in similar conditions, leads mainly to monoether **17b**.

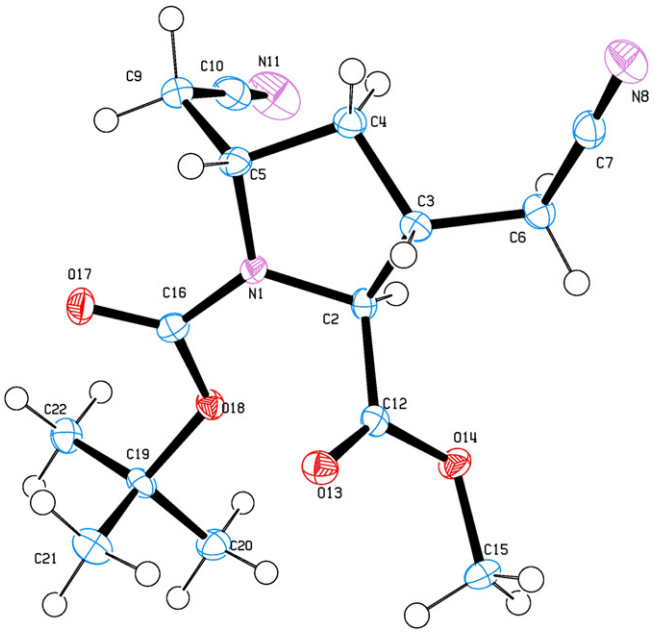


Figure 3. X-ray crystallographic structure of **15**.

3. Conclusion

The procedure here described is a contribution to the enlargement of the pool of available proline-like structures—a currently very active field with the aim of obtaining new proline-mimetic therapeutic agents. While exploring synthetic approaches to 3,5-disubstituted prolines from a readily available *N*-benzyl protected derivative, **1**, we found that *N*-Boc derivatives are better intermediates than *N*-benzyl derivatives in terms of cleanness and overall yield of the synthetic procedure, as it can be seen comparing reaction sequence (**1**→**7**→**10a**→**11**→**12**) with (**1**→**2a**→**3a**→**4**). In addition, a variety of functional group modifications of the hydroxymethyl groups of **7** have been carried out, thus enabling the preparation of compounds **9**, **13**–**18** and showing diol **7** as a versatile synthon for the preparation of a variety of 3,5-disubstituted proline-mimetics.

4. Experimental

4.1. General methods

All chemicals were of reagent grade and were obtained from Aldrich Chemical Co. and used without further purification. All air-sensitive reactions were carried out under argon. Flash chromatography was performed on silica gel (Merck 60, 230–240 mesh) and analytical TLC was carried out on pre-coated silica gel plates (Merck 60 F_{254}, 0.25 mm). Melting points were measured on a Reichert Kofler Thermopan apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 1640 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 300 spectrometer at 300 and 75.47 MHz, respectively, using TMS as an internal standard (chemical shifts in δ values, J in hertz). Mass spectra were recorded on Hewlett-Packard HP5988A or Micromass Autospec spectrometers. Microanalyses were performed on a FISONS EA 1108 Elemental Analyser at the University of Santiago Microanalysis Service; all results shown are within ±0.4% of the theoretical values.

4.1.1. Methyl (2R,3R*,5R*)-1-benzyl-3,5-[[bis(methylsulfonyl)oxy]methyl]pyrrolidine-2-carboxylate (**2a**).* MsCl chloride (250 μL, 3.22 mmol) was added dropwise over 5 min to a cooled (0 °C), stirred solution of **1** (0.30 g, 1.07 mmol), dry Et_3N (225 μL) and a catalytic amount of 4-DMAP in dry CH_2Cl_2 (10 mL) under argon. On completion of the addition the mixture was stirred at room temperature until the starting material had been consumed (TLC, about 1 h). The mixture was diluted with CH_2Cl_2 (70 mL) and the organic layer was washed successively with saturated aqueous $NaHCO_3$ (2×20 mL), H_2O (2×20 mL) and saturated aqueous NaCl (20 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resulting oil (0.43 g) was purified by column chromatography on silica gel (11 g) (eluent, hexane/EtOAc 1:1). The fractions containing the dimesylated derivative **2a** were concentrated to dryness to give a yellow oil (0.3 g, 64%). IR (film) ν: 3346, 2954, 1726 (CO), 1655, 1459, 1170 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.63–1.69 (m, 1H, 4-*H*H), 2.41–2.57 (m, 2H, 3-H+4-H*H*), 2.93 (s, 3H, SO_2CH_3), 2.95 (s, 3H, SO_2CH_3), 3.53–3.56 (m, 1H), 3.64–3.75 (m, 2H), 3.68 (s, 3H, CO_2CH_3), 3.74 and 4.0 (part A ABM system, J=13.6 Hz, 2H, $PhCH_2$), 4.07–4.31 (m, 4H, $2OCH_2$), 7.21–7.32 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 29.26 (CH_2), 37.57 (CH_3), 37.86 (CH_3), 40.64 (CH), 51.96 (CO_2CH_3), 52.98 (CH_2), 60.10 (CH), 65.56 (CH), 69.79 (CH_2), 71.25 (CH_2), 127.86 (CH), 127.98 (CH), 128.81 (CH), 128.92 (CH), 129.40 (CH), 138.88 (C), 173.23 (CO_2CH_3). MS (ESI-TOF, %): 358.14 (M−Ph, 100). HRMS m/z calcd for $C_{17}H_{25}NO_8S_2$, 435.1022; found, 435.1039.

4.1.2. Methyl (2R,3R*,5R*)-1-benzyl-3,5-bis[[(p-tolylsulfonyl)oxy]methyl]pyrrolidine-2-carboxylate (**2b**) and methyl (2R*,3R*,5R*)-1-benzyl-5-(hydroxymethyl)-3-[[(p-tolylsulfonyl)oxy]methyl]pyrrolidine-2-carboxylate (**2′b**).* TsCl (1.35 g, 7.10 mmol) was added to a cooled (ice bath) solution of **1** (1.24 g, 4.44 mmol) in dry CH_2Cl_2 (6 mL) and dry pyridine (1.2 mL). The mixture was stirred at room temperature under argon for 4 h and was then diluted with CH_2Cl_2 (40 mL). The organic solution was washed with saturated aqueous $NaHCO_3$ (2×20 mL), saturated aqueous NH_4Cl (2×20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting brown oil (1.85 g) was purified by column chromatography on silica gel (60 g) (eluent, hexane/EtOAc 1:3). Compound **2b** (0.56 g, 22%) was eluted first and the mono-tosylated compound **2′b** (0.17 g, 9%) was eluted second as a brownish oil after removal of the solvent.

Compound **2b**. Brownish oil. IR (film) ν: 3141, 2953, 1734 (CO), 1598, 1454, 1360, 1315, 1178, 1113, 1096, 964, 819, 667, 556 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.39–1.45 (m, 1H, 4-*H*H), 2.28–2.42 (m, 2H, 2-H+4-H*H*), 2.44 (s, 3H, $C_6H_4CH_3$), 2.46 (s, 3H, $C_6H_4CH_3$), 3.44–3.47 (m, 1H), 3.49–3.53 (m, 1H), 3.58–3.65 (m, 1H), 3.61 (s, 3H, CO_2CH_3), 3.66–3.69 (m, 1H), 3.77–3.90 (m, 3H), 4.03–4.09 (m, 1H), 7.05–7.08 (m, 2H), 7.22–7.25 (m, 3H), 7.30–7.37 (m, 4H), 7.70–7.79 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 22.06 (CH_3), 22.09 (CH_3), 29.71 (CH_2), 40.71 (CH), 51.83 (CO_2CH_3), 52.01 (CH_2), 59.83 (CH), 65.69 (CH), 70.90 (CH_2), 71.89 (CH_2), 127.73 (CH), 128.25 (CH), 128.54 (CH), 128.77 (CH), 130.34 (CH), 133.03 (C), 133.06 (C), 138.73 (C), 138.76 (C), 145.26 (C), 173.13 (CO_2CH_3). HRMS m/z calcd for $C_{29}H_{33}NO_8S_2$, 587.1648; found, 587.1665.

Compound **2′b**. Brownish oil. IR (film) ν: 3418, 2955, 2860, 1741, 1456, 1173, 1119, 679 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.52 (ddd, J=13.3, 5.8, 3.1 Hz, 1H, 4-*H*H), 2.21 (br s, 1H, D_2O exchange, OH), 2.26 (dd, J=9.2, 4.2 Hz, 1H, 4-H*H*), 2.32–2.43 (m, 1H), 2.40 (s, 3H, $C_6H_4CH_3$), 3.33–3.41 (m, 2H), 3.52 (dd, J=11.3, 3.3 Hz, 1H), 3.56–3.63 (m, 1H), 3.61 (s, 3H, CO_2CH_3), 3.66 and 3.85 (part A ABM system, J=13.9 Hz, 2H, $CH_2C_6H_5$), 3.92 (dd, J=9.6, 6.0 Hz, 1H), 3.99–4.05 (m, 1H), 7.14–7.17 (m, 2H), 7.19–7.30 (m, 5H), 7.70–7.73 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 22.02 (CH_3), 29.58 (CH_2), 40.43 (CH), 51.78 (CO_2CH_3), 52.84 (CH_2), 61.83 (CH_2), 62.83 (CH), 66.24 (CH), 72.16 (CH_2), 127.73 (CH), 128.26 (CH), 128.53 (CH), 128.96 (CH), 130.30 (CH), 133.27 (C), 139.19 (C), 145.29 (C), 173.42 (CO_2CH_3). MS (FAB m/z): 434.25 (M+1, 100). HRMS m/z calcd for $C_{22}H_{27}NO_6S$, 433.1559; found, 433.1578.

4.1.3. Methyl (2R,3S*,5R*)-3,5-bis(azidomethyl)-1-benzylpyrrolidine-2-carboxylate (**3a**) and methyl (2R*,3S*,5R*)-3-(azidomethyl)-1-benzyl-5-(hydroxymethyl)pyrrolidine-2-carboxylate (**3b**). Method A.* NaN_3 (0.22 g, 3.40 mmol) was added to a stirred solution of **2b** (0.2 g, 0.34 mmol) in dry DMF (11 mL) under argon at room temperature. The reaction mixture was heated at 90 °C for 14 h. The DMF was removed under reduced pressure and the residue was partitioned between H_2O (100 mL) and Et_2O (50 mL). The aqueous phase was extracted with EtOAc (3×25 mL) and the combined organic phases were washed with saturated aqueous NaCl (25 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to give an oily residue (0.11 g), which was purified by column chromatography on silica gel (25 g) (hexane/EtOAc 6:1). Compound **3a** (0.06 g, 54%) was isolated as a colourless solid after removal of the solvent, mp 75–78 °C. IR (KBr) ν: 3.444, 2947, 2874, 2097, 1733, 1436, 1255, 1164, 1131 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.55–1.62 (m, 1H, 4-*H*H), 2.28–2.44 (m, 2H, 2-H+4-H*H*), 3.24 and 3.40 (ABM system, J=2.8, 3.2, 12.8 Hz, 2H, $PhCH_2$), 3.35–3.41 (m, 1H), 3.43–3.51 (m, 2H), 3.53–3.59 (m, 1H), 3.66 (s, 3H, CO_2CH_3), 3.73 (d, J=13.6 Hz, 1H), 4.0 (d, J=13.6 Hz, 1H), 7.25–7.35 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 31.59 (CH_2), 41.28 (CH), 51.79 (CO_2CH_3), 52.87 (CH_2), 53.66 (CH_2), 55.53 (CH_2), 61.30 (CH), 66.50 (CH), 127.75 (CH), 128.69 (CH), 128.91 (CH), 138.94 (C), 173.70 (CO_2CH_3). MS (ESI-TOF m/z %): 330.17 (M+1, 100). Anal. Calcd for $C_{15}H_{19}N_7O_2$ (329.36): C 54.70, H 5.81, N 29.77; found C 55.01, H 5.72, N, 29.95.

Method B. A method similar to that described above was used but dimesylated derivative **2a** (1.13 g, 2.59 mmol) was used as the starting material. The standard procedure and subsequent chromatographic separation of the crude product (eluent hexane/EtOAc 8:1) gave compound **3a** (0.35 g, 41%) as the first eluted product. The physical and spectroscopic data for this compound are identical to those for the compound obtained using method A. Removal of the solvent from the second eluted compound gave **3b** as a yellowish oil (0.1 g, 13%). IR (film) ν: 3.419, 2929, 2100, 1733, 1452, 1205 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.64–1.69 (m, 1H, 4-*H*H), 2.11 (br s, 1H, D_2O exchange, OH), 2.30–2.41 (m, 2H, 2-H+4-H*H*), 3.35–3.38 (m, 2H), 3.42–3.46 (m, 1H), 3.50–3.55 (m, 1H), 3.56–3.59 (m, 1H), 3.66 (d, J=3.1 Hz, 1H), 3.69 (s, 3H, CO_2CH_3), 3.72 and 3.92 (AB system, J=13.7 Hz, 2H), 7.23–7.36 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 30.91 (CH_2), 41.05 (CH), 51.84 (CO_2CH_3), 52.63 (CH_2), 55.57 (CH_2), 61.37 (CH_2), 63.05 (CH), 66.97 (CH), 127.85 (CH), 128.59 (CH), 129.08 (CH), 138.19 (C), 174.06 (CO_2CH_3). MS (ESI-TOF m/z %): 305.16 (M+1, 100). HRMS m/z calcd for $C_{15}H_{20}N_4O_3$, 304.1535; found, 304.1552.

4.1.4. Methyl (2R,3S*,5R*)-1-benzyl-3,5-bis[(4-phenyltriazol)-1-ylmethyl]pyrrolidine-2-carboxylate (**4**).* Phenylacetylene (0.25 g, 2.44 mmol), CuI (0.09 g, 0.043 mmol) and DIPEA (0.26 g, 2.04 mmol) were added to a solution of **3a** (0.31 g, 1.02 mmol) in toluene (4.2 mL) and *tert*-butanol (1.2 mL) under argon at room temperature. The reaction mixture was stirred for 18 h at 70 °C. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (50 mL). The solution was washed with H_2O (2×25 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to give **4** (0.29 g, 54%) as a colourless solid, mp 140–142 °C. IR (KBr) ν: 3125, 1730, 1484, 1436, 1364, 1226, 1204, 1150, 1073, 765, 735, 710 cm^{-1}. ^1H NMR (acetone-d_6) δ: 1.84 (ddd, J=7.1, 5.1, 4.7 Hz, 4-*H*H), 2.40 (dt J=13.9, 9.2 Hz, 4-*H*H), 2.79–2.82 (m, 1H), 3.57 (s, 3H, CH_3), 3.81 (d, J=13.8 Hz, 1H), 3.94 (dd, J=10.1, 4.7 Hz, 1H), 4.13–4.31 (m, 3H), 4.64–4.78 (m, 2H), 7.24–7.37 (m, 5H), 7.39–7.46 (m, 6H), 7.80–7.78 (m, 2H), 7.88–7.92 (m, 2H), 8.15 and 8.38 (2s, 2H, 5-H pyrazole+5′H pyrazole). ^{13}C NMR (acetone-d_6) δ: 30.36 (CH), 41.55 (CH_2), 50.69 (CH_2), 52.13 (CH), 52.21 (CH), 52.88 (CH), 61.13 (CH_2), 65.62 (CH_2), 120.81 (CH), 120.87 (CH), 121.94 (CH), 125.30 (CH), 125.38 (CH), 127.18 (CH), 127.72 (CH), 127.76 (CH), 128.41 (CH), 128.47 (CH), 128.72 (CH), 128.78 (CH), 131.24 (C), 131.31 (C), 138.95 (C), 146.87 (C), 146.92 (C), 172.30 (CO_2CH_3). MS (EI m/z %): 534 (3), 533 (M, 7), 443 (20), 442 (69), 376 (11), 375 (44), 329 (15), 297 (13), 256 (13), 230 (9), 184 (8), 172 (15), 130 (11), 103 (10), 102 (8), 82 (9), 91 (100). Anal. Calcd for $C_{31}H_{31}N_7O_2$ (5533.62): C 69.77, H 5.86, N 18.37; found C 69.97, H 6.03, N, 18.59.

4.1.5. Methyl (2R,3R*,5R*)-1-benzyl-3,5-bis[[(tert-butyldimethylsilyl)oxy]methyl]pyrrolidine-2-carboxylate (**5**).* A mixture of **1** (2.45 g, 8.77 mmol), imidazole (2.63 g, 38.59 mmol) and TBDMSCl (4.23 g, 28.06 mmol) in dry CH_2Cl_2 (185 mL) was stirred under argon at room temperature for 2 h. The mixture was partitioned between CH_2Cl_2 (300 mL) and saturated aqueous $NaHCO_3$ (400 mL). The aqueous phase was extracted with CH_2Cl_2 (3×100 mL) and the combined organic extracts were washed with saturated aqueous NH_4Cl (3×125 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to give an oily residue (4.20 g), which was purified by column chromatography on silica gel (120 g) (eluent, hexane/EtOAc 10:1). The disilyl ether **5** was eluted first and was isolated as a colourless oil (3.55 g, 92%). IR (film) ν: 2932, 2858, 1736, 1463, 1361, 1255, 1101, 840 cm^{-1}. ^1H NMR ($CDCl_3$) δ: −0.02 (s, 3H, CH_3), 0.0 (s, 3H, CH_3), 0.2 (s, 3H, CH_3), 0.3 (s, 3H, CH_3), 0.84 (s, 9H, $(CH_3)_3$), 0.88 (s, 9H, $(CH_3)_3$), 1.38–1.43 (m, 1H, 4-*H*H), 2.20–2.26 (m, 2H, 2-H+4-H*H*), 3.41–3.51 (m, 3H), 3.56–3.60 (m, 3H), 3.63 (s, 3H, CO_2CH_3), 3.71 and 4.07 (AB system, J=14.2 Hz, 2H), 7.20–7.28 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: −5.0 (2 CH_3), −4.9 (2 CH_3), 20.91 (C), 26.24 [$(CH_3)_3$], 26.32 [$(CH_3)_3$], 30.21 (CH_2), 44.66 (CH), 51.38 (CO_2CH_3), 53.40 (CH_2), 63.07 (CH), 66.11 (CH_2), 66.40 (CH), 66.57 (CH_2), 127.07 (CH), 128.49 (CH), 129.68 (CH), 140.36 (C), 175.56 (CO_2CH_3). MS (EI m/z %): 448 (M−CO_2CH_3, 1), 363 (23), 362 (82), 91 (100), 89 (21), 73 (39). HRMS m/z calcd for $C_{27}H_{49}NO_4Si_2$, 507.3200; found, 507.3216.

4.1.6. Methyl (2R,3R*,5R*)-1-(tert-butoxycarbonyl)-3,5-bis[[(tert-butyldimethylsilyl)oxy]methyl]pyrrolidine-2-carboxylate (**6**).* A solution of **5** (3.95 g, 7.78 mmol) in dry EtOAc (100 mL) was added to a suspension of 20% $Pd(OH)_2/C$ (0.55 g, 3.89 mmol) and Boc_2O (2.21 g, 10.11 mmol) in dry EtOAc (40 mL). The reaction mixture was stirred at room temperature under a positive pressure of hydrogen for 18 h. The catalyst was filtered off and washed with EtOAc. The filtrate was washed with aqueous NaCl (5×25 mL). The organic extract was dried over Na_2SO_4 and the solvent was removed under reduced pressure to give an oily residue (4.20 g), which was purified by column chromatography on silica gel (100 g) (eluent, hexane/EtOAc 10:1). Compound **6** (3.10 g, 77%) was isolated as a colourless oil. IR (film) ν: 3356, 2953, 2860, 1750, 1707, 1467, 1370, 1254, 1177, 1100, 840 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.04 (s, 6H, 2 CH_3), 0.05 (s, 6H, 2 CH_3), 0.87 (s, 6H, 2 CH_3), 0.88 (s, 6H, 2 CH_3), 0.89 (s, 6H, 2 CH_3), 1.38 (s, 6H, 2 CH_3), 1.46 (s, 3H, CH_3), 1.83–1.93 (m, 1H, 4-*H*H), 2.11–2.19 (m, 1H), 2.29–2.33 (m, 1H), 3.54–3.59 (m, 1H), 3.63–3.67 (m, 1H), 3.71 (s, 3H, CO_2CH_3), 3.80–3.82 (m, 2H), 4.0–4.02 (m, 1H), 4.06 (d, 1H, J=4.7 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ: −4.97 (2 CH_3), −4.91 (CH_3), −4.87 (CH_3), 18.65 (2C), 26.23 ($3CH_3$), 26.31 ($3CH_3$), 28.63 ($3CH_3$), 28.83 (CH), 29.37 (CH_2), 45.62 (CH), 52.22 (CO_2CH_3), 60.0 (CH), 63.52 (CH_2), 64.97 (CH_2), 80.27 (C), 154.91 (C), 174.93 (CO_2CH_3). MS (EI m/z %): 444 (M−O-*t*-Bu, 2), 405 (11), 404 (37), 360 (31), 300 (25), 273 (20), 272 (100), 168 (16), 140 (23), 73 (60). HRMS m/z calcd for $C_{25}H_{51}NO_6Si_2$, 517.8465; found, 517.8482.

4.1.7. Methyl (2R,3R*,5R*)-1-(tert-butoxycarbonyl)-3,5-bis(hydroxymethyl)pyrrolidine-2-carboxylate (**7**). Method A*: A 1 M solution of TBAF in THF (12.11 mL, 12.11 mmol) was added to a cooled (ice bath) solution of **6** (2.85 g, 5.50 mmol) in dry THF (30 mL) under argon. The cooling bath was removed and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the resulting oily residue (5.2 g) was purified by column chromatography on silica gel (160 g) (eluent, CH_2Cl_2/MeOH 30:1). Compound **7** was eluted first and, after removal of the

solvent, was isolated as an oil that crystallised slowly (1.40 g, 88%), mp 88–92 °C. IR (KBr) ν: 3409, 2981, 1742, 1667, 1384, 1320, 1204, 1180, 1141, 1081, 1037, 785 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.46 [s, 9H, C(CH_3)$_3$], 1.48–1.53 (m, 1H, 4-H*H*), 1.82 (br s, 2H, D_2O exchange, 2OH), 2.15–2.25 (m, 1H, 4-*H*H), 2.29–2.40 (m, 1H, 3-H), 3.61–3.70 (m, 4H, 2×OCH_2), 3.74 (s, 3H, CH_3), 4.04–4.13 (m, 1H, 5-H), 4.19 (d, J=5.4 Hz, 1H, 2-H). ^{13}C NMR ($CDCl_3$) δ: 28.15 [(CH_3)$_3$], 30.57 (CH_2), 43.96 (CH), 52.17 (CH_3), 61.97 (CH), 63.86 (CH_2), 63.92 (CH), 66.68 (CH_2), 81.85 (C), 156.42 (NCO_2), 173.84 (CO_2CH_3). MS (EI m/z %): 258 (M−OCH_3, 7), 159 (8), 158 (100), 142 (8), 130 (17), 100 (9), 98 (11), 68 (17), 57 (42). HRMS m/z calcd for $C_{13}H_{23}NO_6$, 289.1525; found, 289.1534.

Method B. A solution of **1** (9.01 g, 32.26 mmol) in EtOAc (250 mL) was added to a suspension of 20% $Pd(OH)_2$/C (2.26 g, 16.13 mmol) and Boc_2O (9.15 g, 41.94 mmol) in dry EtOAc (30 mL). The reaction mixture was stirred at room temperature under a positive pressure of hydrogen for 114 h. The catalyst was filtered off and washed with EtOAc. The solvent was removed under reduced pressure to give an oily residue (10.5 g), which was purified by column chromatography on silica gel (240 g) (hexane/EtOAc 1:3). Compound **7** (7.84 g, 84%) was isolated as a colourless solid. The physical and spectroscopic data for **7** were identical to those for the product obtained using method A.

4.1.8. Methyl (2R,3R*,5R*)-3,5-bis[[(tert-butyldimethylsilyl)oxy]methyl]pyrrolidine-2-carboxylate (**8**).* A solution of **5** (150 mg, 0.296 mmol) in dry EtOAc (3 mL) was added to a suspension of 20% $Pd(OH)_2$/C (21 mg, 0.15 mmol) in dry EtOAc (3 mL). The reaction mixture was stirred at room temperature under a hydrogen atmosphere for 7 days. The catalyst was filtered off (Celite) and washed with EtOAc. The solvent was removed under reduced pressure to give an oily residue (125 mg), which was purified by column chromatography on silica gel (eluent, hexane/EtOAc 10:1). Compound **8** (52 mg, 42%) was isolated as a yellow oil. IR (film) ν: 2932, 2859, 1739, 1465, 1387, 1254, 1207, 1097, 777 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.05 (s, 12H, 4CH_3), 0.89 [s, 18H, 2C(CH_3)$_3$], 1.25–1.37 (m, 3H, one of them D_2O exchange, 4-H_2 and NH), 1.91–2.04 (m, 1H), 2.39–2.47 (m, 1H), 3.39–3.42 (m, 1H), 3.50–3.69 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: −2.37 (2 CH_3), −5.9 (2 CH_3), 22.79 (C), 25.42 [(CH_3)$_3$], 25.76 [(CH_3)$_3$], 30.21 (CH_2), 43.09 (CH), 51.65 (CO_2CH_3), 62.08 (CH), 66.51 (CH_2), 66.40 (CH), 67.57 (CH_2), 176.89 (CO_2CH_3). HRMS m/z calcd for $C_{20}H_{43}NO_4Si_2$, 417.2737; found, 417.2744.

4.1.9. Methyl (2R,3R*,5R*)-1-(tert-butoxycarbonyl)-3,5-bis[[(methylsulfonyl)oxy]methyl]pyrrolidine-2-carboxylate (**10a**).* Compound **10a** was prepared using the procedure described for **2a** but with diol **7** (0.40 g, 1.38 mmol) as the starting material. The standard work-up procedure and separation by column chromatography (silica gel, hexane/EtOAc 1:3) gave **10a** as a yellowish oil (0.59 g, 96%). IR (film) ν: 2977, 1746, 1699, 1355, 1174, 961, 829, 747 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.40 [s, 6H, C(CH_3)$_2$], 1.48 [s, 3H, C(CH_3)], 1.89 (dt, 1H, J=13.8, 4.6 Hz, 4-*H*H), 2.43 (dt, 1H, J=13.8, 8.9 Hz, 4-H*H*), 2.62–2.67 (m, 1H, 3-H), 3.02 (s, 3H, SO_2CH_3), 3.07 (s, 3H, SO_2CH_3), 3.75 (s, 3H, OCH_3), 4.19–4.38 (m, 5H, 2OCH_2+5-H), 4.70 (dd, J=10.1, 4.6 Hz, 1H, 2-H). ^{13}C NMR ($CDCl_3$) δ: 28.51 [(CH_3)$_3$], 28.91 (CH_2), 37.44 (SO_2CH_3), 37.85 (SO_2CH_3), 41.77 (CH), 52.83 (CO_2CH_3), 57.25 (CH), 63.45 (CH), 69.70 (CH_2), 70.04 (CH_2), 83.45 (C), 154.02 (NCO_2), 172.50 (CO_2CH_3). MS (EI m/z %): 389 ((M+1)−*t*-Bu, 1), 344 (M−Boc, 4), 286 ((M+1)−(Boc+CO_2CH_3), 63), 236 (79), 234 (19), 190 (74), 140 (15), 138 (23), 91 (100), 80 (34), 79 (72), 68 (15), 67 (31), 59 (23), 58 (95), 57 (99). HRMS m/z calcd for $C_{15}H_{27}NO_{10}S_2$, 445.1076; found, 445.1088.

4.1.10. Methyl (2R,3R*,5R*)-1-(tert-butoxycarbonyl)-5-(hydroxymethyl)-3-[[(methylsulfonyl)oxy]methyl]pyrrolidine-2-carboxylate (**10b**).* When a **7**/MsCl molar ratio of 1:2 was used compound **10b** was obtained as a minor product. The standard work-up procedure and separation by column chromatography (silica gel, hexane/EtOAc 1:3) gave **8a** in 72% yield (spectroscopic data were identical to those described previously) from the first fractions and **10b** in 25% yield from later fractions.

Compound **10b**. Colourless oil. IR (film) ν: 3433, 2976, 1744, 1691, 1361, 1174, 1087, 1048, 960, 839 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.41 (s, 9H, C(CH_3)$_3$), 1.48–1.60 (m, 2H, one of them D_2O exchange, 4-*H*H+OH), 2.28 (dt, 1H, J=13.2, 7.6 Hz, 4-H*H*), 2.56–2.59 (m, 1H), 3.07 (s, 3H, SO_2CH_3), 3.61–3.68 (m, 1H), 3.76 (s, 3H, CO_2CH_3), 4.09–4.15 (m, 2H), 4.25 (ddd, J=21.2, 10.2, 6.6 Hz, 2H), 5.38 (m, 1H, 2-H). ^{13}C NMR ($CDCl_3$) δ: 28.54 [(CH_3)$_3$], 30.83 (CH_2), 37.96 (SO_2CH_3), 41.79 (CH), 52.77 (CO_2CH_3), 62.18 (CH), 63.73 (CH), 66.53 (CH_2), 69.59 (CH_2), 82.13 (C), 156.09 (C), 173.22 (CO_2CH_3). MS (EI m/z %): 336 (M−OCH_3, 5), 237 (9), 236 (88), 208 (10), 140 (19), 126 (17), 112 (29), 91 (14), 82 (13), 80 (37), 79 (15), 68 (15), 57 (100). HRMS m/z calcd for $C_{14}H_{25}NO_8S$, 367.4152; found, 367.4166.

4.1.11. Methyl (2R,3S*,5R*)-3,5-bis(azidomethyl)-1-(tert-butoxycarbonyl)pyrrolidine-2-carboxylate (**11**).* NaN_3 (0.95 g, 14.6 mmol) was added to a stirred solution of **10a** (0.65 g, 1.46 mmol) in dry DMF (55 mL) under argon at room temperature and the mixture was heated at 90 °C for 1 h. The DMF was removed under reduced pressure and the residue was partitioned between H_2O (100 mL) and Et_2O (50 mL). The aqueous phase was extracted with EtOAc (3×25 mL) and the combined organic phases were washed with saturated aqueous NaCl (2×25 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to give an oily residue (0.39 g), which was purified by column chromatography on silica gel (15 g) (eluent, hexane/EtOAc 3:1). Compound **11** (0.35 g, 71%) was obtained as a yellow solid, mp 45–48 °C. IR (KBr) ν: 2977, 2103, 1748, 1703, 1448, 1366, 1262, 1204, 1174, 1138 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.40 [s, 6H, C(CH_3)$_2$], 1.49 [s, 3H, C(CH_3)], 1.72–1.79 (m, 1H, 4-*H*H), 2.24–2.31 (m, 1H), 2.35–2.42 (m, 1H), 3.43–3.56 (m, 4H), 3.76 (s, 3H, CH_3), 3.98 (dd, J=12.4, 5.5 Hz, 1H), 4.08–4.12 (m, 1H). ^{13}C NMR ($CDCl_3$) δ: 28.56 [(CH_3)$_3$], 31.42 (CH_2), 42.20 (CH), 52.69 (CH_2), 52.79 (CH_3), 53.92 (CH_2), 58.0 (CH), 64.38 (CH), 82.35 (C), 155.21 (NCO_2), 173.50 (CO_2CH_3). MS (EI m/z %): 283 [(M+1)−*t*-Bu, 8], 183 (68), 126 (12), 80 (12), 68 (21), 59 (11), 57 (100). Anal. Calcd for $C_{13}H_{21}N_7O_4$ (339.3503): C 46.01, H 6.24, N 28.89; found C 46.33, H 6.19, N, 29.07.

4.2. General procedure for the synthesis of the bitriazolyl derivatives 12 through a copper(I)-catalyzed Huisgen reaction

Aryl alkyne (200 mg, 2.05 mmol), CuI (0.043 mmol) and DIPEA (1.75 mmol) were added to the diazide **11** (0.85 mmol) solution in toluene (3.5 mL) and *tert*-butanol (1 mL) under argon. The reaction mixture was stirred at room temperature or reflux for 15–72 h. The solvents were removed under reduced pressure to afford a solid residue, which was purified by recrystallization or on column chromatography on silica gel.

4.2.1. Methyl (2R,3S*,5R*)-1-(tert-butoxycarbonyl)-3,5-bis-[(4-phenyl-1H-1,2,3-triazol-1-yl)methyl]pyrrolidine-2-carboxylate (**12a**).* Aryl alkyne: ethynylbenzene, room temperature 15 h, white solid, yield 87%, mp 238–242 °C (recrystallized from EtOAc). IR (KBr) ν: 3084, 2978, 1746, 1711, 1354, 1305, 1202, 1177, 767, 696 cm^{-1}. ^1H NMR (TFA-d_1) δ: 1.48 [s, 9H, C(CH_3)$_3$], 2.34–2.44 (m, 1H, 4-H*H*), 2.80–2.90 (m, 1H, 4-*H*H), 3.79 (s, 3H, CH_3), 4.78–4.87 (m, 2H), 5.04–5.36 (m, 5H), 7.46–7.67 (m, 10H, 2phenyl), 8.64 (s, 2H, 5-H pyrazole+5′H pyrazole). ^{13}C NMR (TFA-d_1) δ: 25.66 [(CH_3)$_3$], 32.90 (CH_2), 40.90 (CH), 52.29 (CH_2), 54.03 (CH_2), 54.44 (CH_3), 59.70 (CH), 62.07 (CH), 90.10 (C), 120.17 (C), 120.04 (C), 125.41 (CH), 125.66 (CH), 126.12 (CH), 129.60 (CH), 132.72 (CH), 144.64 (C), 144.98 (C), 160,15 (NCO_2), 167.52 (CO_2CH_3). MS (EI m/z %): 544 (M+1, 100), 516

(25), 489 (29), 488 (81), 444 (31), 416 (14), 299 (21), 186 (16), 174 (46), 160 (15), 146 (15), 132 (24), 130 (17), 120 (19), 119 (15), 118 (68), 105 (23), 104 (63), 103 (46), 93 (20), 91 (40). Anal. Calcd for $C_{29}H_{33}N_7O_4$ (543.62): C 64.07, H 11.6, N 18.04; found C 64.31, H 11.54, N, 18.26.

4.2.2. Methyl (2R,3S*,5R*)-1-(tert-butoxycarbonyl)-3,5-bis-[[4-(4-hidroxymethyl)phenyl]-1H-1,2,3-triazol-1-ylmethyl]pyrrolidine-2-carboxylate (**12b**).* Aryl alkyne: (4-ethynylphenyl)methanol, reflux 17 h, white solid, yield 60%, mp 227–229 °C (recrystallized from EtOAc/EtOH). IR (KBr) *ν*: 3327, 2941, 1744, 1704, 1401, 1350, 1176, 1141, 1044, 1009, 978, 802 cm^{-1}. ^1H NMR (TFA-d_1) *δ*: 1.73 [s, 9H, C$(CH_3)_3$], 2.65–2.69 (m, 1H, 4-H*H*), 3.09–3.11 (m, 1H, 4-*H*H), 3.76–3.88 (m, 1H), 4.07 (s, 3H, CH_3), 4.99–5.10 (m, 3H), 5.29–5.35 (m, 2H), 5.42–5.49 (m, 2H), 5.52–5.66 (m, 3H), 7.75–7.83 (m, 4H, 2-H+6-H phenyl+2′-H+6′-H phenyl), 7.86–7.98 (m, 4H, 3-H+5-H phenyl+3′-H+5′-H phenyl), 8.93 and 8.95 (2s, 2H, 5-H pyrazole+5′-H pyrazole). ^{13}C NMR (TFA-d_1) *δ*: 25.34 (CH_2), 25.61 [$CH_3C(CH_3)_2$], 25.87 [2 $CH_3C(CH_3)_2$], 33.17 (CH_2), 41.11 (CH), 52.55 (CH_2), 54.32 (CH_3), 54.69 (CH_2), 59.89 (CH), 62.28 (CH), 63.97 (CH_2), 68.51 (C), 108.73 (CH), 112.47 (CH), 119.99 (CH), 121.49 (CH), 126.94 (CH), 127.13 (CH), 128.52 (CH), 128.58 (CH), 129.41 (CH), 129.48 (CH), 138.47 (2C), 144.84 (C), 144.25 (C), 160.15 (NCO_2), 167.73 (CO_2CH_3). MS (EI *m*/*z* %): 604 (M+1, 100), 604 (52), 309 (18), 278 (18), 263 (14), 231 (72), 197 (12), 156 (10), 155 (34), 154 (98), 139 (14), 137 (100), 19 (24), 105 (12). Anal. Calcd for $C_{31}H_{37}N_7O_6$ (603.67): C 61.68, H 6.18, N 16.24; found C 61.97, H 6.31, N, 16.53.

4.2.3. Methyl (2R,3S*,5R*)-1-(tert-butoxycarbonyl)-3,5-bis-[[4-(2-nitromethyl)phenyl]-1H-1,2,3-triazol-1-ylmethyl]pyrrolidine-2-carboxylate (**12c**).* Aryl alkyne: 1-ethynyl-2-nitrobenzene, reflux 17 h, yellow-white solid, yield 57%, mp 118–119 °C (purified by column chromatography on silica gel, eluent hexane/EtOAc 1/1). IR (KBr) *ν*: 3108, 1736, 1700, 1528, 1348, 1217, 1176, 1143, 854, 751 cm^{-1}. ^1H NMR ($CDCl_3$) *δ*: 1.46 [s, 9H, C$(CH_3)_3$], 1.91 (dt, *J*=14.0, 5.5 Hz, 1H, 4-H*H*), 2.42 (dt, *J*=14.1, 9.0 Hz, 1H, 4-*H*H), 2.88–2.94 (m, 1H, 3-H), 3.68 (s, 3H, CH_3), 3.71–3.76 (m, 1H, 5-H), 4.0 (dd, *J*=13.8, 7.7 Hz, 1H), 4.05–4.10 (m, 1H), 4.41–4.47 (m, 1H), 4.73 (d, *J*=13.8 Hz, 1H), 5.21 (dd, *J*=14.0, 4.9 Hz, 1H), 7.46–7.54 (m, $2H_{aromatic}$), 7.62–7.70 (m, $3H_{aromatic}$), 7.80–7.86 (m, $2H_{aromatic}$), 7.93–7.98 (m, $3H_{aromatic}$). ^{13}C NMR ($CDCl_3$) *δ*: 28.07 ($C(CH_3)_3$), 30.09 (CH_2), 41.73 (CH), 51.13 (CH_2), 52.23 (CH_2), 52.45 (CH_3), 57.92 (CH), 64.03 (CH), 81.88 (C), 124.04 (CH), 124.14 (CH), 124.27 (CH), 124.65 (CH), 128.95 (CH), 129.24 (CH), 131.16 (CH), 131.21 (CH), 132.44 (CH), 132.67 (CH), 142.70 (2C), 148.36 (C), 148.43 (C), 154.65 (NCO_2), 172.07 (CO_2CH_3). MS (FAB) *m*/*z* (%): 634.20 (M+1, 100%). Anal. Calcd for $C_{29}H_{31}N_9O_8$ (633.61): C 54.97, H 4.93, N 19.90; found C 55.32, H 5.08, N, 19.65.

4.3. Cleavage of the *tert*-butyldiphenylsilyl group from compounds 7, 11 and 12a. General procedure

CF_3CO_2H (1.54 mL; 20.00 mmol) was added to a stirred suspension of the compound to be deprotected (1 mmol) in CH_2Cl_2 cooled (ice bath). The reaction mixture was stirred at room temperature for 2–6 h, after which the solvent was removed under reduced pressure to give a solid residue, that was dissolved in NaOH 1 N (15 mL) and extracted with EtOAc (3×20 mL). The organic phase was concentrated to dryness and purified by column chromatography or washed with AcOEt/Et_2O.

4.3.1. Methyl (2R,3R*,5R*)-3,5-bis(hydroxymethyl)pyrrolidine-2-carboxylate (**9**).* Compound to be deprotected, **7**, reaction time 6 h, the brown residue was purified by column chromatography on silica gel (eluent CH_2Cl_2/MeOH 20/1) to give **9** as a yellow oil (76%). IR (KBr) *ν*: 3350, 2969, 2493, 1742, 1669, 1439, 1373, 1200, 1180, 1129, 1057, 837, 799, 722 cm^{-1}. ^1H NMR (pyridine-d_5) *δ*: 2.41 (dt, *J*=12.6, 9.1 Hz, 1H, 4-H*H*), 2.63 (dt, *J*=12.6, 7.3 Hz, 1H 4-*H*H), 3.21–3.29 (m, 1H, 3-H), 3.96 (s, 3H, CH_3), 4.33–4.50 (m, 5H, H-5+2CH_2OH), 5.09 (dd, *J*=6.7, 2.6 Hz, 1H, H-2), 6.45 (br s, 3H, D_2O exchange, NH+2OH). ^{13}C NMR (pyridine-d_5) *δ*: 30.43 (CH_2), 46.44 (CH), 52.79 (CH_3), 61.21 (CH), 62.22 ($2CH_2$), 62.33 (CH), 171.84 (CO). MS (EI *m*/*z* %): 189 (M, 11), 159 (35), 158 (60), 131 (14), 130 (100), 126 (10), 118 (10), 112 (21), 108 (11), 100 (70), 99 (11), 95 (11), 94 (12), 82 (49), 82 (14), 81 (69), 71 (18), 70 (93), 69 (84), 68 (30), 58 (18), 57 (34), 56 (43), 55 (18), 54 (17), 52 (41). HRMS *m*/*z* calcd for $C_8H_{15}NO_4$, 189.1001; found, 189.1013.

4.3.2. Methyl (2R,3S*,5R*)-3,5-bis[(4-phenyl-1H-1,2,3-triazol-1-yl)methyl]pyrrolidine-2-carboxylate (**13a**).* Compound to be deprotected, **12a**, reaction time 3 h, the brown oily residue was treated with a mixture of EtOAc/Et_2O to give **13a** as a colourless solid (88%), mp 159–161 °C. IR (KBr) *ν*: 3411, 2953, 1751, 1675, 1658, 1439, 1233, 1200, 1143, 770, 762, 698 cm^{-1}. ^1H NMR (acetone-d_6) *δ*: 1.15 (dt, *J*=26.3, 7.04 Hz, 1H, 4-H*H*), 2.45–2.53 (m, 1H, 4-*H*H), 3.30–3.41 (m, 2H, one of them D_2O exchange NH), 3.70 (s, 3H, CH_3), 4.46–4.48 (m, 1H), 4.68–4.70 (m, 1H), 4.76–4.83 (m, 1H), 4.89–5.04 (m, 3H), 7.31–7.35 (m, 2H, 4-H C_6H_5+4′-H C_6H_5), 7.41–7.46 (m, 4H, 3-H+5-H C_6H_5 and 3′-H+5′-H C_6H_5), 7.86–7.91 (m, 4H, 2-H+6-H C_6H_5 and 2′-H+6′-H C_6H_5), 8.46 and 8.48 (2s, 2H, 5-H pyrazole+5′-H pyrazole). ^{13}C NMR (acetone-d_6) *δ*: 32.45 (CH_2), 41.68 (CH), 49.61 (CH_2), 50.84 (CH_2), 53.23 (CH_3), 58.57 (CH), 58.55 (CH), 122.1 (CH), 122.38 (CH), 125.22 (CH), 127.99 (CH), 128.072 (CH), 128.96 (CH), 128.99 (CH), 130.51 (C), 130.64 (C), 146.46 (C), 146.48 (C), 168.36 (CO). MS (FAB) *m*/*z* (%): 443.24 (M, 40%). Anal. Calcd for $C_{24}H_{25}N_7O_2$ (443.50): C 65.0, H 5.68, N 22.11; found C 64.91, H 5.89, N, 22.34.

4.3.3. Methyl (2R,3S*,5R*)-3,5-bis(azidomethyl)pyrrolidine-2-carboxylate (**14**).* Compound to be deprotected, **11**, reaction time 2 h, the brown oily residue was treated with a mixture of EtOAc/Et_2O to give **14** as a colourless solid (80%), mp 104–106 °C. IR (KBr) *ν*: 2964, 2103, 1682, 1446, 1361, 1261, 1191, 1135, 1044, 834, 796, 723 cm^{-1}. ^1H NMR (DMSO-d_6) *δ*: 1.48–1.52 (m, 1H, 4-H*H*), 2.22–2.28 (m, 1H, 4-*H*H), 2.63–2.69 (m, 1H), 3.53–3.66 (m, 4H), 3.72–3.76 (m, 1H), 3.77 (s, 3H, CH_3), 4.01 (d, *J*=8.3 Hz, 1H, 2-H). ^{13}C NMR (DMSO-d_6) *δ*: 31.24 (CH_2), 41.15 (CH), 50.67 (CH_2), 51.38 (CH_2), 53.25 (CH_3), 58.0 (CH), 60.17 (CH), 168.62 (CO). MS (EI *m*/*z* %): 240 (M+1, 1), 184 (11), 183 (100), 180 (38), 126 (33), 106 (9), 95 (17), 94 (12), 82 (16), 80 (25), 69 (35), 68 (19), 59 (12), 56 (8), 55 (7), 54 (7), 51 (11). Anal. Calcd for $C_8H_{13}N_7O_2$ (239.23): C 40.16, H 5.48, N 40.98; found C 40.41, H 5.74, N, 41.14.

4.3.4. Methyl (2R,3S*,5R*)-1-(tert-butoxycarbonyl)-3,5-bis(cyanomethyl)pyrrolidine-2-carboxylate (**15**).* NaCN (0.26 g, 5.38 mmol) and 18-crown-6 (0.03 g, 0.11 mmol) were added to a stirred solution of **10a** (0.30 g, 0.67 mmol) in dry CH_3CN (3 mL) at room temperature under argon. The mixture was heated at 60 °C for 23 h and allowed to cool down to room temperature. Et_2O (25 mL) was added and the resulting precipitate was filtered off and washed with Et_2O and EtOAc. The solvent was removed from the filtrate under reduced pressure to give an oily residue (0.22 g), which was purified by column chromatography on silica gel (7 g) (eluent, hexane/EtOAc 2:1). Compound **15** (0.126 g, 61%) was isolated as a colourless solid, mp 100–102 °C. IR (KBr) *ν*: 2876, 2372, 2251, 1757, 1695, 1401, 1352, 1208, 1138 cm^{-1}. ^1H NMR ($CDCl_3$) *δ*: 1.37 [s, 6H, C$(CH_3)_2$], 1.46 [s, 3H, C(CH_3)], 1.76–1.80 (m, 1H, 4-*H*H), 2.52–2.68 (m, 4H), 2.86–2.93 (m, 1H), 3.16–3.24 (m, 1H), 3.74 (s, 3H, CO_2CH_3), 4.06–4.14 (m, 2H, 2-H+5-H). ^{13}C NMR ($CDCl_3$) *δ*: 21.73 (CH_2), 22.94 (CH_2), 28.43 [$(CH_3)_3$], 35.76 (CH_2), 38.68 (CH), 52.94 (CO_2CH_3), 54.89 (CH), 65.67 (CH), 82.14 (C), 117.78 and 117.55 (2C≡N), 153.93 (NCO_2), 172.08 (CO_2CH_3). MS (EI *m*/*z* %): 251 [(M+1−*t*-Bu), 1], 206 (19), 167 (13), 149 (10), 148 (100), 59 (9), 57

(63). Anal. Calcd for $C_{15}H_{21}N_3O_4$ (307.34): C 58.62, H 6.89, N 13.67; found C 58.94, H 6.76, N, 13.83.

Single crystals suitable for X-ray diffractometry were obtained by dissolving crystals of **15** in the least possible quantity of cold acetone in an open vial that was then placed in a larger container with a little pentane in its bottom; the container was closed, and after a few days in a cool, dark place free from vibrations afforded the desired single crystals.

4.4. X-ray crystal structure determination of 15 (see Fig. 3)

Single crystals of compound **15** suitable for X-ray diffractometry were obtained by iterative recrystallization of the isolated product using acetone/pentane. The desired single crystals were mounted in an inert oil and transferred to the cold gas stream of the diffractometer. Empirical formula: $C_{15}H_{21}N_3O_4$; formula weight: 307.34; crystal size: $0.49\times0.36\times0.3$ mm^3; crystal colour: colourless; habit: prismatic; crystal system: monoclinic; lattice type: plate; lattice parameters: a=20.7171 (8) Å, b=8.8276 (3) Å, c=18.6784 (5) Å, β=111.073 (2)°, T=100 K, V=3187.50 (18) Å^3; space group: $C2/c$; Z=8; D calcd=1.312 Mg m^{-3}; λ=0.7107 Å. Diffractometer: Smart-1000 BRUKER APPEX-II CCD.

4.4.1. Methyl (2R,3R*,5R*)-1-(tert-butoxycarbonyl)-3,5-bis(methyloxymethyl)pyrrolidine-2-carboxylate (**16a**) and methyl (2R*,3R*,5R*)-1-(tert-butoxycarbonyl)-5-(hydroxymethyl)-3-(methyloxymethyl) pyrrolidine-2-carboxylate (**16b**).* Ag_2O (0.24 g, 1.04 mmol) was added to a solution of **7** (0.10 g, 0.34 mmol) and CH_3I (1 mL) in CH_3CN (6 mL). The mixture was stirred and heated at 40 °C under argon for 60 h and allowed to cool down to room temperature. The solid was filtered off and washed with CH_3CN. The solvent was removed from the filtrate under reduced pressure to give a yellow oil (0.1 g), which was purified by column chromatography on silica gel (7 g) (eluent, hexane/EtOAc 1:0.25). Compound **16b** (0.05 g, 34%) was eluted first followed by **16a** (0.04 g, 36%).

Compound **16a**. Yellow oil. IR (film) ν: 2977, 2931, 1749, 1705, 1446, 1367, 1258, 1199, 1177, 1139 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.47 [s, 3H, C*H*$_3$C(CH$_3$)$_2$], 1.47 [s, 6H, CH$_3$C(C*H*$_3$)$_2$], 1.80 (t, 1H, J=13.3, 4.1 Hz, 4-*H*H), 2.26 (dd, J=8.9, 13.3 Hz, 1H, 4-H*H*), 2.40–2.48 (m, 1H, 5-H), 3.33 (s, 2H, OCH$_3$), 3.37 (s, 2H, OCH$_3$), 3.38–3.51 (m, 2H), 3.73 (s, 3H, CO_2CH_3), 3.67–3.80 (m, 2H), 4.06–4.09 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 28.18 [(CH$_3$)$_3$], 29.24 (CH$_2$), 42.49 (CH), 51.96 (CO_2*C*H$_3$), 57.64 (CH), 58.74 (OCH$_3$), 58.91 (OCH$_3$), 63.55 (CH), 73.26 (CH$_2$), 74.24 (CH$_2$), 89.41 (C), 154.30 (CON), 173.53 (CO). MS (EI m/z %): 172 [(M+1)–(O-*t*-Bu+CO_2CH_3), 63], 158 (13), 144 (14), 80 (20), 59 (7), 58 (7), 57 (100). MS (EI m/z %): 172 [(M+1)–(O-*t*-Bu+2OCH$_3$), 12], 216(18), 202 (16), 173 (9), 172 (100), 80 (11), 57 (48), 45 (17), 41 (11). HRMS m/z calcd for $C_{15}H_{27}NO_6$, 317.1838; found, 317.1845.

Compound **16b**. Colourless oil. IR (film) ν: 3424, 2977, 2952, 2932, 1747, 1701, 1392, 1367, 1176, 1138 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.40 [s, 9H, C(CH$_3$)$_3$], 1.43–1.50 (m, 1H, 4-*H*H), 2.20 (dt, J=12.9, 7.7 Hz, 1H, 4-H*H*), 2.34–2.40 (m, 1H, 5-H), 3.30–3.33 (m, 1H), 3.35 (s, 3H, OCH$_3$), 3.38–3.44 (m, 1H), 3.64–3.72 (m, 2H), 3.73 (s, 3H, CO_2CH_3), 4.08–4.14 (m, 2H), 5.08 (br s, 1H, D_2O exchange, OH). ^{13}C NMR ($CDCl_3$) δ: 28.14 [(CH$_3$)$_3$], 30.89 (CH$_2$), 41.81 (CH), 52.08 (CO_2*C*H$_3$), 58.94 (CH), 62.08 (OCH$_3$), 63.77 (CH), 67.19 (CH$_2$), 73.37 (CH$_2$), 81.43 (C), 156.23 (CON), 173.18 (CO). MS (EI m/z %): 172 [(M+1)–(Boc+OCH$_3$), 63], 158 (13), 144 (14), 80 (20), 59 (7), 58 (7), 57 (100). HRMS m/z calcd for $C_{14}H_{25}NO_6$, 303.1682; found, 303.1695.

4.4.2. Methyl (2R,3R*,5R*)-1-(tert-butoxycarbonyl)-5-(hydroxymethyl)-3-(ethyloxymethyl)pyrrolidine-2-carboxylate (**17b**).* It was obtained from **7** in the same way as **16**, with CH_3CH_2I and purified by column chromatography on silica gel (eluent, hexane/EtOAc 1:1). Colourless oil. IR (film) ν: 3419, 2976, 2871, 1744, 1698, 1392, 1365, 1255, 1169, 1136 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.18 (dt, J=1.2, 7.1 Hz, 3H, CH$_3$), 1.39 [s, 9H, C(CH$_3$)$_3$], 1.43–1.50 (m, 1H, 4-*H*H), 1.86 (br s, 1H), 2.17–2.24 (m, 1H, 4-H*H*), 2.35–2.41 (m, 1H, 5-H), 3.32–3.38 (m, 1H), 3.42–3.51 (m, 2H, C*H*$_2$CH$_3$), 3.64–3.67 (m, 2H), 3.71 (s, 3H, CO_2CH_3), 4.07–4.12 (m, 2H), 5.09 (m, 1H, D_2O exchange, OH). ^{13}C NMR ($CDCl_3$) δ: 28.14 [(CH$_3$)$_3$], 30.13 (CH$_2$), 41.99 (CH), 52.02 (CH$_2$), 52.20 (CO_2CH_3), 62.17 (OCH$_3$), 63.92 (CH), 66.54 (CH$_2$), 67.15 (CH$_2$), 71.21 (CH$_2$), 81.39 (C), 156.17 (CON), 173.21 (CO). MS (EI m/z %): 318 [(M+1), 10], 263 (14), 262 (100), 218 (54), 186 (18), 80 (14), 68 (18), 59 (23), 57 (65). HRMS m/z calcd for $C_{15}H_{27}NO_6$, 317.1838; found, 317.1854.

4.4.3. Methyl (2R,3R*,5R*)-1-(tert-butoxycarbonyl)-5-[[(3,5-dinitrobenzoyl)oxy]methyl]-3-(methoxymethyl)pyrrolidine-2-carboxylate (**18**).* A solution of **16b** (160 mg, 0.53 mmol) in dry THF (9 mL) was added to a mixture of 3,5-dinitrobenzoyl chloride (182 mg, 0.79 mmol), DMAP (4.0 mg) and Et_3N (0.08 mL). The reaction mixture was stirred at 70 °C for 45 h under argon, and allowed to cool down to room temperature. The THF removed under reduced pressure and the residue was partitioned between $NaHCO_3$ (50 mL) and EtOAc (30 mL). The aqueous phase was extracted with EtOAc (3×30 mL) and the combined organic phases were washed with saturated aqueous NaCl (2×30 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to give an oily residue (0.18 g), which was purified by column chromatography on silica gel (15 g) (eluent, hexane/EtOAc 6:1). Compound **18** (0.12 g, 46%) was isolated as an oil. IR (film) ν: 2977, 1743, 1694, 1438, 1393, 1352, 1168, 1141, 955, 830 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.34 [s, 6H, CH$_3$C(C*H*$_3$)$_2$], 1.47 [s, 3H, C*H*$_3$C(CH$_3$)$_2$], 1.74–1.83 (m, 1H, 4-*H*H), 2.32–2.42 (m, 1H, 4-H*H*), 2.44–2.51 (m, 1H, 5-H), 3.34 (s, 3H, OCH$_3$), 3.38–3.54 (m, 2H), 3.73 (s, 3H, CO_2CH_3), 4.18–4.24 (m, 1H), 4.43–4.56 (m, 2H), 4.63–4.71 (m,1H), 9.13–9.21 (m, 3H). ^{13}C NMR ($CDCl_3$) δ: 28.07 [(CH$_3$)$_3$], 29.08 (CH$_2$), 42.51 (CH), 52.16 (CO_2*C*H$_3$), 56.37 (CH), 58.89 (CH), 63.51 (CH), 67.79 (CH$_2$), 73.76 (CH$_2$), 80.88 (C), 122.32 (CH), 122.52(CH), 129.53 (CH), 133.94 (C), 148.61(C), 154.41 (CON), 162.39 (CO), 173.18 (CO). HRMS m/z calcd for $C_{21}H_{27}N_3O_{11}$, 497.1646; found, 497.1661.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.06.053.

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