Synthesis and evaluation of 2-(2-fluoro-4-hydroxymethyl-5-methoxyphenoxy)acetic acid as a linker in solid-phase synthesis monitored by gel-phase ¹⁹F NMR spectroscopy[†]

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Gel-phase ¹⁹F NMR spectroscopy is a useful monitoring technique for solid-phase organic chemistry due to the high information content it delivers and swift acquisition times, using standard NMR spectrometers. This paper describes the synthesis of the novel linker 2-(2-fluoro-4-hydroxymethyl-5-methoxy-phenoxy)acetic acid in 29% yield over seven steps, using nucleophilic aromatic substitutions on 2,4,5-trifluorobenzonitrile as key steps. Following standard solid-phase synthesis a peptide could be cleaved from the linker using 20% TFA in CH_2Cl_2 in 30 minutes, in contrast to a previously described monoalkoxy linker that requires 90% TFA in water at elevated temperature. A resin-bound peptide could be successfully glycosylated using only two equivalents of a thioglycoside donor, activated with *N*-iodosuccinimide and trifluoromethanesulfonic acid, and subsequent cleavage and deprotection gave the target glycopeptide. Direct glycosylation of the linker itself followed by mild acidic cleavage gave a fully protected hemiacetal for further chemical manipulation.

Introduction

Solid-phase syntheses of peptides¹ and oligonucleotides² have become standard procedures, with automated methods and synthesizers. Non-peptidic solid-phase organic synthesis is also developing strongly, but mostly for rather simple chemistry with few steps and good regio- and diastereoselectivity. For more complex targets, e.g. oligosaccharides, the lack of methods for reaction monitoring still hampers progress.3 One method that overcomes this problem is gel-phase ¹⁹F NMR spectroscopy using fluorine containing linkers, reactants and/or protecting groups. Spectra showing the yield, and in many cases also the diastereomeric ratio, can be recorded in a few minutes using standard NMR spectroscopy equipment. This method has been successfully used in both oligosaccharide and small molecule solid phase organic synthesis.⁴⁻¹⁸ In order to accomplish this, several linkers e.g. 1,¹⁷ 2,^{6,15} and 3¹⁴ (Fig. 1) have been developed that can be cleaved under acidic, basic or oxidative conditions respectively. However, linkers 1 and 2 demand rather harsh conditions for cleavage, such as 90% trifluoroacetic acid (TFA) in water at 60 $^{\circ}\mathrm{C}$ for six hours for 1.6,17 Even though more advanced automated or semi-automated synthesizers have the ability to warm and cool the reaction, solid-phase synthesis is greatly simplified if the reactions can be performed at room temperature. To this end, and to further extend the range of linkers and enable more gentle cleavage methods, more acid labile linkers are needed. In non-fluorinated



Fig. 1 Examples of fluorinated linkers and the Wang resin 4. Carboxylic acids attached to 1, 2, and 4 are cleavable under acidic or basic conditions, those attached to 3 are cleavable under oxidative conditions.

versions, alkoxy substituted benzyl alcohols have been widely used as linkers, with the classical Wang-linker¹⁹ **4** as the most common one.²⁰ The Wang-linker is normally cleaved with TFA at room temperature, but the introduction of an electron withdrawing fluorine atom (*cf.* linker **1**) decreases the acid lability of the linker.⁶ Increasing the number of electron donating alkoxy groups on the linker conversely increases the acid lability,²⁰ indicating that the introduction of a methoxy group into the fluorinated linker **1** could give the desired properties. In this study we describe the synthesis and application of the fluorinated dialkoxy linker 2-(2fluoro-4-hydroxymethyl-5-methoxy-phenoxy)acetic acid.

Results and discussion

To synthesize the new linker 2-(2-fluoro-4-hydroxymethyl-5methoxy-phenoxy)acetic acid **12**, a method based on nucleophilic aromatic substitutions was envisioned. Wells *et al.* demonstrated this method with both *tert*-butyl trifluorobenzoate and trifluorobenzonitrile **5**.²¹ Our first strategy was based on the *tert*butyl trifluorobenzoate pathway with two nucleophilic aromatic

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Scheme 1 Synthesis of linker 12. Reagents and conditions: a) t-BuOK, BnOH, THF, -78 °C-room temperature. b) t-BuOK, MeOH, THF, -50 °C-0 °C. c) NaOH, EtOH, reflux. d) H₂ (g), Pd/C, AcOH. e) BH₃·DMS, B(OMe)₃, THF. f) Ethyl bromoacetate, DBU, MeCN, reflux. g) LiOH, THF-MeOH-water 3:1:1.

substitutions as initial steps, first with methyl glycolate in the para position and then methoxide in the ortho position. The nucleophilic aromatic substitutions, especially the second one, gave low yields due to decomposition of the starting material and transesterification. Instead the linker 12 was prepared from the trifluorobenzonitrile 5 in 29% yield over seven steps (Scheme 1). Nucleophilic aromatic substitutions with potassium benzoxide and methoxide, followed by hydrolysis of the nitrile and hydrogenolysis of the benzyl ether gave the benzoic acid 9 in 53% yield over 4 steps. 9 is an analogue of 3-fluoro-4-hydroxybenzoic acid, the starting material in our previous synthesis of linker 14.17 Unfortunately, the increased electron density of 9 disturbed both the reduction to alcohol 10 and the following alkylation. The reduction could however be performed using the same conditions as before in a good yield but the product was very sensitive, most likely due to oxidation, and the product was only briefly characterized before continuation of the synthesis. Alkylation of the phenolic oxygen using ethyl bromoacetate and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) proceeded faster than for the non-alkoxy version. During the reaction the product was surprisingly dimerized on the benzylic position to form 13 (Fig. 2). Since 11 and 13 have very similar spectroscopic properties, and both give the benzylic cation as main ion in LC-MS analysis, X-ray crystallography was used to distinguish them (supporting information§). By reducing the reaction time, the formation of 13 could be suppressed. Finally, basic ester hydrolysis furnished the



Fig. 2 Dimer formed in the alkylation of 10.

§ CCDC reference numbers 644117 and 644118. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b704472k

linker 12 after chromatographic purification. With the linker in hand, studies to confirm its acid lability were performed.

Linker 12 and its monoalkoxy analogue 14 were attached to TentaGel HL-NH₂ resin through amide bonds to form linker resins 15 and 1 respectively (Scheme 2). Amides were formed in DMF using N, N'-diisopropyl-carbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) and monitored with bromophenol blue. Fmoc-glycine was then connected to the benzylic alcohols of 15 and 1 through ester bonds formed with 1-(2-mesitylenesulfonyl)-3nitro-1H-1,2,4-triazole (MSNT) and N-methyl-imidazole (MeIm) to give resin 16 and 17. Continued amide formations with Fmoc-4fluorophenylalanine and 2,4-difluorobenzoic acid gave the resinattached peptides 18 and 19. Carboxylic acids attached to nonfluorinated versions of linker 12 are cleavable with 1% TFA in CH_2Cl_2 . Based on this, and the electron withdrawing character of the fluorine atom in 12, the cleavage study was performed using 5% and 20% TFA in CH₂Cl₂ (Fig. 3).



Fig. 3 Cleavage of peptide from resins 19 (black circles) and 18 (black triangles) using 5% TFA in CH2Cl2 and 18 (black squares) using 20% TFA in CH₂Cl₂.

The resins 18 and 19 were treated with the 5% cleavage solution for 30 minutes and gel-phase ⁹F NMR spectroscopy revealed $\sim 10\%$ cleavage from resin 18 and none from resin 19. Another



Scheme 2 Synthesis and cleavage of test peptide *Reagents and conditions*: a) TentaGel HL-NH₂, DIC, HOBt, DMF. b) Fmoc-Gly-OH, MSNT, MeIm, CH₂Cl₂. c) i. Piperidine, 20% in DMF. ii. Fmoc-*p*-F-Phe-OH, DIC, HOBt, DMF. iii. Piperidine, 20% in DMF. iv. 2,4-Difluorobenzoic acid, DIC, HOBt, DMF. d) 5% TFA in CH₂Cl₂ (from **18**).

portion of cleavage solution was added and the mixture was shaken for 90 minutes and gel-phase ¹⁹F NMR spectroscopy then showed ~40% cleaved from resin **18**. The procedure was repeated and after a total of four hours ~90% was cleaved from resin **18** while resin **19** still was intact. Prolonged treatment for up to seven hours of **19** did not result in any cleavage. When resin **18** was treated with 20% TFA in CH₂Cl₂ for 30 minutes ~90% of the peptide was cleaved. Interestingly, about 5% of the resin bound peptide could not be cleaved with either 5% or 20% TFA despite increased reaction time. This indicates that some of the peptide is bound to the resin matrix in a non-cleavable way. Cleavage of resin **18** with both 5% and 20% TFA in CH₂Cl₂ gave peptide **20** in ~95% yield based on the loading capacity of resin **16**. Resin **19** was then cleaved using the previously described method of TFA–water at 60 °C.¹⁸ Analytical data for **20** was in agreement with those published.¹⁸ In a preliminary study, the Tentagel linker resin 2, a regioisomer of 14, seemed to have an acid lability in between 12 and 14 (data not shown).

To test the utility of linker **12** during solid-phase glycosylations, a glycopeptide was assembled. The peptide **21** was prepared as described by Mogemark *et al.*¹⁵ The serine hydroxyl was then glycosylated to give **23** in a remarkable 89% yield using only two equivalents of the galactose donor **22**¹⁵ under promotion by *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) without loss of peptide from the resin (Scheme 3). The yield was determined by comparison of the gel-phase ¹⁹F NMR signals from the fluorine containing protecting groups on the glycosyl donor (Fig. 4). From the gel-phase ¹⁹F NMR spectra it was concluded that the glycosylation was β-selective since no signals from the



Scheme 3 Synthesis of glycopeptide 25. *Reagents and conditions:* a) i) Fmoc-Val-OH, MSNT, MeIm, CH_2Cl_2 , room temperature, 14 h. ii) Peptide synthesis according to previously reported procedure.²² b) NIS, TfOH, CH_2Cl_2 . c) TFA–water 9 : 1, room temperature, 2 h. d) LiOH (20 mM in MeOH–water 4 : 1).



 α -anomer were seen. Cleavage and simultaneous deprotection of the benzylidene protecting group with TFA-water (9 : 1) at room temperature followed by preparative HPLC gave partially protected glycopeptide **24** in a modest 29% yield based on the resin loading. Debenzoylation with LiOH in MeOH-water (4 : 1) finally yielded glycopeptide **25** in 47% yield.

To test the potential for direct glycosylation the linker resins 15 and 1 were both subjected to four equivalents of galactosyl donor 22, NIS and a catalytic amount of TfOH (Scheme 4). The donor carries fluorinated versions of common protective groups in carbohydrate chemistry i.e. a benzylidene acetal and two benzoates. Examination of the resins with gel-phase ¹⁹F NMR spectroscopy revealed that 100% of the more acid stable resin 1 was glycosylated to give 26, while 40% of the new acid labile linker 15 was glycosylated under the same conditions to give 27. Resin 27 was obtained in 63% yield after a second glycosylation. The glycosylation gave resin 27 as an anomeric mixture (α : β , 1 : 10) as determined by gel-phase ¹⁹F NMR spectroscopy. Obviously resin 15 is more difficult to glycosylate than 1 using our standard protocol. When the glycosylated resins 26 and 27 were treated with the mild cleavage solution 5% TFA in CH₂Cl₂ gel-phase ¹⁹F NMR spectroscopy revealed that nothing



Scheme 4 Glycosylation of the linkers 1 and 15. *Reagents and conditions:* a) 4 or 6 equivalents of 22 and NIS, TfOH (catalytic amount), CH_2Cl_2 , room temperature, 4 h or 6 hours. b) 5% TFA in CH_2Cl_2 , room temperature, 7 h.

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was cleaved from resin 26 while the spectra for resin 27 indicated that approximately 72% was cleaved to give the hemiacetal 28. Despite another seven hours with the cleavage solution a small amount of the galactose moiety was not cleaved. The isolated total yield of 28 was 58% based on loading capacity of the linker resin 16. The mild cleavage conditions make it possible to maintain sensitive protecting groups such as benzylidene acetals during the cleavage step from the solid-phase. An attractive strategy would be to assemble oligosaccharides on the linker and the protected hemiacetals obtained after cleavage can be transformed into various donators *e.g.* trichloroacetamides and phosphates for further glycosylations, in a convergent manner. This strategy is currently under investigation in our laboratory.

Conclusions

Gel-phase ¹⁹F NMR spectroscopy is a technique well suited for monitoring solid-phase organic synthesis due to its simplicity and sensitivity. Fluorinated linkers with diverse cleavage methods are of great use for this technique. We have prepared a new acid labile fluorinated linker analogue to the commercial 4-hydroxymethylphenoxy acetic acid (HMPA) linker. The synthesis was based on nucleophilic aromatic substitutions and the acid lability of the resulting linker was evaluated. It was shown that a test peptide could be cleaved from the resin using 5% TFA in CH₂Cl₂ and after four hours $\sim 90\%$ of the peptide was cleaved. Under the same conditions a previously described linker, an analogue to the Wang-linker, was not affected at all. When the peptide was treated with 20% TFA in $CH_2Cl_2 \sim 90\%$ was cleaved from the new acidlabile linker in only 30 minutes. In addition, the new linker is stable during glycosylation with thioglycosides with NIS-TfOH, as demonstrated by the synthesis of a glycopeptide. It was also shown that the new linker could be directly glycosylated with a thioglycoside using NIS and TfOH as promoters. This implies that the linker can be used for the synthesis of oligosaccharides. The process for glycosylation of the linker however needs to be optimized since six equivalents of glycosyl donor were required. The glycosylation protocol is currently being optimized by our group. The protected hemiacetal was cleaved from the new acid labile resin under mild conditions using 5% TFA in CH₂Cl₂ for a total of 14 hours leaving the benzylidene protective groups intact. The resulting hemiacetal can be transformed into a new glycosyl donor allowing this approach to be employed in a convergent manner. The yield and diastereomeric ratio could easily be determined from the gel-phase ¹⁹F NMR spectrum pointing out the value and applicability of this linker for solid-phase glycoconjugate synthesis.

Experimental

Solid-phase synthesis was performed on TentaGel HL-NH₂ resin (0.42 mmol g⁻¹) from Rapp Polymere. CH₂Cl₂ was distilled from calcium hydride, THF from potassium and DMF was distilled under vacuum. Before concentration, all organic solvents were dried over Na₂SO₄ and filtered. Solvent mixtures are reported as v/v ratios. TLC was run on Silica Gel 60 F₂₅₄ (Merck) and the spots were detected in UV-light and stained with phosphomolybdic acid (12 g in 250 ml ethanol) and heat. Silica gel (Matrex, 60 Å, 35–70 µm, Grace Amicon) and solvents of analytical grade were

used for flash column chromatography. The NMR-spectra were recorded on a Bruker DRX-400 or ARX-500 spectrometer with CDCl₃, MeOH-d₄ or DMSO-d₆ as solvents and residual CHCl₃ ($\delta_{\rm H}$ 7.27 ppm), MeOH-d₃ ($\delta_{\rm H}$ 3.30 ppm) or DMSO-d₅ ($\delta_{\rm H}$ 2.50 ppm) as internal standard for ¹H and CDCl₃ ($\delta_{\rm C}$ 77.23 ppm) or MeOH-d₄ ($\delta_{\rm C}$ 49.15 ppm) as internal standard for ¹³C. CFCl₃ ($\delta_{\rm F}$ 0.00 ppm) was added to the solvents as internal standard for ¹⁹F. Peaks that could not be assigned are not reported. Jvalues are given in Hz. J values for the glycopeptide 25 were determined from DQF-COSY. ¹³C-NMR resonances from the fluorine labeled protective groups are split by J_{C-F} couplings, leading to complex spectra. Since the coupling patterns and hence the actual shift, could not be assigned unambiguously, the peaks are not reported. Gel-phase proton decoupled ¹⁹F NMR spectra were recorded on resin suspensions in CDCl₃ or MeOH-d₄ with CFCl₃ ($\delta_{\rm F}$ 0.00 ppm) as internal standard. Two peaks appear in the spectra around 0 ppm. One originates from CFCl₃ inside the polymer and one from CFCl₃ outside the polymer. The peak with higher shift was used as internal standard. Preparative reversed phase LC-MS was performed on an XTerra C-18 column (50 \times 19 mm, 5 µm, 125 Å), eluted with a linear gradient of MeCN in water, both of which contained formic acid (0.2%). A flow rate of 25 ml min⁻¹ was used and detection was at 214 and 254 nm and with positive and negative electrospray mass analysis. Preparative HPLC separations were performed on a Beckman System Gold HPLC, using a Supelco Discovery Biowide Pore C18 column $(250 \times 212 \text{ mm}, 5 \mu\text{m})$ with a flow rate of 11 ml min⁻¹ and detection at 214 nm. Analytical HPLC were performed on a Beckman System Gold HPLC, using a Supelco Discovery Biowide Pore C18 column (250 \times 46 mm, 5 μ m) with a flow rate of 1.5 ml min⁻¹ and detection at 214 nm. Mass spectra were performed on a Waters Micromass ZG 2000 with positive electrospray. Positive fast-atom-bombardment mass spectra were recorded on a Jeol SX102 mass spectrometer. Ions were produced by a beam of Xenon atoms (6 keV) from a matrix of 3-nitrobenzyl alcohol. IR spectra were recorded on an ATI Mattson Infinity Series FTIRTM spectrometer.

4-Benzyloxy-2,5-difluorobenzonitrile (6)

Potassium tert-butoxide (1.57 g, 12.9 mmol) was suspended in THF (15 ml) and the mixture was cooled to 0 °C. Benzyl alcohol (2.64 ml, 25.5 mmol) was added and the solution was stirred at 0 °C for 30 min followed by dropwise addition to 2,4,5trifluorobenzonitrile (2.00 g, 12.7 mmol) in THF (15 ml) at -78 °C. The solution was stirred at -78 °C for 3 h, warmed to room temperature over 1.5 h and stirred at room temperature for 20 h 15 min. The solution was diluted with EtOAc (100 ml) and washed with water (2 \times 70 ml). The water phases were reextracted with CH₂Cl₂ (100 ml) and the combined organic phases were concentrated yielding 4.4 g crude product. Recrystallization from EtOH resulted in 6 (2.63 g, 84%). Mp 118–119 °C; $v_{\rm max}/{\rm cm}^{-1}$ (solid) 2240 (C \equiv N); ¹H NMR (CDCl₃) δ 7.48–7.36 (m, 5H, C₆H₅), 7.32 (dd, 1H, J = 9.9, 6.0, H-6), 6.84 (dd, 1H, J = 10.0, 6.7, H-3),5.19 (s, 2H, PhCH₂); ¹⁹F NMR (CDCl₃) δ –108.5 (d, 1F, J = 14.3, F-2), -136.4 (d, 1F, J = 13.3, F-5); ¹³C NMR (CDCl₃) δ 134.6, 129.1, 129.0, 127.6, 71.8; HRMS (FAB) calcd for C₁₄H₁₀F₂NO 2460730 (M + H)+, found 246.0724.

4-Benzyloxy-5-fluoro-2-methoxybenzonitrile (7)

Potassium tert-butoxide (1.65 g, 13.5 mmol) was suspended in THF (15 ml), cooled to 0 °C, and methanol (545 µl, 13.5 mmol) was added. The slurry was stirred at 0 °C for 30 min and added to a solution of difluorobenzonitrile 6 (2.36 g, 9.62 mmol) in THF (30 ml) at -50 °C. The mixture was stirred at -50 °C for 1 h, warmed to 0 °C over 30 min, and stirred at 0 °C for 21 h. The solution was diluted with EtOAc (100 ml), washed with water $(2 \times 100 \text{ ml})$, and the water phases were extracted with CH_2Cl_2 (100 ml). The combined organic phases were concentrated and recrystallization from EtOH gave 7 (1.96 g, 79%). Mp 125 °C; v_{max} /cm⁻¹ (solid) 2213 (C=N); ¹H NMR (CDCl₃) δ 7.49–7.35 (m, 5H, C_6H_5), 7.26 (d, 1H, J = 10.2, H-6), 6.57 (d, 1H, J = 6.7, H-3), 5.22 (s, 2H, PhCH₂), 3.85 (s, 3H, CH₃); ¹⁹F NMR (CDCl₃) δ -142.2; ¹³C NMR (CDCl₃) δ 135.3, 129.0, 128.8, 127.6, 71.7, 56.7; HRMS (FAB) calcd for $C_{15}H_{13}FNO_2$ 258.0930 (M + H)⁺, found 258.0931.

4-Benzyloxy-5-fluoro-2-methoxybenzoic acid (8)

Benzonitrile 7 (499 mg, 1.94 mmol) was suspended in EtOH (5 ml), NaOH (2 M aq, 3.9 ml, 7.8 mmol) was added and the mixture was refluxed for 39 h and concentrated. The residue was separated between EtOAc and NaOH (1 M aq). The water phase was washed with EtOAc, acidified (pH 1) with HCl (conc. aq), and extracted twice with EtOAc. The combined organic phases from the acidic extraction were concentrated yielding **8** (494 mg, 92%). Mp 126– 127 °C; v_{max} /cm ⁻¹ (solid) 2942 (OH), 1662 (C=O); ¹H NMR (MeOH-d₄) δ 7.61 (d, 1H, J = 11.8, H-6), 7.50–7.43 (m, 2H, C₆H₅), 7.42–7.30 (m, 3H, C₆H₅), 6.85 (d, 1H, J = 6.8, H-3), 5.26 (s, 2H, PhCH₂), 3.87 (s, 3H, CH₃); ¹⁹F NMR (MeOH-d₄) δ –144.3; ¹³C NMR (MeOH-d₄) δ 168.1, 137.7, 129.8, 129.5, 128.9, 72.4, 57.3; HRMS (FAB) calcd for C₁₅H₁₄FO₄ 277.0876 (M + H)⁺, found 277.0870.

5-Fluoro-4-hydroxy-2-methoxybenzoic acid (9)

Benzoic acid **8** (597 mg, 2.16 mmol) and palladium (10% on carbon, 607 mg) were dissolved/suspended in AcOH (300 ml) and stirred under H₂ (g, 1 atm) for 23 h at room temperature. The mixture was filtered through Celite and the solvents were evaporated and coevaporated with MeOH–hexane yielding 0.4 g crude product. The residue was dissolved in EtOAc and washed with water. The water phase was reextracted with EtOAc and the combined organic phases were concentrated yielding **9** (347 mg, 86%). Mp 170 °C; v_{max}/cm^{-1} (solid) 3220 (OH), 1689 (C=O); ¹H NMR (MeOH-d₄) δ 7.58 (d, 1H, J = 11.7, H-6), 6.62 (d, 1H, J = 7.1, H-3), 3.85 (s, 3H, CH₃O); ¹⁹F NMR (MeOH-d₄) δ -147.0; ¹³C NMR (MeOH-d₄) δ 57.1; HRMS (FAB) calcd for C₈H₇FO₄ 187.0407 (M + H)⁺, found 187.0411.

5-Fluoro-4-hydroxy-2-methoxybenzyl alcohol (10)

Benzoic acid **9** (150 mg, 806 μ mol) was dissolved in THF (3 ml) and added slowly to trimethyl borate (0.73 ml, 6.4 mmol) and borane–dimethyl sulfide complex (0.5 ml, 5 mmol) in THF (6 ml). The solution immediately became milky with a white precipitate and was then stirred at room temperature for 24 h. After 4 h the mixture was clear. The reaction was quenched with MeOH (10 ml)

and the solvents were evaporated and the residue was evaporated from MeOH. Flash column chromatography (heptane–EtOAc 2 : 1) yielded **10** (128 mg, 92%) as an oil. $v_{\text{max}}/\text{cm}^{-1}$ (oil) 3297 (OH); ¹H NMR (MeOH-d₄) δ 7.01 (d, 1H, J = 11.5, H-6), 6.51 (d, 1H, J = 7.3, H-3), 4.49 (s, 2H, PhCH₂), 3.75 (s, 3H, CH₃); ¹⁹F NMR (MeOH-d₄) δ –148.8; HRMS (FAB) calcd for C₈H₉FO₃ 172.1537 (M)⁺, found 172.0546.

Ethyl (2-fluoro-4-hydroxymethyl-3-methoxyphenoxy)acetate (11)

Phenol 10 (305 mg, 1.77 mmol) and ethyl bromoacetate (395 µl, 3.56 mmol) in MeCN (22 ml) were stirred at room temperature and DBU (400 µl, 2.65 mmol) was added dropwise. The solution was refluxed for 4.5 h. The yellow solution was allowed to reach room temperature, poured into EtOAc (100 ml), and washed with HCl (0.05 M aq, 100 ml) and brine (100 ml). The organic phase was evaporated yielding 0.5 g crude material as a yellow oil. A first chromatography (heptane-EtOAc 3:1) gave 0.36 g partly purified material. A second chromatography (CH2Cl2-MeOH 100:1) gave 11, sufficiently pure for further use (329 mg, 72%). Mp 47 °C; $v_{\rm max}$ /cm⁻¹ (solid) 3282 (OH), 1766 (C=O); ¹H NMR (CDCl₃) δ 7.03 (d, 1H, J = 11.6, H-6), 6.54 (d, 1H, J = 6.8, H-3), 4.66 (s, 2H, $C(O)CH_2O$, 4.55 (s, 2H, PhCH₂), 4.24 (q, 2H, J = 7.1, CH₃CH₂), 3.76 (s, 3H, CH₃O), 2.57 (br s, 1H, OH), 1.27 (t, 3H, J = 7.1, CH₂CH₃); ¹⁹F NMR (CDCl₃) δ –143.6; HRMS (FAB) calcd for C₁₂H₁₅FO₅ 258.2429 (M)⁺, found 258.0907.

2-(2-Fluoro-4-hydroxymethyl-5-methoxyphenoxy)acetic acid (12)

Acetate **11** (321 mg, 1.24 mmol) was dissolved in THF–MeOH– water (3 : 1 : 1, 25 ml) and cooled to 0 °C. LiOH (1 M aq, 1.74 ml) was added slowly and the resulting solution was stirred at 0 °C for 30 min. The solution was allowed to reach room temperature and stirred for additional 2 h 15 min. After cooling to 0 °C the solution was acidified with HCl (1 M aq) and extracted with EtOAc (2 × 25 ml). The organic phases were washed with brine (25 ml) and evaporated yielding 0.36 g crude product. Chromatography (heptane–EtOAc 1 : 1 + 2.5% AcOH) gave **12** (233 mg, 81%). Mp 124 °C; v_{max} /cm ⁻¹ (solid) 3370 (OH), 1978 (C=O); ¹H NMR (MeOH-d₄) δ 7.10 (d, 1H, J = 11.9, H-6), 6.67 (d, 1H, J = 7.0, H-3), 4.72 (s, 2H, C(O)CH₂O), 4.52 (s, 2H, PhCH₂), 3.79 (s, 3H, CH₃O); ¹⁹F NMR (MeOH-d₄) δ –144.5; ¹³C NMR (MeOH-d₄) δ 172.5, 67.9, 59.6, 56.6; HRMS (FAB) calcd for C₁₀H₁₁FO₅ 230.1897 (M)⁺, found 230.0598.

Resin 1, 17 and 19

Preparation and analytical data in agreement with those previously reported.^{18,23}

Resin 15. Tentagel-HL-NH₂ (600 mg, 0.260 mmol) was swelled in distilled DMF (4 ml) and filtered. Linker **12** (88 mg, 0.382 mmol) and HOBt (55 mg, 0.407 mmol) were dissolved in distilled DMF (3 ml) and DIC (61 μ l, 0.392 mmol) was added. After stirring at room temperature for 5 min bromophenol blue (2 mM in DMF, 20 μ l) was added and the solution was added to the resin. The mixture was mechanically agitated for 15 h (until the mixture became yellow). The resin was washed with DMF and CH₂Cl₂ (5 × 5 ml each). CH₂Cl₂–Ac₂O–pyridine (1 : 1 : 1, 6 ml) was added to the resin and the mixture was agitated mechanically for 5 h. The resin was washed with DMF, CH₂Cl₂, DMF (5 × 5 ml each) and dry MeOH (2 × 5 ml). NaOMe (0.2 M in MeOH, 5 ml, 1.0 mmol) was added to the resin and the mixture was agitated mechanically for 15 h. The resin was washed with DMF (2 × 5 ml), MeOH (5 ml), and CH₂Cl₂ (5 × 5 ml) to give **15**. ¹⁹F NMR (CDCl₃) δ –144.7.

Resin 16. Fmoc-Gly-OH (232 mg, 0.780 mmol) and MSNT (233 mg, 0.786 mmol) was suspended in dry CH₂Cl₂ (3 ml) and *N*-methylimidazole (MeIm, 60 µl, 0.753 mmol) was added. The mixture was stirred for 5 min until the solution was clear. The solution was added to resin 1 (0.260 mmol), prewashed with dry CH₂Cl₂, and the mixture was agitated mechanically for 22 h. The resin was washed with CH₂Cl₂, DMF (3 × 5 ml each), and CH₂Cl₂ (2 × 5 ml). ¹⁹F NMR (CDCl₃) δ –144.5. Fmoc determination²⁴ was performed on 16 in order to determine the loading capacity of the resin bound linker yielding a loading capacity of 0.35 mmol g⁻¹ (given as a mean value of three independent measurements) instead of 0.42 mmol g⁻¹ given by the supplier.

Resin 18

Resin 16 (211 mmol) was treated with piperidine (20% in DMF, 2×7 ml, 5 and 15 min respectively). The resin was washed with DMF (3 \times 5 ml) and distilled DMF (3 \times 3 ml). FmocpF-L-Phe-OH (422 mg, 1.041 mmol) and HOBt (211 mg, 1.562 mmol) were dissolved in distilled DMF (3 ml). DIC (160 µl, 1.027 mmol) was added and the solution was stirred at room temperature for 5 min before bromophenol blue (2 mM in DMF, 20 µl) was added. The solution was added to the resin and the mixture was agitated mechanically for 2 h (until the mixture became yellow). The resin was washed with DMF and CH_2Cl_2 (3 × 5 ml each). ¹⁹F NMR (CDCl₃) δ -116.4, -144.4. The resin was treated with piperidine (20% in DMF, 2×7 ml, 5 and 15 min respectively) and washed with DMF (3×5 ml) and distilled DMF (3×3 ml). *o*,*p*-Difluorobenzoic acid (165 mg, 1.044 mmol) and HOBt (211 mg, 1.562 mmol) were dissolved in distilled DMF (3 ml). DIC (160 μ l, 1.027 mmol) was added and the solution was stirred at room temperature for 5 min before bromophenol blue (2 mM in DMF, 20 µl) was added. The solution was added to the resin and the mixture was agitated mechanically for 1 h 30 min (until the mixture became yellow). The resin was washed with DMF and CH_2Cl_2 (3 × 5 ml each) to give resin 18. ¹⁹F NMR (CDCl₃) δ -104.0, -108.8, -116.3, -144.5.

[N-(2,4-Difluorobenzoyl)-4-fluorophenylalanyl]glycine (20)

To resin **18** (53 µmol) TFA (5% or 20% in CH_2Cl_2 , 2 ml) was added and the mixture was mechanically agitated for 30 min. After extensive washings and ¹⁹F NMR spectroscopy the procedure was repeated three times. After a total of 7 h the combined filtrates were concentrated and preparative LC-MS gave **20** (20 mg, 96% based on Fmoc determination²⁴ of the linker resin **16**). Analytical data in agreement with previously reported.¹⁸

Resin 21

Preparation and analytical data in agreement with previously reported.¹⁵

Resin 23

Resin **21** (302 µmol), glycosyl donor **22** (387 mg, 608 µmol) and NIS (140 mg, 622 µmol) were dried under vacuum overnight in the absence of light. Distilled CH₂Cl₂ (4 ml) was added, followed by TfOH (1 µl, 0.01 mmol). The mixture was agitated mechanically for 3.5 h in the absence of light. The resin was washed with CH₂Cl₂, THF, piperidine (20% in DMF), DMF, and CH₂Cl₂ (3 × 15 ml each). Gel-phase ¹⁹F NMR spectroscopy indicated that resin **23** was prepared in a yield of 89%. ¹⁹F NMR (CDCl₃) δ –105.1, –105.4, –108.6, –113.4 and –144.5.

4-Fluorobenzoyl-L-glycyl-*O*-(β-D-galactopyranosyl)-L-seryl-L-valine (25)

A solution of TFA in H₂O (9 : 1, 10 ml) was added to resin **23** (150 µmol). The mixture was agitated mechanically at room temperature for 2 h. The resin was removed by filtration and washed with H₂O, THF and CH₂Cl₂. The filtrate was concentrated. Preparative HPLC yielded the partially deprotected glycopeptide **24** (34 mg, 29%). **24** was dissolved in MeOH (5 ml) and aqueous LiOH (0.81 ml, 0.1 M) was added dropwise. After 1 h the solution was neutralized with AcOH, concentrated, diluted with AcOH (2 ml), and lyophilized. Preparative HPLC yielded **25** (10.8 mg, 47%) with analytical data in agreement with those previously reported¹⁵ except for the data for the galactose hydrogens H-1 to H-4 which instead should be as follows. ¹H NMR (DMSO-*d*₆) Gal: 4.15–4.12 (m, 1H, J = 9.2, H-1), 3.97–3.93 (m, 1H, H-4), 3.64–3.62 (m, 1H, H-3), 3.31–3.28 (m, 1H, H-2) ¹⁹F NMR (DMSO-*d*₆) δ –109.2.

Resin 26

Resin 1 (0.120 mmol) and the glycosyl donor 22 (0.305 g, 0.479 mmol) were put under vacuum for one hour followed by addition of NIS (0.102 g, 0.453 mmol) in the absence of light. Distilled CH₂Cl₂ (4 ml) and a catalytic amount of TfOH were added. The mixture was stirred at room temperature for 3.5 h in the absence of light. The resin was washed with CH₂Cl₂, THF, 20% piperidine in DMF, DMF, and CH₂Cl₂ (3 × 15 ml). According to gel-phase ¹⁹F NMR spectroscopy the yield was 100% and only one isomer was formed. ¹⁹F NMR (CDCl₃) δ –105.0 (s, 1F, 4-*F*PhCO₂), –105.4 (s, 1F, 4-*F*PhCO₂), –113.5 (s, 1F, 3-*F*PhCHO₂), –134.0 (s, 1F, linker *F*).

Resin 27

Resin **15** (0.120 mmol) and the glycosyl donor **22** (0.301 g, 0.473 mmol) were put under vacuum for 1 h followed by addition of NIS (0.108 g, 0.480 mmol) in the absence of light. Distilled CH₂Cl₂ (4 ml) and TfOH (1.0 M in CH₂Cl₂, 0.017 mmol, 17 µmol) were added. The mixture was stirred at room temperature for 4 h in absence of light. The resin was washed with CH₂Cl₂, THF, 20% piperidine in DMF, DMF and CH₂Cl₂ (3 × 15 ml). According to gel-phase ¹⁹F NMR spectroscopy the yield was ~40%. When the glycosylation was repeated with glycosyl donor **22** (0.153 g, 0.240 mmol), NIS (0.051 g, 0.227 mmol) and catalytic amount of TfOH (1.0 M in CH₂Cl₂, 0.017 mmol, 17 µmol) for 2 h, the yield was increased to 63%. According to gel-phase ¹⁹F NMR spectroscopy two isomers were formed with the ratio 1 : 10. ¹⁹F

NMR (CDCl₃) δ major isomer -105.0 (s, 1F, 4-*F*PhCO₂), -104.5 (s, 1F, 4-*F*PhCO₂), -113.5 (s, 1F, 3-*F*PhHCO₂), -144.6 (s, 1F, linker *F*); minor isomer -105.7 (s, 1F, 4-*F*PhCO₂), -106.0 (s, 1F, 4-*F*PhCO₂), -113.2 (s, 1F, 3-*F*PhHCO₂), -145.0 (s, 1F, linker *F*).

2,3-Di-*O-p*-fluorobenzoyl-4,6-*O-m*-fluorobenzylidene-D-galactose (28)

The resin 27 (0.060 mmol) was treated with 5% TFA in CH_2Cl_2 for 7 h. The filtrate was washed with CH₂Cl₂ and the procedure was repeated. After extensive washing with CH₂Cl₂ the filtrates were combined and concentrated. Preparative LC-MS gave 15.4 mg 28 (58% total yield based on Fmoc determination of the linker resin 16). According to NMR spectroscopy two isomers were formed with the ratio $3.8 : 1.^{1}$ H NMR (CDCl₃) $\delta 8.11-7.99$ (m, 4H, ArH), 7.42-7.24 (m, 3H, ArH), 7.14-7.04 (m, 5H, ArH); major isomer 5.87 (dd, 3.4, 1H, J = 3.4, 10.6, H-3), 5.33-5.75 (m, 2H, H-2, H-1),5.59 (s, 1H, 3-FPhCHO₂), 4.68 (d, 1H, J = 3.3, H-4), 4.36 (dd, 1H, J = 1.2, 12.5, H-6, 4.23–4.15 (m, 2H, H-5, H-6); minor isomer 5.66 (dd, 1H, J = 8.0, 10.3, H-2), 5.60 (s, 1H, 3-FPhCHO₂), 5.49(dd, 1H, J = 3.6, 10.4, H-3), 4.61 (d, 1H, J = 3.4, H-4), 4.48 (dd, 1H, J = 1.4, 12.4, H-6), 3.77 (s-br, 1H, J = 10.2, H-5); ¹⁹F NMR $(CDCl_3) \delta$ major isomer -105.1 (s, 1F, 4-FPhCO₂), -105.2 (s, 1F, 4-FPhCO₂), -113.6 (s, 1F, 3-FPhHCO₂); minor isomer -104.7 (s, 1F, 4-FPhCO₂), -104.8 (s, 1F, 4-FPhCO₂), -113.5 (s, 1F, 3-FPhCHO₂); ¹³C NMR (CDCl₃) δ 99.9, 91.5, 74.4, 69.3, 69.1, 68.9, 62.4; HRMS (FAB) calcd form C₂₇H₂₁F₃NaO₈ 553.4358 (M + Na)⁺, found 553.1089.

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Notes and references

- 1 G. B. Fields, Z. Tian and G. Barany, in *Synthetic Peptides: A User's Guide*, ed. G. A. Grant, W. H. Freeman and Company, New York, 1992, pp. 77–183.
- 2 M. H. Caruthers, Science, 1985, 230, 281-285.
- 3 P. H. Seeberger and W.-C. Haase, Chem. Rev., 2000, 100, 4349-4393.
- 4 A. Svensson, K.-E. Bergquist, T. Fex and J. Kihlberg, *Tetrahedron Lett.*, 1998, **39**, 7193–7196.
- 5 J. M. Salvino, N. V. Kumar, E. Orton, J. Airey, T. Kiesow, K. Crawford, R. Mathew, P. Krolikowski, M. Drew, D. Engers, D. Krolikowski, T. Herpin, M. Gardyan, G. McGeehan and R. Labaudiniere, *J. Comb. Chem.*, 2000, 2, 691–697.
- 6 A. Svensson, T. Fex and J. Kihlberg, J. Comb. Chem., 2000, 2, 736-748.
- 7 M. Mogemark, M. Elofsson and J. Kihlberg, Org. Lett., 2001, 3, 1463–1466.
- 8 Y. Pan and C. P. Holmes, Org. Lett., 2001, 3, 2769-2771.
- 9 Y. Pan, B. Ruhland and C. P. Holmes, *Angew. Chem., Int. Ed.*, 2001, **40**, 4488–4491.
- 10 J. M. Salvino, S. Patel, M. Drew, P. Krowlikowski, E. Orton, N. V. Kumar, T. Caulfield and R. Labaudiniere, J. Comb. Chem., 2001, 3, 177–180.
- 11 H. Emtenäs, K. Åhlin, J. S. Pinkner, S. J. Hultgren and F. Almqvist, J. Comb. Chem., 2002, 4, 630–639.
- 12 M. Mogemark, M. Elofsson and J. Kihlberg, *ChemBioChem*, 2002, 3, 1266–1269.
- 13 M. Mogemark, M. Elofsson and J. Kihlberg, J. Org. Chem., 2003, 68, 7281–7288.
- 14 M. Mogemark, L. Gustafsson, C. Bengtsson, M. Elofsson and J. Kihlberg, Org. Lett., 2004, 6, 4885–4888.
- 15 M. Mogemark, F. Gårdmo, T. Tengel, J. Kihlberg and M. Elofsson, Org. Biomol. Chem., 2004, 2, 1770–1776.

- 16 M. Hourdin, G. Gouhier, A. Gautier, E. Condamine and S. R. Piettre, J. Comb. Chem., 2005, 7, 285–297.
- 17 F. K. Wallner, H. A. Norberg, A. I. Johansson, M. Mogemark and M. Elofsson, *Org. Biomol. Chem.*, 2005, **3**, 309–315.
- 18 F. K. Wallner and M. Elofsson, J. Comb. Chem., 2006, 8, 150-152.
- 19 S.-S. Wang, J. Am. Chem. Soc., 1973, 95, 1328-1333.
- 20 F. Guillier, D. Orain and M. Bradley, Chem. Rev., 2000, 100, 2091–2157.
- 21 K. M. Wells, Y.-J. Shi, J. E. Lynch, G. R. Humphrey, R. P. Volante and P. J. Reider, *Tetrahedron Lett.*, 1996, **37**, 6439–6442.
- 22 J. Broddefalk, M. Forsgren, I. Sethson and J. Kihlberg, J. Org. Chem., 1999, 64, 8948–8953.
- 23 F. K. Wallner and M. Elofsson, J. Comb. Chem., 2006, 8, 440-440.
- 24 M. Gude, J. Ryf and P. D. White, Lett. Pept. Sci., 2002, 9, 203-206.