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PAPER

Photorelease of tyrosine from α-carboxy-6-nitroveratryl (αCNV) derivatives†‡

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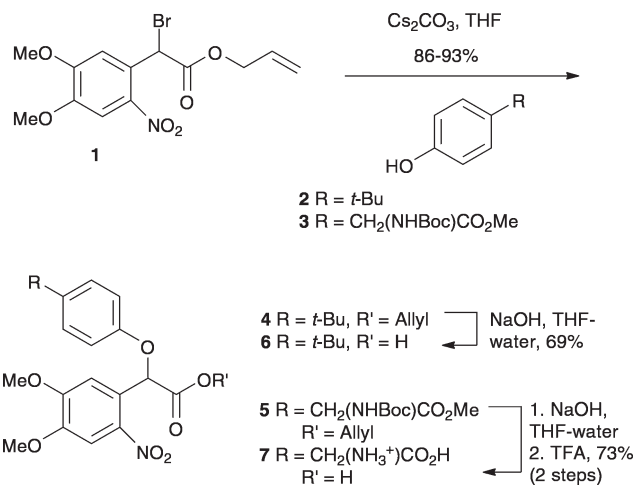
The synthesis of photolabile tyrosine derivatives protected on the phenolic oxygen by the α-carboxy-6-nitroveratryl (αCNV) protecting group is described. The compounds undergo rapid photolysis at wavelengths longer than 300 nm to liberate the corresponding phenol in excellent yield (quantum yield for the deprotection of tyrosine = 0.19). Further protection of caged tyrosine is possible, yielding *N*-Fmoc protected derivatives suitable for direct incorporation of caged tyrosine in solid-phase peptide synthesis.

Photolabile protecting groups have found application in a number of different areas, such as the synthesis of complex organic molecules,[1] the preparation of spatially-addressable arrays of macromolecules[2] and microlithography.[3] In particular, the temporal and spatial resolution offered by the photorelease of biomolecules has led to a very fertile area of research in which biomolecules rapidly released from a 'caged' form are used to initiate a biological reaction that may be studied by fast X-ray diffraction,[4] infrared spectroscopy,[5] voltage clamp, patch clamp or other physiological recording techniques.[6] Photolabile protecting groups have been available for some time for carboxylic acids, phosphates and metal ions,[7] while more recently attention has turned to the photoliberation of phenols.[8–13] This area is of considerable interest, as it includes several families of biologically-active phenols including steroids, catecholamines[14] such as epinephrine and dopamine, as well as tyrosine and a host of tyrosine-containing peptides.[15] For the latter, the development of caged tyrosine derivatives compatible with automated peptide synthesis that can be photo-deprotected in high yields and with rapid kinetics using long-wavelength irradiation remains a challenge.

Derivatives of the 2-nitrobenzyl group have been applied to the caging of phenols. The 6-nitroveratryl protecting group may be removed by photolysis at wavelengths up to 420 nm, but the timescale for phenol release is only 220 s^{-1}.[14a] By contrast, the α-carboxy-2-nitrobenzyl (αCNB) protecting group shows good photolysis kinetics of 2000 s^{-1},[14a] but its lower excitation wavelength can lead to protein damage in biological samples.

The α-carboxy-6-nitroveratryl (αCNV) protecting group has good kinetics for cleavage by light at wavelengths >350 nm and it has been applied to the caging of amines,[16] thiols[17] and carboxylic acids.[18] It has also been used to cage the phenol capsaicin for use in physiological experiments, although the kinetics for capsaicin release have not been reported.[19] Given the potential importance of caged tyrosine and tyrosine-containing peptides, we set out to determine the applicability of αCNV to the protection of tyrosine. For comparison, and to allow us to carry out photolysis experiments in aqueous and organic media, we studied the photoprotection of 4-*tert*-butylphenol in parallel.

To introduce the protecting group we used the previously prepared bromide **1** (Scheme 1).[18] The allyl ester has the advantage that it may be removed by saponification or under the mild conditions of palladium(0) catalysis. Alkylation of 4-*tert*-butylphenol **2** and *N*-(*tert*-butoxycarbonyl)tyrosine methyl ester **3** by **1** in



Scheme 1 Synthesis of monoacids **6** and **7**.

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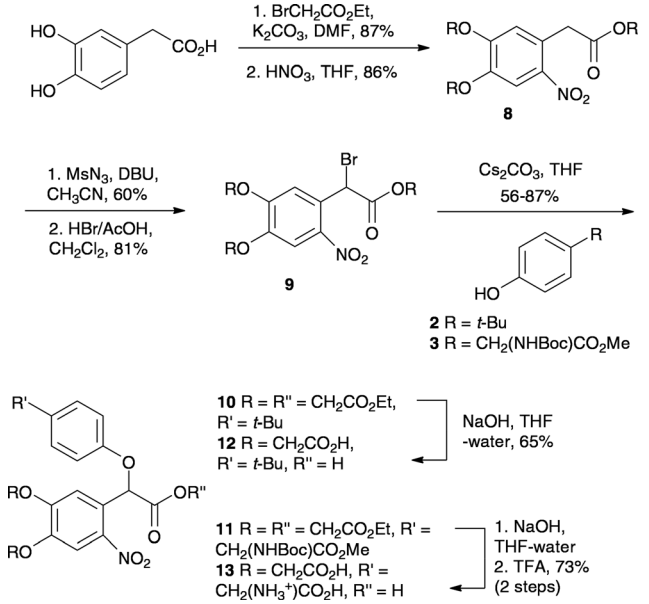
‡ Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra for all compounds, details of photolysis kinetics and quantum yield determinations. See DOI: 10.1039/c2pp05320a

THF with cesium carbonate as base proceeded smoothly, affording **4** and **5** in 93% and 86% yields, respectively, the latter as a 1 : 1 mixture of diastereoisomers. Ester saponification and, in the case of **5**, TFA treatment to remove the *N*-*tert*-butoxycarbonyl group, gave good yields of the two target compounds **6** and **7**.

The triacid derivatives **12** and **13** (Scheme 2) were also prepared by a similar route. Trialkylation of 3,4-dihydroxyphenylacetic acid by ethylbromoacetate, followed by nitration, gave **8** in 75% yield. Diazotransfer followed by treatment with HBr in acetic acid proceeded in good yield to give **9**, which smoothly reacted with **2** and **3** in the presence of cesium carbonate to provide **10** and **11** in 87% and 56% yields, respectively. Saponification of **10** gave **12**, while saponification and further TFA treatment in the case of **11** afforded the tyrosine derivative **13**.



Scheme 2 Synthesis of triacids **12** and **13**.

Photolysis of esters **4**, **5**, **10** and **11** (in acetone) and acids **6**, **7**, **12** and **13** (in D_2O or in acetone) was performed with the sample in a 5 mm NMR tube using a Pyrex-filtered 400 W medium-pressure mercury lamp, with monitoring by ^{1}H NMR or HPLC (Table 1). The tyrosine derivatives exhibited good aqueous solubility at pH 7.4, and in all cases the compounds were comfortably soluble at the 30 mM concentration used for the photolysis experiments. While the limits of solubility were not determined, triacid **13** did not appear to show a significant solubility advantage over **7**, although the additional carboxylates may engender improved solubility to peptides incorporating a caged tyrosine residue. The photolyses proceeded smoothly, with typically 50% product release within 20–30 min, and a plateau of between 80–90% released product being achieved after 1–2 h (Fig. 1).[20] Although the products are cleanly released, prolonged photolysis leads to darkening of the solution. It is assumed that the plateau results from the production of UV-absorbing by-products that act as light filters as it is known that the nitroso carbonyl compounds that are released readily dimerize to form azodioxy compounds,[21] and these have been observed to reduce to diazo compounds under photolytic conditions;[22] both would be strongly UV-absorbing.

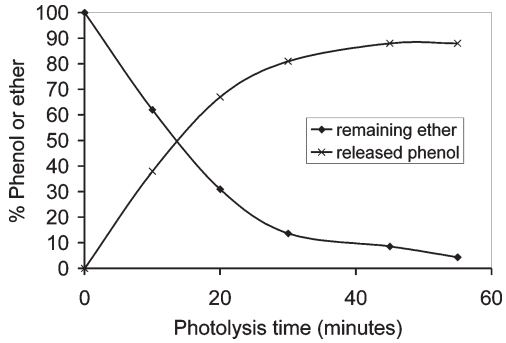


Fig. 1 Photolysis of **10** in acetone-d_6 monitored by ^{1}H NMR.

Table 1 Photolysis results

Entry	Compound photolyzed	Photolysis product[a]	Photolysis yield[b]
1	**4**	**2**	85%
2	**6**	**2**	79%
3	**5**	**3**	89%
4	**7**	tyrosine[c]	90%
5	**10**	**2**	75%
6	**12**	**2**	88%
7	**11**	**3**	65%
8	**11**	**3**[d]	80%[e]
9	**13**	tyrosine[c]	92%

[a] Photolysis performed in acetone using a 400 W high pressure mercury lamp and a Pyrex filter, unless otherwise stated. [b] Yield determined by ^{1}H NMR, using an internal standard. [c] Photolysis performed in D_2O. [d] Photolysis performed in acetonitrile. [e] Yield determined by HPLC.

The quantum yields for release of tyrosine from **7** and **13** in water were determined by chemical actinometry to be 0.19 and 0.11, respectively. Laser flash photolysis (λ_{ex} = 355 nm) of solutions of **7** and **13** in water revealed the rapid formation of a transient absorption with a maximum at 420 nm (see Fig. 2 for the data from **13**), assigned to the *aci*-nitro intermediate based on its similarity with previously reported spectra. The rate of disappearance of **7** was determined by monitoring the transient absorption at 420 nm, which decayed monoexponentially on the microsecond timescale (k = 2.4 × 10^4 s^{-1}) towards the initial absorbance level. The *aci*-nitro intermediate from **13** decayed at a similar rate of 2.3 × 10^4 s^{-1}.[23] These values are two orders of magnitude faster than nitroveratryl-protected phenols and an order of magnitude greater than CNB-protected phenols.[14a]

The decay of the *aci*-nitro transient has previously been assumed to correlate with product release, and this has been confirmed in the case of ATP release from 'caged ATP', the disodium salt of adenosine-5′- triphosphate-[P^3-(1-(2-nitrophenyl) ethyl)] ester,[24] for pH > 6.[25] However, detailed investigations by Corrie and co-workers[26] and by Wirz and co-workers[24] on the release of alcohols from nitrobenzyl ether derivatives showed that the hydrolysis of the hemi-acetal can be rate-limiting for poor leaving groups, with the rate-limiting step being dependent on solvent, pH and the nature of the leaving group. Although we believe that the phenoxide anion can be considered a moderately good leaving group for which the decay of the *aci*-nitro intermediate may be correlated to product release,[7e] further experiments are needed to corroborate this assumption. Thus, the observed kinetic rates should be taken as an upper limit for the rate of product release.

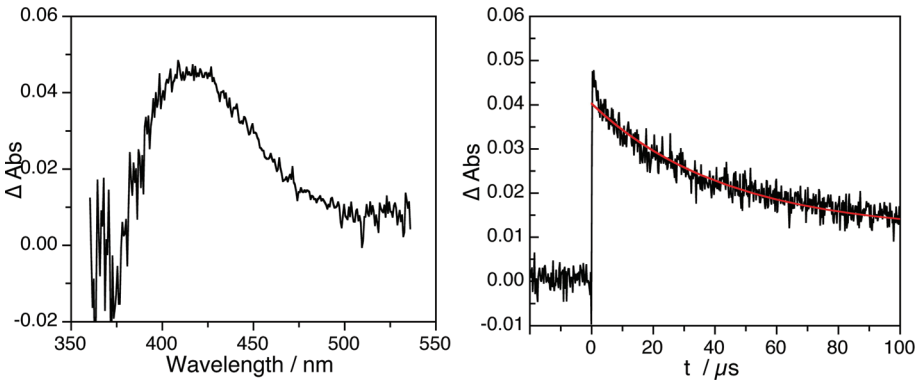
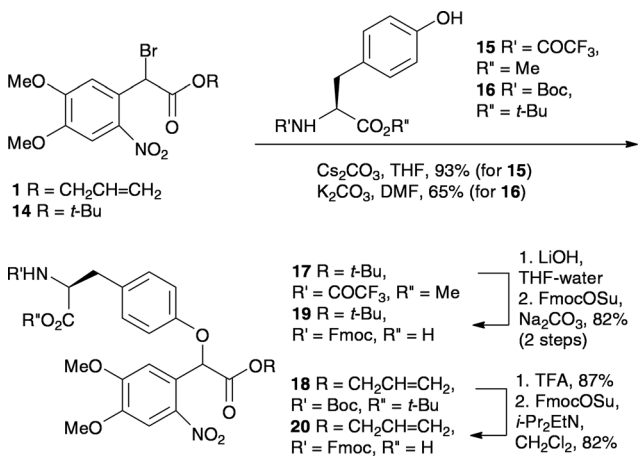


Fig. 2 Transient absorption spectrum of **13** in water 10 μs after pulsed excitation at 355 nm and decay of the transient absorption monitored at 420 nm. The red line represents the best fit according to a mono-exponential decay with $k = 2.3 \times 10^4$ s^{-1}.

Photolysis of the Mosher's amide of **7** cleanly released the Mosher's amide of tyrosine. Comparison of the ^{19}F NMR spectra of this material with those from the Mosher's amide of racemic tyrosine indicated that, within the limits of detection, the tyrosine released by photolysis was a single enantiomer.

The caging of tyrosine residues within peptides is of considerable potential interest, and so we set about the synthesis of two orthogonally-protected, caged tyrosine building blocks, **19** and **20** (Scheme 3), that would be compatible with Fmoc solid-phase peptide synthesis.



Scheme 3 Synthesis of caged tyrosine derivatives **19** and **20**.

Synthesis of *tert*-butyl ester **20** required alkylating agent **14**. Treatment of 3,4-dimethoxy-6-nitrophenylacetic acid with *O*-*tert*-butyl-*N*,*N*-dicyclohexylisourea afforded the *tert*-butyl ester in 97% yield. Diazo transfer and bromination as before gave **14**, which was used to alkylate *N*-trifluoroacetamidotyrosine methyl ester **15** to give **17** in 93% yield. Basic hydrolysis of the methyl ester and the trifluoroacetamide, followed by Fmoc protection of the amine gave the orthogonally-protected tyrosine derivative **19** in 82% yield. In a similar fashion, *N*-Boc tyrosine *tert*-butyl ester **16** was alkylated using the allyl ester **1**, affording **18**; treatment with TFA, followed by Fmoc protection gave **20**.

In conclusion, we have developed a convenient route to αCNV phenolic ethers, applicable to a range of phenols including tyrosine derivatives. These compounds photolyse two orders of magnitude faster than nitroveratryl-protected phenols and an order of magnitude faster than αCNB-protected phenols,[14a] as judged by decay of the *aci*-nitro intermediate. The methodology should be applicable to the protection and release of neurotransmitters such as catecholamines, while the orthogonally-protected, caged tyrosine building blocks **19** and **20** will allow for the Fmoc solid-phase synthesis of caged tyrosine-containing peptides.

Experimental

Allyl 2-(4-(*t*-butyl)phenoxy)-2-(4,5-dimethoxy-2-nitrophenyl) acetate (4)

4-*t*-Butylphenol (0.068 g, 0.45 mmol) and Cs_2CO_3 (0.150 g, 0.46 mmol) were stirred in THF (20 mL) at 0 °C for 30 min before **1** (0.150 g, 0.42 mmol) in THF (20 mL) was added. The reaction was stirred at rt overnight before the THF was removed *in vacuo* and the residue partitioned between water and EtOAc. The organic phase was washed with 1 M NaOH and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc, 7 : 3) afforded ether **4** (0.180 g, 93%) as a yellow oil: R_f 0.50; ν_{max}/cm^{-1} (film) 3099, 2963, 2868, 1751, 1649, 1609, 1583, 1524, 1512; δ_H (300 MHz, $CDCl_3$) 1.25 (9 H, s), 3.91 (3 H, s), 3.94 (3 H, s), 4.65 (2 H, dd, J = 5.5, 1.2 Hz), 5.19 (1 H, dd, J = 10.7, 1.1 Hz), 5.25 (1 H, dd, J = 17.3, 1.1 Hz), 5.77–5.92 (1 H, m), 6.62 (1 H, s), 6.92 (2 H, d, J = 8.8 Hz), 7.27 (2 H, d, J = 8.8 Hz), 7.33 (1 H, s), 7.70 (1 H, s); δ_C (75 MHz, $CDCl_3$) 31.4, 34.2, 56.4, 56.5, 66.3, 75.7, 108.2, 109.7, 115.5, 118.6, 126.5, 126.8, 131.3, 140.3, 145.2, 148.7, 153.8, 155.1, 168.1; *m*/*z* (electrospray) 452 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{23}H_{27}NO_7Na$: 452.1685. Found: 452.1679.

(2*S*)-Methyl 3-(4-(2-(allyloxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)-2-((*t*-butoxycarbonyl)amino)propanoate (5)

N-Boc-L-tyrosine methyl ester (0.271 g, 0.92 mmol) and Cs_2CO_3 (0.300 g, 0.92 mmol) were stirred in THF (20 mL) at 0 °C for 30 min before **1** (0.299 g, 0.83 mmol) in THF (20 mL) was added. The reaction was stirred at rt overnight before the THF was removed *in vacuo* and the residue partitioned between water and EtOAc. The organic phase was washed with 1 M NaOH and brine, dried with $MgSO_4$ and concentrated *in vacuo*.

Purification by column chromatography (hexane/EtOAc, 7 : 3) afforded ether **5** (0.410 g, 86%) as a yellow solid: R_f 0.25; mp 49–52 °C; ν_{max}/cm^{-1} (film) 3388, 3062, 2978, 2939, 2851, 1748, 1715, 1649, 1612, 1583, 1524, 1510; δ_H (300 MHz, $CDCl_3$) 1.32 (9 H, s), 2.82–3.04 (2 H, m), 3.62 (3 H, s), 3.85 (3 H, s), 3.89 (3 H, s), 4.41–4.50 (1 H, m), 4.60 (2 H, d, J = 5.5 Hz), 4.99 (1 H, d, J = 8.1 Hz), 5.14 (1 H, d, J = 11.4 Hz), 5.19 (1 H, d, J = 17.3 Hz), 5.72–5.87 (1 H, m), 6.56 (1 H, s), 6.86 (2 H, d, J = 8.3 Hz), 6.98 (2 H, d, J = 8.3 Hz), 7.23 (1 H, s), 7.65 (1 H, s); δ_C (75 MHz, $CDCl_3$) 28.2, 37.4, 52.1, 54.4, 56.35, 56.44, 66.3, 75.4, 79.8, 108.2, 109.6, 115.9, 118.6, 126.3, 130.1, 130.5, 131.2, 140.2, 148.8, 153.7, 155.0, 156.3, 167.8, 172.2; *m/z* (electrospray) 597 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{28}H_{34}N_2O_{11}Na$: 597.2060. Found: 597.2037.

2-(4-(*t*-Butyl)phenoxy)-2-(4,5-dimethoxy-2-nitrophenyl)acetic acid (6)

2 M NaOH (10 mL) and **4** (0.184 g, 0.43 mmol) were stirred in THF (20 mL) overnight before the THF was removed *in vacuo*. The aqueous phase was acidified to pH 2 with 2 M HCl and extracted with EtOAc. The organic phase was washed with brine, dried with $MgSO_4$ and evaporated to afford acid **6** (0.115 g, 69%) as a yellow oil: ν_{max}/cm^{-1} (film) 3421, 3019, 1729, 1524; δ_H (300 MHz, acetone-d_6) 1.26 (9 H, s), 3.91 (3 H, s), 3.95 (3 H, s), 6.60 (1 H, s), 7.01 (2 H, d, J = 8.8 Hz), 7.33 (2 H, d, J = 8.8 Hz), 7.38 (1 H, s), 7.73 (1 H, s); δ_C (75 MHz, acetone-d_6) 31.7, 34.6, 56.6, 56.7, 76.0, 109.1, 110.9, 116.1, 127.19, 127.21, 141.5, 145.5, 149.9, 154.6, 156.2, 169.5; *m/z* (electrospray) 412 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{20}H_{23}NO_7Na$: 412.1372. Found: 412.1358.

(1*S*)-1-Carboxy-2-(4-(carboxy(4,5-dimethoxy-2-nitrophenyl) methoxy)phenyl)ethanaminium 2,2,2-trifluoroacetate (7)

2 M NaOH (10 mL) and **5** (0.144 g, 0.25 mmol) were stirred in THF (20 mL) overnight before the THF was removed *in vacuo*. The aqueous phase was acidified to pH 2 with 2 M HCl and extracted with EtOAc. The organic phase was washed with brine, dried with $MgSO_4$ and evaporated to leave a yellow oil. TFA (1 mL) was added and the solution was stirred for 24 h. Removal of the TFA *in vacuo* afforded product **7** (0.097 g, 73%) as a yellow solid: mp 137–141 °C; ν_{max}/cm^{-1} (film) 2931, 2856, 1727, 1665, 1611, 1584, 1509; δ_H (300 MHz, acetone-d_6, mixture of diastereoisomers) 3.35–3.53 (2 H, m), 3.92 and 3.93 (3 H, 2 × s), 3.97 (3 H, s), 4.45–4.55 and 5.13–5.20 (1 H, 2 × m), 6.61 and 6.64 (1 H, 2 × s), 7.04–7.12 (2 H, m), 7.30–7.38 (3 H, m), 7.74 (1 H, s); δ_C (75 MHz, acetone-d_6, mixture of diastereoisomers) 35.4, 36.6, 54.9, 56.3, 56.4, 62.9, 75.6, 75.7, 108.8, 110.8, 116.5, 116.76, 116.82, 126.5, 126.6, 128.7, 129.6, 131.5, 131.6, 141.3, 149.6, 154.2, 157.5, 157.6, 168.9, 169.4, 170.4; *m/z* (electrospray) 443 (18%, $[M+Na]^+$), 421 (100%, $[M+H]^+$); HRMS (electrospray) Calcd for $C_{19}H_{20}N_2O_9Na$: 443.1067. Found: 443.1070.

2-(3,4-Bis(ethoxycarbonylmethoxy)phenyl)acetic acid ethoxycarbonylmethyl ester

K_2CO_3 (7.64 g, 55.4 mmol), ethyl bromoacetate (6.14 mL, 9.25 g, 55.4 mmol) and 2-(3,4-dihydroxyphenyl)acetic acid (3.00 g, 17.8 mmol) were stirred in anhydrous DMF (80 mL) at rt for 7 d. The mixture was poured into water, extracted with Et_2O and the organic phase was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated *in vacuo* to leave a pale orange oil (6.56 g, 87%). The crude product was of sufficient purity (>95%) to be used directly, but a portion was purified by column chromatography (hexane/EtOAc, 3 : 2) to give 2-(3,4-bis(ethoxycarbonylmethoxy)phenyl)acetic acid ethoxycarbonylmethyl ester as a colorless oil: R_f 0.41; ν_{max}/cm^{-1} (film) 2984, 2940, 1744, 1594, 1514; δ_H (300 MHz, $CDCl_3$) 1.10–1.18 (9 H, m), 3.53 (2 H, s), 4.00–4.17 (6 H, m), 4.49 (2 H, s), 4.58 (2 H, s), 4.60 (2 H, s), 6.65–6.80 (3 H, m); δ_C (75 MHz, $CDCl_3$) 13.8, 13.9, 39.8, 60.8, 60.9, 61.1, 66.4, 66.5, 115.5, 116.4, 123.0, 127.6, 147.0, 147.8, 167.3, 168.6, 168.7, 170.5; *m/z* (electrospray) 449 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{20}H_{26}O_{10}Na$: 449.1424. Found: 449.1433.

2-(4,5-Bis(ethoxycarbonylmethoy-2-nitro)phenyl)acetic acid ethoxycarbonylmethyl ester (8)

Fuming nitric acid (1.2 mL) and 2-(3,4-bis(ethoxycarbonylmethoxy)phenyl)acetic acid ethoxycarbonylmethyl ester (6.17 g, 14.5 mmol) were stirred in anhydrous CH_2Cl_2 (100 mL) at 0 °C for 30 min. Saturated aqueous $NaHCO_3$ was added until effervescence ceased. The layers were separated and the organic phase was washed with water, dried over $MgSO_4$ and concentrated *in vacuo* to yield an off-white solid (5.88 g, 86%). The crude product was of sufficient purity (>95%) to be used directly, but a portion was purified by column chromatography (hexane/EtOAc, 3 : 2) to give product **8** as a white solid: R_f 0.30; mp 76–78 °C (from EtOAc); ν_{max}/cm^{-1} (film) 2984, 2940, 1749, 1619, 1584, 1526; δ_H (300 MHz, $CDCl_3$) 1.18–1.35 (9 H, m), 4.06 (2 H, s), 4.16–4.31 (6 H, m), 4.63 (2 H, s), 4.76 (2 H, s), 4.81 (2 H, s), 6.79 (1 H, s), 7.72 (1 H, s); δ_C (75 MHz, $CDCl_3$) 14.15, 14.18, 14.21, 39.4, 61.3, 61.6, 61.7, 61.8, 66.4, 66.6, 112.2, 117.9, 125.1, 141.8, 146.9, 152.0, 167.6, 167.8, 167.9, 169.5; *m/z* (electrospray) 494 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{20}H_{25}NO_{12}$ Na: 494.1274. Found: 494.1269.

Diethyl 2,2′-((4-(1-diazo-2-(2-ethoxy-2-oxoethoxy)-2-oxoethyl)-5-nitro-1,2-phenylene)bis(oxy))diacetate

DBU (0.79 mL, 5.3 mmol) was added to a solution of **8** (2.26 g, 4.8 mmol) in anhydrous acetonitrile (40 mL). After stirring for 5 min, methanesulfonyl azide (0.64 g, 5.3 mmol) was added and the resulting mixture was stirred in the dark for 3 h. The solution was concentrated *in vacuo* and the residue partitioned between CH_2Cl_2 and water. The organic phase was dried over $MgSO_4$ and evaporated *in vacuo*. Purification by column chromatography (hexane/EtOAc, 7 : 3) afforded diethyl 2,2′-((4-(1-diazo-2-(2-ethoxy-2-oxoethoxy)-2-oxoethyl)-5-nitro-1,2-phenylene) bis(oxy))diacetate (1.43 g, 60%) as a yellow solid: R_f 0.23; mp 92–96 °C; ν_{max}/cm^{-1} (film) 3058, 2986, 2118, 1757, 1708, 1578, 1526; δ_H (300 MHz, $CDCl_3$) 1.18–1.33 (9 H, m), 4.18–4.31 (6 H, m), 4.71 (2 H, s), 4.78 (2 H, s), 4.80 (2 H, s), 7.09 (1 H, s), 7.69 (1 H, s); δ_C (75 MHz, $CDCl_3$) 14.1, 14.2,

61.2, 61.7, 61.8, 66.3, 66.5, 112.0, 115.8, 117.2, 140.5, 147.4, 151.8, 164.5, 167.6, 167.76, 167.77; *m/z* (electrospray) 520 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{20}H_{23}N_3O_{12}Na$: 520.1179. Found: 520.1172.

2-(4,5-Bis(ethoxycarbonylmethoxy)-2-nitrophenyl)-2-bromoacetic acid ethoxycarbonylmethyl ester (9)

HBr (48% in acetic acid, 0.62 mL) was added to a solution of diethyl 2,2′-((4-(1-diazo-2-(2-ethoxy-2-oxoethoxy)-2-oxoethyl)-5-nitro-1,2-phenylene)bis(oxy))diacetate (1.30 g, 2.61 mmol) in anhydrous CH_2Cl_2 (40 mL). After stirring for 30 min, the solution was washed with saturated aqueous $NaHCO_3$, dried with $MgSO_4$ and concentrated *in vacuo* to afford bromide **9** (1.16 g, 81%) as a yellow oil: ν_{max}/cm^{-1} (film) 2985, 1751, 1655, 1613, 1582, 1527; δ_H (300 MHz, $CDCl_3$) 1.18–1.32 (9 H, m), 4.16–4.31 (6 H, m), 4.67 (2 H, s), 4.77 (2 H, s), 4.86 (2 H, s), 6.35 (1 H, s), 7.39 (1 H, s), 7.59 (1 H, s); δ_C (75 MHz, $CDCl_3$) 13.78, 13.85, 13.88, 41.8, 61.74, 61.77, 61.78, 62.3, 66.3, 66.5, 111.8, 117.5, 126.2, 141.1, 148.3, 152.6, 167.5, 167.7, 168.1, 168.3; *m/z* (electrospray) 574 (90%, $[M+Na]^+$), 572 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{20}H_{24}NO_{12}BrNa$: 572.0380. Found: 572.0360.

Diethyl 2,2′-((4-(1-(4-(*t*-butyl)phenoxy)-2-(2-ethoxy-2-oxoethoxy)-2-oxoethyl)-5-nitro-1,2-phenylene)bis(oxy))diacetate (10)

4-*t*-Butylphenol (0.030 g, 0.2 mmol) and Cs_2CO_3 (0.065 g, 0.2 mmol) were stirred in THF (20 mL) at 0 °C for 30 min before **9** (0.100 g, 0.18 mmol) in THF (20 mL) was added. After stirring overnight at rt, the THF was removed *in vacuo* and the residue partitioned between water and EtOAc. The organic phase was washed with 1 M NaOH and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc, 11 : 9) afforded ether **10** (0.097 g, 87%) as a yellow oil: R_f 0.58; ν_{max}/cm^{-1} (film) 3064, 2965, 2908, 2871, 1756, 1608, 1584, 1527, 1511; δ_H (300 MHz, $CDCl_3$) 1.06–1.32 (18 H, m), 4.04 (2 H, q, J = 7.1 Hz), 4.14 (2 H, q, J = 7.2 Hz), 4.23 (2 H, q, J = 7.1 Hz), 4.52–4.65 (2 H, m), 4.66–4.75 (4 H, m), 6.62 (1 H, s), 6.88 (2 H, d, J = 8.8 Hz), 7.24 (2 H, d, J = 8.8 Hz), 7.28 (1 H, s), 7.68 (1 H, s); δ_C (75 MHz, $CDCl_3$) 14.0, 14.1, 31.4, 34.1, 61.52, 61.56, 61.61, 61.62, 66.0, 66.4, 75.0, 111.9, 112.6, 115.3, 126.5, 127.0, 140.8, 145.3, 147.3, 152.3, 155.0, 166.8, 167.4, 167.6, 167.8; *m/z* (electrospray) 642 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{30}H_{37}NO_{13}Na$: 642.2163. Found: 642.2169.

Diethyl 2,2′-((4-(1-(4-((*S*)-2-((*t*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)phenoxy)-2-(2-ethoxy-2-oxoethoxy)-2-oxoethyl)-5-nitro-1,2-phenylene)bis(oxy))diacetate (11)

N-Boc-L-tyrosine methyl ester (0.233 g, 0.79 mmol) and Cs_2CO_3 (0.258 g, 0.79 mmol) were stirred in THF (20 mL) at 0 °C for 30 min before **9** (0.402 g, 0.73 mmol) in THF (20 mL) was added. After stirring overnight the THF was removed *in vacuo* and the residue partitioned between water and EtOAc. The organic phase was washed with 1 M NaOH and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (hexane/EtOAc, 7 : 3) afforded ether **11** (0.310 g, 56%) as a yellow oil: R_f 0.36; ν_{max}/cm^{-1} (film) 3430, 3061, 2984, 1753, 1714, 1611, 1585, 1526, 1510; δ_H (300 MHz, $CDCl_3$) 1.06–1.29 (9 H, m), 1.33 (9 H, s), 2.84–3.02 (2 H, m), 3.61 (3 H, s), 4.00–4.08 (4 H, m), 4.13 (2 H, q, J = 7.1 Hz), 4.40–4.50 (1 H, m), 4.55–4.69 (2 H, m), 4.73–4.79 (4 H, m), 4.96 (1 H, broad d, J = 7.7 Hz), 6.58 (1 H, s), 6.85 (2 H, d, J = 8.3 Hz), 6.96 (2 H, d, J = 8.3 Hz), 7.22 (1 H, s), 7.63 (1 H, s); δ_C (75 MHz, $CDCl_3$) 14.0, 14.1, 28.2, 37.3, 52.2, 54.4, 61.5, 61.60, 61.61, 66.0, 66.4, 74.9, 79.8, 111.8, 112.8, 116.0, 126.6, 130.2, 130.5, 140.8, 147.3, 152.3, 156.3, 166.7, 167.3, 167.52, 167.54, 167.8, 172.2; *m/z* (electrospray) 787 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{35}H_{44}N_2O_{17}Na$: 787.2538. Found: 787.2552; Anal. calcd for $C_{35}H_{44}N_2O_{17}$: C, 54.97; H, 5.80; N, 3.66. Found: C, 54.86; H, 5.52; N, 3.29.

2,2′-((4-((4-(*t*-Butyl)phenoxy)(carboxy)methyl)-5-nitro-1,2-phenylene)bis(oxy))diacetic acid (12)

2 M NaOH (5 mL) and **10** (0.1 g, 0.16 mmol) in THF (10 mL) were stirred overnight before the THF was removed *in vacuo*. The aqueous phase was acidified to pH 2 with 2 M HCl and extracted with EtOAc. The organic phase was washed with brine, dried with $MgSO_4$ and evaporated to leave acid **12** (0.050 g, 65%) as a yellow oil: ν_{max}/cm^{-1} (film) 3023, 2964, 2870, 1755, 1584, 1521, 1292, 1192; δ_H (300 MHz, acetone-d_6) 1.26 (9 H, s), 4.95 (2 H, s), 4.98 (2 H, s), 6.54 (1 H, s), 7.01 (2 H, d, J = 8.8 Hz), 7.33 (2 H, d, J = 8.8 Hz), 7.44 (1 H, s), 7.80 (1 H, s); δ_C (75 MHz, acetone-d_6) 31.1, 34.0, 65.86, 65.92, 75.6, 111.8, 113.4, 115.7, 126.6, 127.4, 141.4, 145.0, 147.8, 152.5, 155.7, 168.7, 168.8, 169.1; *m/z* (electrospray) 500 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{22}H_{23}NO_{11}Na$: 500.1169. Found: 500.1152.

(1*S*)-2-(4-((4,5-Bis(carboxymethoxy)-2-nitrophenyl)(carboxy)methoxy)phenyl)-1-carboxyethanaminium 2,2,2-trifluoroacetate (13)

2 M NaOH (10 mL) and **11** (0.120 g, 0.16 mmol) in THF (20 mL) were stirred overnight before the THF was removed *in vacuo*. The aqueous phase was acidified to pH 2 with 2 M HCl and extracted with EtOAc. The organic phase was washed with brine, dried with $MgSO_4$ and evaporated to leave a yellow oil. TFA (1 mL) was added and the solution was stirred for 24 h. Removal of the TFA *in vacuo* afforded product **13** (0.066 g, 68%) as an orange gum: ν_{max}/cm^{-1} (film) 3430, 3055, 2987, 1732, 1511; δ_H (300 MHz, D_2O) 2.83–3.06 (2 H, m), 4.02–4.10 (1 H, m), 4.54 (3 H, s), 4.60 (3 H, s), 6.23 (1 H, s), 6.71 (2 H, d, J = 8.1 Hz), 6.88–6.97 (3 H, m), 7.43 (1 H, s); δ_C (75 MHz, D_2O) 34.0, 53.4, 64.6, 64.9, 74.2, 110.3, 112.0, 115.7, 125.3, 127.4, 130.1, 140.0, 145.8, 150.6, 155.1, 170.7, 170.8, 171.0, 171.1; *m/z* (electrospray) 531 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{21}H_{20}N_2O_{13}Na$: 531.0863. Found: 531.0847.

t-Butyl 2-(4,5-dimethoxy-2-nitrophenyl)acetate

A mixture of *N*,*N*-dicyclohexylcarbodiimide (35.40 g, 172 mmol), *t*-BuOH (20.1 mL, 15.6 g, 210 mmol) and CuCl

(181 mg) was stirred at rt for 5 d to afford *O*-*t*-butyl-*N*,*N*-dicyclohexylisourea. The mixture was diluted with CH_2Cl_2 (50 mL) and added to a solution of 2-(4,5-dimethoxy-2-nitrophenyl) acetic acid (5.00 g, 20.75 mmol) in CH_2Cl_2 (150 mL). After stirring at rt for 5 d the mixture was diluted with CH_2Cl_2 and the precipitate removed by filtration and washed with EtOAc. The organic phase was evaporated *in vacuo*. Purification by column chromatography (hexane/EtOAc, 5 : 1) gave *t*-butyl 2-(4,5-dimethoxy-2-nitrophenyl)acetate (6.16 g, 97%) as a colorless crystalline solid: R_f 0.1; mp 78–81 °C (from hexane); ν_{max}/cm^{-1} (film) 2977, 1731, 1618, 1524; δ_H (300 MHz, $CDCl_3$) 1.42 (9 H, s), 3.86 (2 H, s), 3.92 (3 H, s), 3.94 (3 H, s), 6.69 (1 H, s), 7.70 (1 H, s); δ_C (75 MHz, $CDCl_3$) 28.0, 41.4, 56.3, 81.5, 108.4, 114.6, 125.4, 141.0, 147.9. 153.1, 169.5; *m*/*z* (electrospray) 320 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{14}H_{19}NO_6Na$: 320.1110. Found: 320.1098.

t-Butyl 2-bromo-2-(4,5-dimethoxy-2-nitrophenyl)acetate (14)

Mesyl azide (1.62 g, 13.4 mmol), DBU (2.00 mL, 2.04 g, 13.4 mmol) and *t*-butyl 2-(4,5-dimethoxy-2-nitrophenyl)acetate (3.98 g, 13.4 mmol) were stirred in anhydrous acetonitrile (50 mL) at 0 °C to rt for 3 h. Acetonitrile was removed *in vacuo*, and the residue dissolved in EtOAc. The solution was washed with water, dried over $MgSO_4$ and concentrated *in vacuo* to give a brown gum. Column chromatography (hexane/EtOAc, 7 : 3) yielded *t*-butyl 2-diazo-2-(4,5-dimethoxy-2-nitrophenyl)acetate (2.67 g) as a bright yellow solid that still contained impurities (>90% pure) and so was used immediately in the next step.

Crude *t*-butyl 2-diazo-2-(4,5-dimethoxy-2-nitrophenyl)acetate (2.67 g) in CH_2Cl_2 (100 mL) at 0 °C was treated with 45% HBr in acetic acid (2.41 mL, 13.4 mmol). Vigorous effervescence was observed and the bright yellow color quickly faded. The reaction was warmed to rt and stirred for 20 min before being poured into saturated aqueous $NaHCO_3$. The phases were separated and the organic phase was washed with water, dried over $MgSO_4$ and concentrated *in vacuo* to yield a yellow oil which was purified by column chromatography (hexane/EtOAc, 6 : 1) to afford bromide **14** (2.73 g, 54% over 2 steps) as a colorless oil: R_f 0.4; ν_{max}/cm^{-1} (film) 1732, 1612, 1524; δ_H (300 MHz, $CDCl_3$) 1.45 (9 H, s), 3.93 (3 H, s), 3.98 (3 H, s), 6.06 (1 H, s), 7.39 (1 H, s), 7.57 (1 H, s); δ_C (75 MHz, $CDCl_3$) 25.8, 43.4, 54.6, 54.7, 81.5, 106.0, 112.5, 123.6, 138.4, 147.2, 151.4, 164.9; *m*/*z* (electrospray) 400 (85%, $[M+Na]^+$), 398 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{14}H_{18}NO_6BrNa$: 398.0215. Found: 398.0218.

(2*S*)-Methyl 3-(4-(2-(*t*-butoxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)-2-(2,2,2-trifluoroacetamido)propanoate (17)

N-Trifluoroacetyl-L-tyrosine methyl ester (0.94 g, 3.24 mmol) and Cs_2CO_3 (1.06 g, 3.24 mmol) were stirred in THF (140 mL) at rt for 30 min before **14** (1.11 g, 2.94 mmol) in THF (70 mL) was added. After stirring for 36 h the reaction was quenched by the addition of water and concentrated *in vacuo*. The residue was partitioned between EtOAc and water. The organic phase was dried with $MgSO_4$, evaporated *in vacuo* and the residue purified by column chromatography (Et_2O/hexane, 1 : 1) to afford (2*S*)-methyl ether **17** (1.60 g, 93%) as a yellow oil: R_f 0.1; ν_{max}/cm^{-1} (film) 3338, 2980, 1727, 1612, 1584, 1510; δ_H (300 MHz, acetone-d_6) 1.40 (9 H, s), 3.04 (1 H, dd, *J* = 14.1, 9.6 Hz), 3.25 (1 H, dd, *J* = 14.1, 5.2 Hz), 3.70 (3 H, s), 3.90 (3 H, s), 3.95 (3 H, s), 4.69–4.78 (1 H, m), 6.42 (1 H, s), 7.03 (2 H, d, *J* = 8.5 Hz), 7.24 (2 H, d, *J* = 8.5 Hz), 7.31 (1 H, s), 7.72 (1 H, s), 8.70 (1 H, br s); δ_C (75 MHz, acetone-d_6) 27.9, 36.4, 52.8, 55.0, 56.6, 76.90, 76.96, 82.9, 109.0, 110.6, 116.73 (q, *J* = 287 Hz), 116.74, 127.0, 131.0, 131.2, 141.3, 149.7, 154.5, 157.4 (q, *J* = 37 Hz), 157.5, 167.7, 171.1; *m*/*z* (electrospray) 609 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{26}H_{29}N_2O_{10}F_3Na$: 609.1672. Found: 609.1680.

(2*S*)-*t*-Butyl 3-(4-(2-(allyloxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)-2-((*t*-butoxycarbonyl)amino)propanoate (18)

N-Boc-L-tyrosine *t*-butyl ester (0.152 g, 0.45 mmol) and K_2CO_3 (0.062 g, 0.45 mmol) were stirred in DMF (5 mL) for 30 min before **1** (0.107 g, 0.30 mmol) in DMF (3 mL) was added. The reaction was stirred overnight, after which time starting material was still visible by TLC, and so a further portion of K_2CO_3 (0.035 g, 0.25 mmol) and allyl 2-bromo-2-(4,5-dimethoxy-2-nitrophenyl)acetate (0.091 g, 0.25 mmol) were added and stirring continued overnight. The reaction mixture was poured into water and extracted with Et_2O. The organic phase was washed with 1 M NaOH and water, dried over $MgSO_4$ and concentrated. Purification by column chromatography (hexane/EtOAc, 2 : 1) afforded ether **18** as a yellow oil (0.180 g, 65%): R_f 0.4; ν_{max}/cm^{-1} (film) 2976, 2937, 1742, 1711, 1612, 1583, 1522, 1507; δ_H (300 MHz, $CDCl_3$) 1.37 (9 H, s), 1.396 (5 H, s), 1.404 (4 H, s), 1.42 (2 H, s), 2.97 (2 H, d, *J* = 4.2 Hz), 3.91 (3 H, s), 3.96 (3 H, s), 4.38 (1 H, dd, *J* = 13.9, 6.0 Hz), 4.66 (2 H, dq, *J* = 5.6, 1.3 Hz), 4.92–5.00 (1 H, m), 5.17–5.32 (2 H, m), 5.85 (1 H, ddt, *J* = 17.2, 10.5, 5.6 Hz), 6.63 (1 H, s), 6.90 (2 H, d, *J* = 8.6 Hz), 7.07 (2 H, d, *J* = 8.6 Hz), 7.28 (1 H, s), 7.70 (1 H, s); δ_C (100 MHz, $CDCl_3$) 28.03, 28.39, 30.42, 37.75, 54.96, 56.51, 56.58, 66.43, 75.49, 79.74, 82.08, 108.34, 109.78, 115.93, 118.78, 125.57, 126.52, 130.64, 130.89, 131.33, 140.42, 148.91, 153.90, 155.13, 156.34, 168.03, 170.93; *m*/*z* (electrospray) 639 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{31}H_{40}N_2O_{11}Na$: 639.2530. Found: 639.2527.

(1*S*)-2-(4-(2-(Allyloxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)-1-carboxyethanaminium 2,2,2-trifluoroacetate

A solution of **18** (0.120 g, 0.195 mmmol) in TFA (2 mL) was stirred at room temperature for 2 h. The TFA was removed *in vacuo* and the residue was triturated with Et_2O to afford (1*S*)-2-(4-(2-(allyloxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy) phenyl)-1-carboxyethanaminium 2,2,2-trifluoroacetate (0.090 g, 87%) as a pale yellow solid: ν_{max}/cm^{-1} (film) 2929, 1742, 1676, 1609, 1583, 1508; δ_H (300 MHz, acetone-d_6) 2.99–3.15 (1 H, m), 3.21–3.35 (1 H, m), 3.91 (3 H, s), 3.97 (3 H, s), 4.68 (2 H, dt, *J* = 5.4, 1.5 Hz), 5.19 (1 H, ddd, *J* = 10.5, 2.8, 1.3 Hz), 5.28 (1 H, ddd, *J* = 17.2, 3.2, 1.6 Hz), 5.91 (1 H, ddt, *J* = 17.2, 10.7, 5.4 Hz), 6.58 (1 H, d, *J* = 1.3 Hz), 7.05 (2 H, d, *J* = 7.4 Hz), 7.29 (2 H, d, *J* = 8.6 Hz), 7.34 (1 H, s), 7.75 (1 H, s); *m*/*z*

(electrospray) 505 (25%, $[M-2H+2Na]^+$), 483 (100%, $[M-H+Na]^+$), 280 (40%); HRMS (electrospray) Calcd for $C_{22}H_{24}N_2O_9Na$: 483.1380. Found: 483.1376.

(2*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(2-(*t*-butoxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl) propanoic acid (19)

A solution of **20** (0.095 g, 0.162 mmol) and LiOH (0.010 g, 0.42 mmol) in THF/H_2O (1 : 1, 2 mL) was stirred overnight before being neutralized using 1 M HCl. To this solution was added Na_2CO_3 (0.026 g, 0.245 mmol) and *N*-(9*H*-fluoren-9-ylmethoxycarbonyloxy)succinimide (0.055 g, 0.162 mmol) and the mixture was stirred overnight. The THF was removed *in vacuo* and the residue acidified to pH 5 with 1 M HCl to afford a white precipitate which was extracted into EtOAc. The organic phase was dried with $MgSO_4$ and the solvent removed *in vacuo*. Purification by column chromatography (hexane/EtOAc/AcOH, 1 : 1 : 0.02) afforded product **19** (93 mg, 82%) as a pale yellow oil: R_f 0.25; ν_{max}/cm^{-1} (film) 3354, 3021, 2980, 2938, 1724, 1611, 1584, 1510; δ_H (300 MHz, $CDCl_3$) 1.39 (9 H, s), 2.98–3.16 (2 H, m), 3.86–3.88 (3 H, m), 3.91–3.93 (3 H, m), 4.14–4.19 (1 H, m), 4.31–4.38 (1 H, m), 4.40–4.46 (1 H, m), 4.59–4.65 (1 H, m), 5.19 (1 H, d, J = 7.0 Hz), 6.50 (1 H, s), 6.92 (2 H, d, J = 8.3 Hz), 7.03 (2 H, d, J = 8.3 Hz), 7.21–7.30 (3 H, m), 7.32–7.39 (2 H, m), 7.48–7.55 (2 H, m), 7.62–7.67 (1 H, m), 7.70–7.76 (2 H, m); δ_C (75 MHz, $CDCl_3$) 27.8, 36.8, 47.1, 54.5, 56.3, 56.5, 67.0, 75.6, 83.2, 108.2, 109.3, 116.1, 120.0, 124.9, 125.0, 126.6, 127.0, 127.7, 129.3, 130.6, 140.4, 141.3, 143.6, 143.7, 148.5, 153.6, 155.8, 156.5, 167.3, 175.3; *m*/*z* (electrospray) 721 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{38}H_{38}N_2O_{11}Na$: 721.2373. Found: 721.2368.

(2*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(2-(allyloxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl) propanoic acid (20)

(1*S*)-2-(4-(2-(Allyloxy)-1-(4,5-dimethoxy-2-nitrophenyl)-2-oxoethoxy)phenyl)-1-carboxyethanaminium 2,2,2-trifluoroacetate (0.050 g, 0.088 mmol), *N*-(9*H*-fluoren-9-ylmethoxycarbonyloxy)succinimide (0.034 g, 0.1 mmol) and diisopropylethylamine (52 μL, 0.039 g, 0.3 mmol) were stirred overnight in CH_2Cl_2 (3 mL). The solution was washed with 1 M HCl, brine and dried ($MgSO_4$). Removal of the solvent *in vacuo* and purification of the residue by column chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH, 9 : 1) afforded product **19** (0.049 g, 82%) as a pale-yellow foam: R_f 0.2 (9 : 1 CH_2Cl_2/MeOH); ν_{max}/cm^{-1} (neat) 2936, 1743, 1719, 1611, 1583, 1507; δ_H (400 MHz, $CDCl_3$) 3.02 (1 H, dd, J = 12.9, 6.6 Hz), 3.13 (1 H, dd, J = 14.0, 4.8 Hz), 3.86 (1 H, s), 3.87 (1 H, s), 3.92 (1 H, s), 3.93 (1 H, s), 4.13–4.20 (1 H, m), 4.28–4.37 (1 H, m), 4.40–4.49 (1 H, m), 4.56–4.70 (3 H, m), 5.16–5.25 (1 H, m), 5.25–5.32 (1 H, m), 5.84 (1 H, ddd, J = 21.8, 10.7, 5.5 Hz), 6.63 (1 H, d, J = 2.8 Hz), 6.91 (2 H, d, J = 7.5 Hz), 7.03 (1 H, d, J = 8.2 Hz), 7.23–7.32 (3 H, m), 7.37 (2 H, dd, J = 13.8, 6.8 Hz), 7.49–7.57 (2 H, m), 7.68 (1 H, d, J = 5.5 Hz), 7.74 (2 H, d, J = 7.5 Hz); δ_C (100 MHz, $CDCl_3$) 37.00, 54.83, 56.51, 56.62, 66.57, 67.06, 75.41, 75.50, 108.34, 109.68, 116.15, 118.91, 120.10, 125.07, 125.15, 126.40, 127.17, 127.85, 129.85, 130.82, 131.27, 140.33, 141.40, 143.71, 143.82, 148.89, 153.91, 155.87, 156.50, 168.16, 175.36; *m*/*z* (electrospray) 705 (100%, $[M+Na]^+$); HRMS (electrospray) Calcd for $C_{37}H_{34}N_2O_{11}Na$: 705.2060. Found: 705.2051.

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