A Novel Approach to the Solid-Phase Synthesis of Peptides with a Tetrazole at the C-Terminus

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Abstract: Peptidomimetics containing a C-terminal tetrazole can be easily prepared using modifications to traditional peptide synthesis protocols.

Key words: bioorganic chemistry, peptides, peptidomimetics, solid-phase synthesis, tetrazole

Tetrazoles have long been recognized as classical carboxylic acid isosteres.[1] The acidity of the tetrazole has been found to correspond well to that of the carboxyl group,[2] and it often exhibits greater metabolic stability.[3] Tetrazole-containing compounds have been explored, amongst others, as antibacterial,[4] antiviral,[5] and antiarrhythmic agents.[6]

As part of a study towards the development of novel inhibitors of peroxisomal protein trafficking we designed C-terminally modified analogues of the pentapeptide YQSKL (**1**), a well-studied ligand of the peroxisomal protein import receptor PEX5.[7] Our targets included those containing a C-terminal tetrazole equivalent of a range of amino acids (**2a**–**d**, Figure 1). Fmoc-protected tetrazole derivatives of leucine, tyrosine, phenylalanine, and cyclohexylalanine were synthesized using variations of literature procedures. Following conversion of the commercially available Fmoc-protected amino acids into the respective amides,[8] dehydration with cyanuric chloride[9] gave the nitrile. The protocol of Sharpless[10] gave a good conversion of the nitrile to the tetrazole for the leucine derivative; however, some optimization of the solvent system was required in order to prepare the tyrosine, phenylalanine, and cyclohexylalanine analogues as the precursors were insoluble in the recommended isopropanol–water mixture. Changing the solvent to a mixture of THF and water (10:1) went some way to address the problem, giving a solvent in which both the nitrile and the sodium azide were soluble and enabling the preparation of Fmoc-protected amino tetrazoles **3a**–**d**.[11]

With the required tetrazole building blocks in hand we needed to assemble the target peptidomimetics. Preparation of the naturally occurring sequence **1** is easily performed using solid-phase peptide synthesis. However, the standard approach to peptide assembly requires attachment of the peptide to the solid support via the C-terminal carboxylic acid. Reported approaches to C-terminal tetrazolyl peptides have employed solution-phase couplings[12] which we found to be very inefficient giving poor yields and difficult purifications. Hallberg has employed inverted N-to-C peptide assembly on solid phase to prepare tetrazole-containing protease inhibitors;[5] however, this methodology risks epimerization of the C-terminal residue during activation to give byproducts which are difficult to separate. We reasoned that if the tetrazole could provide a linkage to the solid support that was stable to peptide synthesis conditions, then assembly of the target sequences would be much more easily achieved.

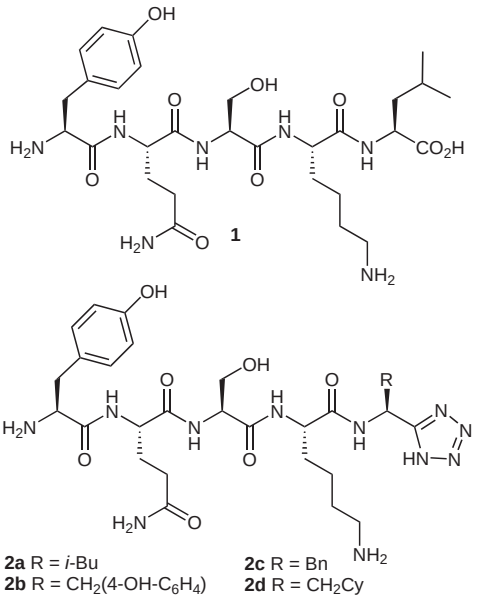

Figure 1 YQSKL **1** and tetrazole-containing peptidomimetic targets **2**.

We were delighted to observe that the Fmoc-protected amino tetrazole was easily coupled to 2-chlorotrityl chloride polystyrene resin under basic conditions[13] and that the trityl tetrazole linkage proved to be stable to standard peptide coupling procedures. The loaded resin was subjected to standard Fmoc deprotection using piperidine followed by chain extension to couple the remainder of the residues in the pentapeptide sequence, (lysine, serine, glutamine, and tyrosine). A cleavage cocktail of TFA

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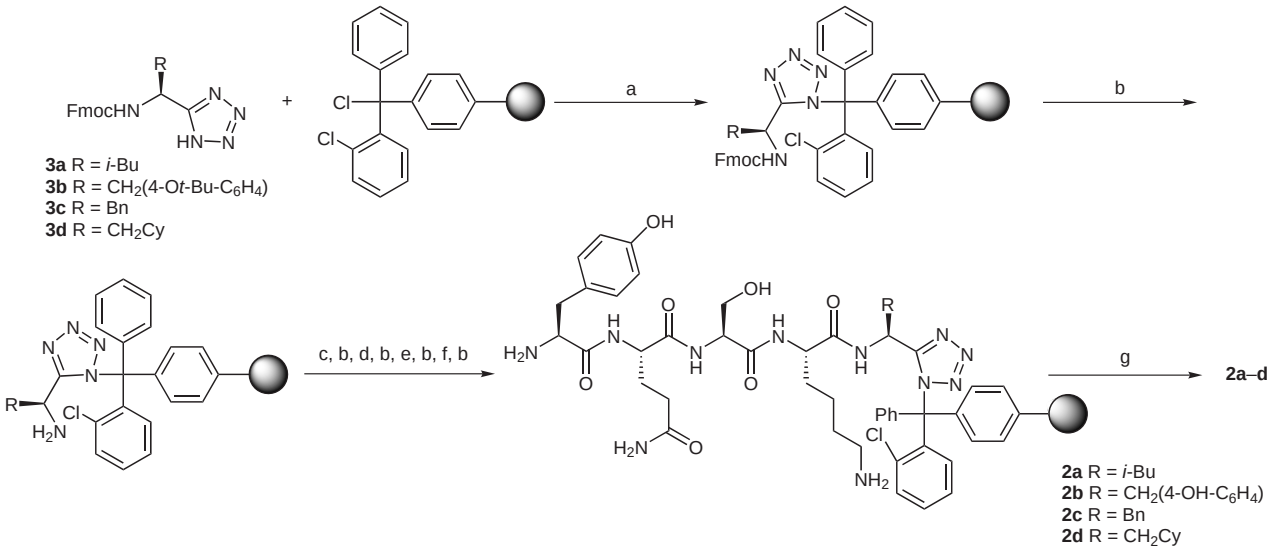

Scheme 1 *Reagents and conditions*: (a) DIPEA, CH_2Cl_2; (b) piperidine, DMF; (c) Fmoc-Lys(Boc)-OH (5 equiv), 2-(6-chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU, 4.9 equiv), HOBt (5 equiv), DIPEA (10 equiv); (d) Fmoc-Ser(*t*-Bu)-OH (5 equiv), HCTU (4.9 eqiuv), HOBt (5 equiv), DIPEA (10 equiv); (e) Fmoc-Gln(Trt)-OH (5 equiv), HCTU (4.9 equiv), HOBt (5 equiv), DIPEA (10 equiv); (f) Fmoc-Tyr(*t*-Bu)-OH (5 equiv), HCTU (4.9 equiv), HOBt (5 equiv), DIPEA (10 equiv); (g) TFA, *i*-Pr_3SiH, H_2O.

Table 1 Preparation of Fmoc-Protected Amino Tetrazoles and their Conversion into Pentapeptides

	Yields (%)			
Fmoc-protected amino acid	Acid to amide	Amide to nitrile	Nitrile to tetrazole	Tetrazole to peptide[c]
leucine	91	72	63[a]	96
tyrosine	91	75	40[b]	79
phenylalanine	90	78	34[b]	95
cyclohexylalanine	96	81	36[b]	78

[a] Solvent: *i*-PrOH–H_2O.
[b] Solvent: THF–H_2O.
[c] Yield of isolated **2** starting from **3**. Typically, approximately 10 mg of peptide were prepared.

containing 2.5% triisopropyl silane and 2.5% water gave efficient cleavage of the peptidomimetics from the resin and simultaneous global deprotection of the side chains to yield pentapeptides with a tetrazole moiety at the C-terminus in excellent purity and isolated yield (Scheme 1, Table 1).[14]

In summary, we have demonstrated that peptides containing a C-terminal tetrazole function can be easily prepared on solid phase by using the tetrazole as a point of attachment. The procedure is simple to perform and provides rapid access to this interesting range of peptidomimetics.

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(11) **Analytical Data of the Fmoc-Protected Tetrazoles**
Compound **3a**: mp 175–177 °C; R_f = 0.8 (EtOAc); $[\alpha]_D$ –32 (*c* 1, MeOH). IR (solid): ν_{max} = 3308, 2961, 1682, 1532 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ = 0.85 (3 H, d, *J* = 6.6 Hz, Lδ_1), 0.87 (3 H, d, *J* 6.6 Hz, Lδ_2), 1.54 (1 H, m, Lγ), 1.69 (1 H, m, Lβ_1), 1.75 (1 H, m, Lβ_2), 4.10 (1 H, t, *J* = 6.5 Hz, CH), 4.27 (1 H, dd, *J* = 10.5, 6.5 Hz CH_{2a}), 4.38 (1 H, dd, *J* = 10.4, 6.8 Hz, CH_{2b}), 4.96 (1 H, dd, *J* = 5.6, 9.8 Hz, Lα), 7.18 (2 H, t, *J* = 7.4 Hz, $Fmoc_{Ar2}$), 7.28 (2 H, t, *J* = 7.4 Hz, $Fmoc_{Ar3}$), 7.54 (2 H, t, *J* = 7.6 Hz, $Fmoc_{Ar1}$), 7.68 (2 H, d, *J* = 7.5 Hz, $Fmoc_{Ar4}$). ^{13}C NMR (75 MHz, CD_3OD): δ = 22.2 (Lδ), 23.5 (Lδ), 26.0 (Lγ), 43.5 (Lα), 43.5 (Lβ), 46.4 (CH_2), 68.1 (CH), 121.2 ($Fmoc_{Ar1}$), 126.4 ($Fmoc_{Ar2}$), 128.4 ($Fmoc_{Ar3}$), 129.1 ($Fmoc_{Ar4}$), 142.9 ($Fmoc_{Ar5}$), 145.4 ($Fmoc_{Ar6}$)

158.6 (CO), 160.5 ($L_{tetrazole}$). MS (ES): *m/z* calcd for $C_{21}H_{22}N_5O_2$: 376.1779; found $[M - H]^-$: 376.1773.
Compound **3b**: mp 97 °C; R_f = 0.13 (10% MeOH in CH_2Cl_2); $[\alpha]_D$ –32 (*c* 2, DMF). IR (solid): ν_{max} = 3308, 2976, 2763, 1679 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ = 1.10 (9 H, s, *t*-Bu), 3.06 (1 H, m, Yβ$_1$), 3.22 (1 H, m, Yβ$_2$), 3.98 (1 H, t, *J* = 7.0 Hz, CH), 4.10 (1 H, m, CH_{2a}), 4.20 (1 H, m, CH_{2b}), 5.11 (1 H, t, *J* = 6.0 Hz, Yα), 6.72 (2 H, d, *J* = 8.1 Hz, Y_{Ar1}), 7.00 (2 H, d, J, = 8.1 Hz, Y_{Ar2}), 7.18 (2 H, t, *J* = 7.2 Hz, $Fmoc_{Ar2}$), 7.27 (2 H, t, *J* = 7.3 Hz, $Fmoc_{Ar3}$), 7.48 (2 H, t, *J* 8.1 Hz, $Fmoc_{Ar1}$), 7.67 (2 H, d, *J* = 7.4 Hz, $Fmoc_{Ar4}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 29.2 (CMe_3), 38.2 (Yβ), 47.5 (CH), 56.3 (Yα), 67.5 (CH_2), 78.9 (OC*t*-Bu), 121.2 ($Fmoc_{Ar1}$), 125.5 ($Fmoc_{Ar2}$), 126.5 (Y_{Ar1}), 128.4 (Y_{ArCH2}), 129.1 ($Fmoc_{Ar3}$), 131.2 ($Fmoc_{Ar4}$), 133.3 (Y_{Ar2}), 142.9 ($Fmoc_{Ar5}$), 145.5 ($Fmoc_{Ar6}$), 155.7 ($Y_{ArOt\text{-}Bu}$), 158.4 (CO), 159.8 ($Y_{tetrazole}$). MS (ES): *m/z* calcd for $C_{28}H_{28}N_5O_3$: 482.2198; found $[M - H]^-$: 482.2180.
Compound **3c**: mp 186–190 °C; R_f = 0.43 (20% MeOH in CH_2Cl_2); $[\alpha]_D$ –28 (*c* 1, DMF). IR (solid): ν_{max} = 3316, 2899, 2469, 1680 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ = 3.19 (1 H, dd, *J* = 10.3,13.6 Hz, Fβ$_1$), 3.30 (1 H, dd, *J* = 6.0,13.6, Fβ$_2$), 4.16–4.25 (3 H, m, CH_2, CH), 5.14 (1 H, t, *J* = 6.3 Hz, Fα), 7.19–7.31 (7 H, m, F_{Ar}, $Fmoc_{Ar2}$), 7.42 (2 H, t, *J* = 7.4 Hz, $Fmoc_{Ar3}$), 7.63 (2 H, d, *J* = 5.3 Hz, $Fmoc_{Ar1}$), 7.89 (2 H, d, *J* = 7.5 Hz, $Fmoc_{Ar4}$). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 38.7 (Fβ), 46.9 (CH), 56.4 (Fα), 66.1 (CH_2), 120.5 ($Fmoc_{Ar1}$), 125.7 ($Fmoc_{Ar2}$), 126.9 (F_{Ar1}), 127.4 (FAr_2), 128.0 (F_{Ar3}), 128.6 ($Fmoc_{Ar3}$), 129.6 ($Fmoc_{Ar4}$), 137.5 ($_{FAr\text{-}CH2}$), 141.0 ($Fmoc_{Ar5}$), 144.1 ($Fmoc_{Ar6}$), 156.0 (CO), 156.6 ($F_{tetrazole}$). MS (ES): *m/z* calcd for $C_{24}H_{20}N_5O_2$: 410.1622; found $[M - H]^-$: 410.1633.
Compound **3d**: mp 178–181 °C. R_f = 0.32 (20% MeOH in CH_2Cl_2); $[\alpha]_D$ +3 (*c* 0.5, DMF). IR (solid): ν_{max} = 3291, 3041, 2924, 2851, 1704 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ = 0.79–1.02 (2 H, m, Cha), 1.07–1.23 (4 H, m, Cha), 1.30 (1 H, m, Chaγ), 1.56–1.87 (6 H, m, Cha, Chaβ), 4.28 (2 H, m, CH_2), 4.39 (1 H, m, CH), 5.00 (1 H, dd, *J* = 7.5, 15.2 Hz, Chaα), 7.33 (2 H, t, *J* = 6.8 Hz, $Fmoc_{Ar2}$), 7.43 (2 H, t, *J* = 8.6 Hz, $Fmoc_{Ar3}$), 7.72 (2 H, t, *J* = 8.9 Hz, $Fmoc_{Ar1}$), 7.90 (2 H, d, *J* = 8.1 Hz, $Fmoc_{Ar4}$). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 25.9 (Cha), 26.1 (Cha), 26.3 (Cha), 32.0 (Cha), 33.2 (Chaγ), 33.7 (Chaβ), 47.0 (CH), 52.5 (Chaα), 66.0 (CH_2), 120.5 ($Fmoc_{Ar1}$), 125.6 ($Fmoc_{Ar2}$), 127.4 ($Fmoc_{Ar3}$), 128.0 ($Fmoc_{Ar4}$), 141.1 ($Fmoc_{Ar5}$), 144.0 ($Fmoc_{Ar6}$), 156.2 (CO), 158.7 ($Cha_{tetrazole}$). MS (ES): *m/z* calcd for $C_{24}H_{26}N_5O_2$: 416.2092; found $[M - H]^-$: 416.2086.

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(13) A substoichiometric quantity of tetrazole was loaded relative to the theoretical loading of the resin. The success of loading was determined by isolation of the target peptide. Yields are calculated from the starting quantity of **3** employed in the loading reaction.

(14) **Typical Procedure**
2-Chlorotrityl chloride resin (Novabiochem, 100–200 mesh, 100 mg, 1.4 mmol/g loading) was suspended in CH_2Cl_2 (1 mL) and agitated (30 min), then drained. A solution of Fmoc-amino tetrazole (0.08 mmol) and DIPEA (78.2 μL, 0.47 mmol) in DMF (1 mL) was added and the mixture agitated (4 h). The solution was removed and the resin was washed (DMF, 2 × 1 mL, 2 min) and then treated with a solution of CH_2Cl_2–MeOH–DIPEA (80:15:5, 2 × 1 mL, 5 min) and then was washed again (DMF, 3 × 1 mL, 2 min). The Fmoc group was removed (25% piperidine in DMF, 2 × 1 mL, 2 min). Finally, the resin was washed with DMF (6 × 1 mL, 2 min), *i*-PrOH (3 × 1 mL, 5 min) and hexane (4 × 1 mL, 2 min), sucked dry in air (10 min) then dried in vacuo, over KOH (16 h) and stored at 5 °C. The loaded resin was subject to standard Fmoc solid-phase peptide synthesis conditions: Couplings were performed with a solution of the amino acid to be coupled (5 equiv), HOBt (5 equiv), HCTU (4.9 equiv), and DIPEA (10 equiv) in DMF for 60 min and Fmoc deprotection was performed using a solution of 20% piperidine in DMF. Cleavage from the resin and side-chain deprotection was effected with TFA containing 2.5% triisopropyl silane and 2.5% H_2O. Peptides were purified by preparative HPLC where necessary (with the exception of **2a**; all the other peptides were single peaks by HPLC following cleavage from the resin, and could be used without further purification).
Compound **2a**: ^1H NMR (500 MHz, D_2O): δ = 0.89 (3 H, d, *J* = 6.6 Hz, Lδ$_1$), 0.94 (3 H, d, *J* = 6.6 Hz, Lδ$_2$), 1.26–1.41 (2 H, m, Kγ), 1.55–1.68 (3 H, m, Lγ, Kδ), 1.70–1.87 (3 H, m, Kβ$_1$, Lβ$_1$, Kβ$_2$,), 1.94 (1 H, m, Qβ$_1$), 2.04 (1 H, m, Qβ$_2$), 2.31 (2 H, at, *J* = 7.6 Hz, Qγ), 2.94 (2 H, at, *J* = 7.6 Hz, Kε), 3.11 (1 H, dd, *J* = 5.6, 14.2 Hz, Yβ$_1$), 3.13 (1 H, dd, *J* = 5.0, 14.2 Hz, Yβ$_2$), 3.83 (1 H, dd, *J* = 5.8, 11.4 Hz, Sβ$_1$), 3.88 (1 H, dd, *J* = 5.9, 11.4 Hz, Sβ$_2$) 4.22 (1 H, t, *J* = 7.3 Hz, Yα), 4.34 (1 H, t, *J* = 7.2 Hz, Kα), 4.38 (2 H, m, Sα, Qα), 5.33 (1 H, dd, *J* = 6.0, 9.6 Hz, Lα), 6.85 (2 H, d, *J* = 8.4 Hz, Y_{Ar1}), 7.12 (2 H, d, *J* = 8.4 Hz, Y_{Ar2}). ^{13}C NMR (125 MHz, D_2O): δ = 21.2 (Lδ$_1$), 22.2 (Lδ$_2$), 22.3 (Kγ), 24.6 (Lγ), 26.6 (Kδ), 27.7 (Qβ), 30.7 (Kβ), 31.3 (Qγ), 36.4 (Yβ), 39.6 (Kε), 41.3 (Lβ), 43.6 (Lα), 53.1 (Qα), 54.0 (Kα), 54.7 (Yα), 56.0 (Sα), 61.5 (Sβ), 116.2 (Y_{Ar1}), 125.7 (Y_{ArCH}), 131.2 (Y_{Ar2}), 155.6 (Y_{ArOH}), 158.6 ($L_{tetrazole}$), 169.3 (Yco), 172.0 (Sco), 172.5 (Qco), 173.8 (Kco), 178.2 (Q_{CONH2}). MS (ES): *m/z* calcd for $C_{29}H_{46}N_{11}O_7$: 660.3587; found $[M - H]^-$: 660.3601.
Compound **2b**: ^1H NMR (500 MHz, D_2O): δ = 1.15–1.23 (2 H, m, Kγ), 1.57–1.61 (4 H, m, Kδ, Kβ$_1$, Kβ$_2$), 1.90 (1 H, m, Qβ$_1$), 2.01 (1 H, m, Qβ$_2$), 2.30 (2 H, t, *J* = 7.5 Hz, Qγ), 2.91 (2 H, t, *J* = 7.5 Hz, Kε), 3.11 (2 H, d, *J* = 7.0 Hz, Y5β), 3.21 (1 H, dd, *J* = 9.4, 14.0 Hz, Y1β$_1$), 3.32 (1 H, dd, *J* = 6.5, 14.0 Hz, Y1β$_2$), 3.75 (1 H, dd, *J* = 5.8, 11.4 Hz, Sβ$_1$), 3.85 (1 H, dd, *J* = 5.8, 11.3 Hz, Sβ$_2$), 4.19–4.27 (2 H, m, Y1α, Kα), 4.36 (2 H, m, Sα, Qα), 5.49 (1 H, t, *J* = 6.5 Hz, Y5α), 6.82 (2 H, d, *J* = 8.3 Hz, $Y1_{Ar1}$), 6.84 (2 H, d, *J* = 8.3 Hz, $Y5_{Ar1}$), 7.08 (2 H, d, *J* = 8.4 Hz, $Y1_{Ar1}$), 7.11 (2 H, d, *J* = 8.4 Hz, $Y5_{Ar1}$). ^{13}C NMR (125 MHz, D_2O): δ = 24.7 (Kγ), 29.1 (Kδ), 30.2 (Qβ), 33.2 (Kβ), 33.7 (Qγ), 38.9 (Yβ), 40.1 (Yβ), 42.0 (Kε), 49.0 (Y_5α), 55.5 (Qα), 56.6 (Kα), 57.2 (Y_1α), 58.4 (Sα), 63.9 (Sβ), 118.4 ($Y1_{Ar1}$), 118.7 ($Y5_{Ar1}$), 128.2 ($Y1_{Ar\text{-}CH}$), 130.6 ($Y5_{Ar\text{-}CH}$), 133.5 ($Y1_{Ar2}$), 133.7 ($Y5_{Ar2}$), 157.4 ($Y1_{ArOH}$), 158.0 ($Y5_{ArOH}$), 160.6 ($Y5_{tetrazole}$) 171.8 (Yco), 174.4 (Sco), 174.9 (Qco), 176.1 (Kco), 180.6 (Q_{CONH2}). MS (ES): *m/z* calcd for $C_{32}H_{46}N_{11}O_8$: 712.3525; found $[M + H]^+$: 712.3538.
Compound **2c**: ^1H NMR (500 MHz, D_2O): δ = 1.15–1.29 (2 H, m, Kγ), 1.56–1.66 (4 H, m, Kδ, Kβ$_1$, Kβ$_2$), 1.94 (1 H, m, Qβ$_1$), 2.02 (1 H, m, Qβ$_2$), 2.30 (2 H, t, *J* = 7.5 Hz, Qγ), 2.91 (2 H, t, *J* = 7.5 Hz, Kε), 3.13 (2 H, d, *J* = 6.7 Hz, Yβ), 3.30 (1 H, dd, *J* = 9.9, 13.6 Hz, Fβ$_1$), 3.40 (1 H, dd, *J* = 7.2, 13.6 Hz, Fβ$_2$), 3.76 (1 H, dd, *J* = 6.4, 11.2 Hz, Sβ$_1$), 3.87 (1 H, dd, *J* = 6.0, 11.2 Hz, Sβ$_2$), 4.23 (1 H, t, *J* = 7.3 Hz, Yα), 4.28 (1 H, t, *J* = 7.0 Hz, Kα), 4.35–4.40 (2 H, m, Sα, Qα), 5.55 (1 H, t, *J* = 7.9 Hz, Fα), 6.87 (2 H, d, *J* = 8.2 Hz, Y_{Ar1}), 7.13 (2 H, d, *J* = 7.9 Hz, Y_{Ar2}), 7.23 (2 H, d, *J* = 6.4 Hz, F_{Ar1}), 7.30–7.38 (3 H, m, F_{Ar2}, F_{Ar3}). ^{13}C NMR (125 MHz, D_2O): δ = 24.7 (Kγ), 29.0 (Kδ), 30.1 (Qβ), 33.2 (Kβ), 33.7 (Qγ), 38.8 (Yβ), 41.1 (Fβ), 41.9 (Kε), 49.1 (Fα), 55.5 (Qα), 56.5 (Kα), 57.1 (Yα), 58.3 (Sα), 63.9 (Sβ), 118.7 (Y_{Ar2}) 128.2 (Y_{ArCH2}), 130.1 (F_{Ar3}), 131.6 (F_{Ar2}), 132.1 (F_{Ar1}), 133.6 (Y_{Ar1}), 138.8

(F_{ArCH2}), 158.0 (Y_{ArOH}), 161.0 ($F_{tetrazole}$), 171.7 (Yco), 174.3 (Sco), 174.9 (Kco), 176.0 (Qco), 180.6 (Q_{CONH2}). MS (ES): *m/z* calcd for $C_{32}H_{46}N_{11}O_7$: 696.3576; found $[M + H]^+$: 696.3587.

Compound **2d**: ^{1}H NMR (500 MHz, D_2O): δ = 0.89–1.04 (2 H, m, Cha), 1.16 (4 H, m, Cha), 1.23–1.31 (1 H, m, Cha), 1.32–1.42 (3 H, m, Kγ, Chaγ), 1.57–1.72 (6 H, m, Cha, Kδ), 1.75 (2 H, m, $K\beta_1$, $Cha\beta_1$), 1.84 (2 H, m, $K\beta_2$, $Cha\beta_2$), 1.94 (1 H, m, $Q\beta_1$), 2.04 (1 H, m, $Q\beta_2$), 2.32 (2 H, t, *J* = 7.6 Hz, Qγ), 2.94 (2 H, t, *J* = 8.1 Hz, Kε), 3.12 (2 H, d, *J* = 7.3 Hz, Yβ), 3.83 (1 H, dd, *J* = 5.7, 11.3 Hz, $S\beta_1$), 3.90 (1 H, dd, *J* = 5.7, 11.3 Hz, $S\beta_2$), 4.22 (1 H, t, *J* = 7.2 Hz, Yα), 4.33 (1 H, dd, *J* = 6.4, 8.2 Hz, Kα), 4.37–4.41 (2 H, m, Sα, Qα), 5.36 (1 H, dd, *J* = 6.4, 9.5 Hz, Chaα), 6.85 (2 H, d, *J* = 8.5 Hz, Y_{Ar1}), 7.12 (2 H, d, *J* = 8.2 Hz, Y_{Ar2}). ^{13}C NMR (125 MHz, D_2O): δ = 24.8 (Kγ), 28.5 (Cha), 28.6 (Cha), 28.8 (Cha), 29.1 (Kδ), 30.2 (Qβ), 33.1 (Kβ), 33.7 (Qγ), 34.5 (Cha), 35.6 (Cha), 36.4 (Chaγ), 38.8 (Yβ), 42.0 (Kε), 42.3 (Chaβ), 45.4 (Chaα), 55.5 (Qα), 56.4 (Kα), 57.1 (Yα), 58.5 (Sα), 63.9 (Sβ), 118.7 (Y_{Ar2}), 128.2 (Y_{ArCH2}), 133.7 (Y_{Ar1}), 158.0 (Y_{ArOH}), 161.3 ($Cha_{tetrazole}$), 171.6 (Y_{CO}), 174.4 (S_{CO}), 174.9 (K_{CO}), 176.2 (Q_{CO}), 180.6 (Q_{CONH2}). MS (ES): *m/z* calcd for $C_{32}H_{51}N_{11}O_7$: 702.4046; found $[M + H]^+$: 702.4048.