Synthesis and Characterisation of Glucosamine-NSAID Bioconjugates

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Complete List of Authors:	Jones, Rachel; University of Florida, Department of Chemistry Thillier, Yann; University of Florida, Department of Chemistry Panda, Siva; University of Florida, Department of Chemistry Rivera Rosario, Nicole; University of Florida, Department of Chemistry Hall, Dennis; University of Florida, Department of Chemistry Katritzky, Alan; University of Florida, Department of Chemistry; King Abdulaziz University, Department of Chemistry

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Rachel A. Jones,[a] Yann Thillier,[a] Siva S. Panda,[a] Nicole Rivera Rosario,[a] C. Dennis Hall,[*a] and Alan R. Katritzky,[‡a,b]

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Strategies to couple non-steroidal anti-inflammatory drugs (NSAIDs) to a glucosamine hydrochloride salt *via* an amino acid linker are investigated and a series of novel NSAID-glucosamine bioconjugates have been prepared.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are an important class of compound used for the management of pain, in particular as a treatment for osteo- and rheumatoid arthritis.[1] NSAIDs reduce pain and swelling associated with arthritis by inhibition of the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). This leads to decreased biosynthesis of prostaglandins (PGs), resulting in a reduced inflammatory response. However, PGs are also implicated in maintaining the integrity of gastric mucosa as well as contributing to the cytoprotection of the gastric epithelium. Thus inhibition of PG biosynthesis, can result in the formation of gastric ulcers.[2] In addition to side effects related to the gastrointestinal (GI) tract,[3] the long term use of NSAIDs is associated with diverse risks,[2] including nephrotoxicity, hepatotoxicity,[2a] and an increased risk of heart disease.[4] Indomethacin **1a**, mefenamic acid **1b** and diclofenac acid **1c** (Figure 1), widely prescribed NSAIDs,[5] are non-selective COX-1 and COX-2 inhibitors; COX-2 is upregulated in inflammatory cells,[6] whereas COX-1 is responsible for the majority of prostaglandin synthesis in normal GI tract.[7]

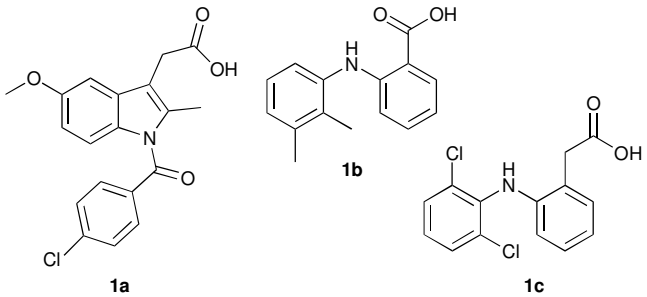


Figure 1 Structure of common NSAIDs

Thus the development of selective COX-2 inhibitors, potentially with fewer side effects related to the GI tract, is a challenging area of continuing scientific study that has met with varying degrees of success.[8] Meanwhile there are several methods to reduce the side effects of NSAIDs, including prescribing the lowest possible dose for the shortest time necessary to control symptoms.[9] Concomitant administration of NSAIDs with gastro-protective drugs or synthetic prostaglandins may also reduce potential side effects.[2a] In addition, the intrinsic acidic nature of most NSAIDs results in local action on the gastric epithelium. Thus masking the carboxylic acid functionality of these drugs as pro-drugs can reduce this topical irritation. Various synthetic strategies to reduce gastric ulceration caused by NSAIDs have been investigated, including substitution of aromatic protons for fluorine,[10] synthesis of ester or amide pro-drugs[11] and conjugation of NSAIDs to carbohydrates,[12] gastro-protective drugs[2c] and acetaminophen.[13] Galanakis *et al.*[14] showed that amidation of indomethacin and diclofenac with L-cysteine ethyl ester resulted in pro-drugs with enhanced anti-inflammatory properties in addition to anti-oxidant properties. Recently, Dang *et al.*[15] coupled ketoprofen to glucosamine as part of their studies on the synthesis and characterisation of *N*-acetyl-tetra-*O*-acyl glucosamine derivatives.

Glucosamine **4** is found in almost all human tissue, and is highly concentrated in connective tissue, particularly in cartilage.[16] The use of glucosamine supplements as a complementary treatment for the symptoms of osteoarthritis is widespread in the US and these supplements are typically taken either as glucosamine sulphate or in combination with chondroitin.[17] The efficacy of glucosamine as a treatment for relieving pain in patients with osteoarthritis has not been proven;[18] but there is evidence that in patients who have moderate to severe knee pain, glucosamine in combination with chondroitin may have some effect.[19] In spite of the conflicting data there is no clear evidence against taking glucosamine,[16] and there are no known, adverse side effects associated with

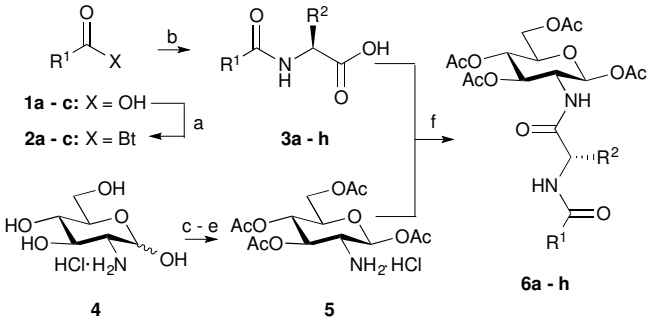
taking this supplement.[20] Furthermore, long-term supplementation with glucosamine and chondroitin may prevent or delay joint surgery associated with the progression of osteoarthritis.[21]

It is known that amino acids can be used as carriers for drugs due to their ability to transport into mammalian tissue. The coupling of amino acids to biologically active compounds resulting in enhanced pharmaceutical activity has been widely explored by the Katritzky group.[13,22] Furthermore, amino acids have been used as synthetic vectors to increase the lipophilicity of the target compounds, thus improving their bioavailability.

It therefore seemed attractive to design new NSAID bioconjugates linked to a glucosamine scaffold *via* an amino acid and we report herein synthetic studies on the preparation of these novel conjugates.

Results and discussion

We designed a convergent route for the preparation of NSAID-glucosamine bioconjugates **6a** - **f** from NSAID-amino acid derivatives **3a** - **d** and glucosamine hydrochloride (GlcN) **5** (Scheme 1).[23]



R^1 = NSAID [indomethacin (**1a**), mefenamic acid (**1b**) or diclofenac (**1c**)]
R^2 = H, CH_3, $CH(CH_3)_2$, or CH_2Ph

Scheme 1 Proposed synthesis of glucosamine-NSAID bioconjugates. (a) BtH, $SOCl_2$, THF or BtH, DCC, CH_2Cl_2 (**2a:** 93%, **2b:** 82%, **2c**: *used as crude product*); (b) glycine, alanine, valine or phenylalanine, Et_3N, H_2O/MeCN (**3a** - **h:** *see Table 1*); (c) *p*-anisaldehyde, NaOH (1M); (d) Ac_2O, py; (e) HCl (5M), acetone;[23] (f) HOBt, EDCI, Et_3N, THF, 0 °C to r.t., 24h (**6a** - **h:** *see Table 3*).

There are numerous methods for the preparation of NSAID-amino acid conjugates, including the use of coupling agents,[11b,14] or the preparation of the corresponding *N*-hydroxysuccinimde esters[24] or acid chloride[25] derivatives of the NSAID. However, these strategies are often used with *C*-protected amino acids, resulting in an additional deprotection step to access the target molecule.[26] It is noteworthy that activation of NSAIDs as *N*-acylbenzotriazoles affords an efficient and straightforward strategy for the *N*-acylation of unprotected amines or amides.[11a,27] This procedure was used to access benzotriazolides **2a** - **c** in good to excellent yields (Scheme 1) and subsequent *N*acylation of amino acids glycine, L-alanine, L-valine and L-phenylalanine proceeded to afford the desired products (**3a** - **d**) in the cases of indomethacin and mefenamic acid (Table 1). All attempts to prepare diclofenac-amino acid conjugates (Table 1, entries 5 and 6) using benzotriazole methodology resulted in cyclisation of the activated diclofenac to form the thermodynamically favoured oxindole;[28] trace quantities of the desired products were observed by ^{1}H NMR of the crude reaction mixture.

Table 1: NSAID-amino acid conjugates

Entry	Product	Isolated Yield (%)
1	Indomethacin-L-Ala-OH, **3a**	91
2	Indomethacin-L-Phe-OH, **3b**	55
3	Mefenamic acid-L-Val-OH, **3c**	86
4	Mefenamic acid-L-Phe-OH, **3d**	84
5	Diclofenac-L-Ala-OH, **3e**	not isolated
6	Diclofenac-L-Val-OH, **3f**	not isolated
7	Indomethacin-Gly-OH, **3g**	91
8	Indomethacin-GABA-OH, **3h**	84

The *N*-aminoacylation of GlcN **5** by *N*-protected amino acids has been achieved previously using dicyclohexylcarbodiimide (DCC) or mixed anhydride methods.[29] In the case of mefenamic acid-amino acid conjugates, these strategies were unsuccessful, however HOBt in combination with EDCI and Et_3N afforded access to indomethacin-carbohydrate conjugates **6a** and **6b** in yields of 36% and 48% respectively.

^{1}H NMR analysis of the desired compounds showed two sets of peaks, which were attributed to the presence of amide rotamers, possibly resulting from the rigidity of the indole ring system and its close proximity to the 'R' groups of the amino acid linker. To confirm this hypothesis, compound **6a** was examined by variable temperature ^{1}H NMR, from which it was shown that coalescence occurred at 95 °C with an average $\Delta_G^{\ddagger}$ = 20.9 kcal mol^{-1} (Figure 2 and ESI).

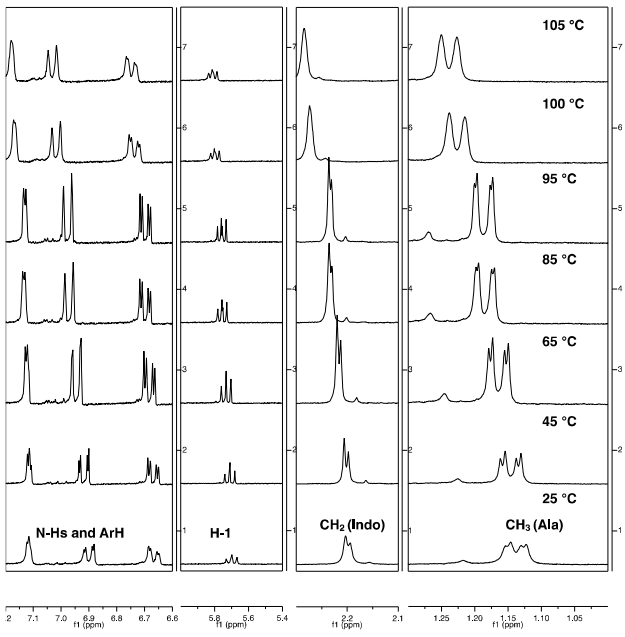
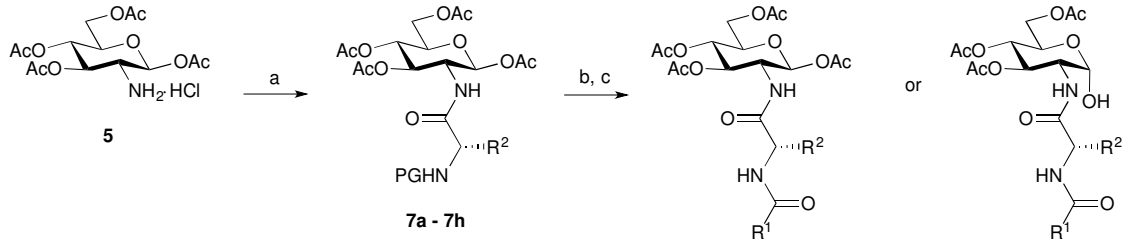


Figure 2 Variable temperature ^{1}H NMR of **6a** (selected peaks) showing coalescence at 95 °C (*see ESI for full spectrum*).

We hypothesised that the absence of the 'R' group on the amino acid or the presence of a longer amino acid linker between the carbohydrate scaffold and indomethacin moieties would

remove the presence of rotamers. Thus, indomethacin-glycine (**3g**) and indomethacin-γ-aminobutyric acid (**3h**) were prepared in yields of 91% and 84% respectively. Reactions with GlcN **5** afforded glycoconjugates **6g** in 65% yield and **6h** in 70% yield. ^{1}H analysis of the final products showed that only one compound was present, indicating that the presence of an amino acid linker bearing a side chain is responsible for the restricted rotation, leading to the observation of rotamers.

Since we were not able to access either the diclofenac or mefenamic acid derived target molecules following the chemical route described in Scheme 1, we decided to explore an alternative strategy. Thus, an *N*-protected amino acid was first coupled to the monosaccharide building block and subsequent deprotection of the amino acid afforded a free amine ready to couple with the desired NSAID (Scheme **2**).



R^1 = NSAID (indomethacin, mefenamic acid or diclofenac), R^2 = H, CH_3, $CH(CH_3)_2$, or CH_2Ph

Scheme 2 (a) *N*-Boc-AA-OH or *N*-Cbz-AA-OH, EDCI, HOBt, Et_3N, THF, 0 °C to r.t., 24 h (**7a - h:** *see Table 2*); (b) H_2, Pd/C, MeOH; (c) **1a**, **1b** or **1c**, EDCI, HOBt, DIPEA, THF, 0 °C to r.t., 24 h (**6a - h:** *see Table 3*).

Table 2: NSAID-amino acid conjugates

Compound	Product	Isolated Yield (%)
7a	*N*-Boc-Gly-GlcN	82
7b	*N*-Boc-L-Ala-GlcN	71
7c	*N*-Boc-L-Val-GlcN	75
7d	*N*-Boc-L-Phe-GlcN	89
7e	*N*-Cbz-Gly-GlcN	63
7f	*N*-Cbz-L-Ala-GlcN	57
7g	*N*-Cbz-L-Val-GlcN	69
7h	*N*-Cbz-L-Phe-GlcN	67

To that end *N*-Boc and *N*-Cbz protected amino acids (glycine, L-alanine, L-valine and L-phenylalanine) were coupled with GlcN **5** in good to excellent yields (Table 2), using modified literature conditions previously used for the successful conjugation of *N*-Fmoc protected amino acids to GlcN **5**.[30] It is note-worthy that the *N*-Boc protected products (**7a** - **d**) were isolated in analytically pure form (passing CHN analysis) without any further purification.

In order to access the free amine of conjugates **7a** - **h** we found that deprotection of the Cbz group under neutral conditions occurred in quantitative yield and was preferable to the acidic conditions required to cleave the Boc group.[31] With the free amine in hand, various acylation conditions were investigated to access the target compounds. We first attempted the reaction of benzotriazolide **2a** with the free amine; but optimised reaction conditions afforded desired product **6a** in only 28% yield.[32] Similarly low yields were observed in the case of previously inaccessible mefenamic acid conjugates **6c** and **6d**. The conjugation of indomethacin **1a**, mefenamic acid **1b** and diclofenac **1c** was achieved under HOBt/EDCI mediated coupling (Table 3), however, when phenylalanine and valine were present in the target compounds, *N*-Cbz deprotection resulted in concomitant anomeric deacetylation to afford hemi-acetals, present as the α-anomer only (Scheme 2). Although unexpected, mutarotation of *N*-amino acyl glucoconjugates under catalytic hydrogenolysis conditions was first reported by Liefländer in 1967.[33] In the case of glycine and alanine, the final products were isolated as tetra-acetates with retention of anomeric stereochemistry, as β-anomers only.

Table 3: NSAID-amino acid-glucosamine conjugates

Entry	NSAID	Amino Acid	Product	Scheme 1: Isolated Yield (%)	Scheme 2: Isolated Yield (%)
1	Indomethacin	L-Ala	**6a**	36	80
2	Indomethacin	L-Phe	**6b**	48	not isolated[a]
3	Indomethacin	L-Phe	**6b'**	not isolated[a]	53
4	Mefenamic Acid	L-Val	**6c'**	*n/a*[b]	52
5	Mefenamic Acid	L-Phe	**6d'**	*n/a*[b]	64
6	Diclofenac	L-Ala	**6e**	*n/a*[c]	57
7	Diclofenac	L-Phe	**6f'**	*n/a*[c]	71
8	Indomethacin	Gly	**6g**	65	n. d.
9	Indomethacin	GABA	**6h**	70	*n/a*[d]

[a] Product **6b'** or **6b** was isolated instead of compound **6b** or **6b'**.
[b] Attempts to prepare mefenamic acid derived conjugates following scheme 1 were unsuccessful.
[c] The synthesis of diclofenac conjugates following scheme 1 was not attempted.
[d] Reaction was not carried out
n.d. = not determined

Conclusions

In conclusion, we have developed methods for the conjugation of NSAIDs to glucosamine hydrochloride *via* an amino acid linker. Using the previously developed benzotriazole methodology, the synthesis of indomethacin or mefenamic acid-amino acid conjugates was achieved in good to excellent yields. Glucosamine-NSAID bioconjugates were accessed using two different protocols. For amino acid linkers bearing no

R groups (Gly and GABA) the coupling of NSAID-amino acid conjugates was found to be most successful. For amino acid linkers bearing larger R groups (Ala, Val and Phe), the reaction of a protected amino acid with GlcN followed by deprotection and coupling with the NSAID was the preferred protocol. Furthermore the presence of rotamers was observed due to a lack of free rotation about amide bonds caused by the close proximity of the amino acid R group to both the NSAID and the carbohydrate moiety.

Experimental

General methods and materials

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, DMSO-d_6, or CD_3OD using a 300 or 500 MHz spectrometer, assignments were made using 2D NMR experiments. The spectra were referenced relative to the residual solvent peak ($CDCl_3$: ^{1}H = 7.27 ppm, ^{13}C = 77.0 ppm; DMSO-d_6: ^{1}H = 2.50 ppm, ^{13}C = 39.5 ppm; CD_3OD: ^{1}H = 3.31 ppm, ^{13}C = 49.0). The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, dd = doublet of doublets, ddd = doublet of doublets of doublets, and dt = doublet of triplets. Mass spectrometry was performed with electrospray ionization (ESI). In the case of rotamers, the MS was obtained from mixed fractions, as it was not always possible to separate the individual product compounds using silica gel column chromatography. Variable temperature ^{1}H NMR spectrum was obtained in the case of compound **6a** and showed the peaks coalescing at elevated temperature. Ether refers to diethyl ether.

General procedure (GP) Ia for the preparation of NSAID-amino acids derivatives

Unprotected amino acid (1 eq) was dissolved in H_2O. Triethylamine (Et_3N) (3 eq) was added, and, if appropriate, MeCN to aid solubility. The appropriate benzotriazolide (**2a** or **2b**) was added and the reaction mixture was diluted with MeCN and H_2O to produce a clear solution. It was stirred at r.t. until complete consumption of the starting materials as observed by TLC (*n*-hexanes/EtOAc, 1:1, v/v). 4 M HCl (2 mL) was added and MeCN was removed under reduced pressure. Crushed ice was added to the reaction vessel the formed crystals were washed sequentially with H_2O (2 x 10 mL) and 4 M HCl (3 x 10 mL). The crystals were collected and dried under high vacuum to yield the desired NSAID-amino acid bioconjugate.

GP Ib for the preparation of NSAID-amino acids derivatives

Repeated as GP Ia, however during w/u, on addition of crushed ice if no crystals were formed, the residue was taken up in EtOAc and washed with 4 M HCl (3 x 20 mL). The combined aqueous layers were back-extracted with EtOAc (1 x 20 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to afford the desired NSAID-amino acid bioconjugate.

GP IIa for the acylation of glucosamine with *N*-protected amino acids

N-protected amino acid (2.6 mmol) was dissolved in anhydrous THF (10 mL) and cooled to 0 °C. HOBt (3.2 mmol) and EDCI (3.2 mmol) were added the reaction mixture was allowed to stir at this temperature for 60 minutes. GlcN **5** (2.6 mmol) was dissolved in anhydrous THF (20 mL) and Et_3N (0.36 mL, 2.6 mmol) was added. After 60 minutes the monosaccharide was added to the reaction vessel containing the *N*-protected amino acid and a further portion of THF (10 mL) was added. The reaction mixture was allowed to warm to r.t. and stirred for a further 16 h until complete consumption of the starting material was observed by TLC (CH_2Cl_2/MeOH, 9:1, v/v). The reaction mixture was concentrated under reduced pressure and the residue was taken up in EtOAc. The organic layer was washed sequentially with 2M HCl (3 x 30 mL), $NaHCO_{3\ (satd.\ aq)}$ (30 mL), H_2O (30 mL) and brine (30 mL). It was then dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford the desired product.

GP IIb for the acylation of glucosamine with indomethacin-amino acid conjugates

Indomethacin-amino acid conjugate (2.6 mmol) was dissolved in anhydrous THF (10 mL) and cooled to 0 °C. HOBt (3.2 mmol) and EDCI (3.2 mmol) were added the reaction mixture was allowed to stir at this temperature for 60 minutes. 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine hydrochloride **5** (2.6 mmol) was dissolved in anhydrous THF (20 mL) and Et_3N (0.36 mL, 2.6 mmol) was added. After 60 minutes the monosaccharide was added to the reaction vessel containing the indomethacin-amino acid and a further portion of THF (10 mL) was added. The reaction mixture was allowed to warm to r.t. and stirred for a further 16 h until complete consumption of the starting material was observed by TLC (CH_2Cl_2/MeOH, 9:1, v/v). The reaction mixture was concentrated under reduced pressure and the residue was taken up in EtOAc. The organic layer was washed sequentially with 4M HCl (3 x 30 mL), $NaHCO_{3\ (satd.\ aq)}$ (30 mL), H_2O (30 mL) and brine (30 mL). It was then dried (Na_2SO_4), filtered and concentrated under reduced pressure. Flash chromatography (CH_2Cl_2 → CH_2Cl_2/MeOH, 95:5, v/v) gave the desired product.

GP III for the *N*-Cbz deprotection and subsequent acylation with NSAIDs

NSAID (2.6 mmol) was dissolved in anhydrous THF (10 mL) and cooled to 0 °C. HOBt (3.6 mmol) and EDCI (3.1 mmol) were added and the reaction mixture was allowed to stir at this temperature for 60 min. Glucosamine-amino acid conjugate (2.6 mmol) was dissolved in anhydrous THF (20 mL) and DIPEA (6.5 mmol) was added. After 60 minutes, this solution was added to the reaction vessel containing the NSAID and a further portion of THF (10 mL) for flask rinse was added. The reaction was allowed to warm to r.t. and stirred for a further 16

h until complete consumption of the starting material was observed by TLC (CH_2Cl_2/MeOH, 9:1, v/v). The reaction mixture was concentrated under reduced pressure and the residue was taken up in EtOAc (50 mL). The organic layer was washed sequentially with 2M HCl (3 x 30 mL), $NaHCO_3$ (satd. aq) (2 x 30 mL), H_2O (30 mL) and brine (30 mL). It was then dried (Na_2SO_4), filtered and concentrated under reduced pressure. Flash chromatography (CH_2Cl_2 → CH_2Cl_2/MeOH, 95:5, v/v) gave the desired product.

(2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl)-L-alanine (3a). Prepared following general procedure IIa from indomethacin-Bt **2a** (459 mg, 1 mmol), L-alanine (96 mg, 1.08 mmol) and Et_3N (0.35 mL) that was stirred in the dark at r.t. in MeCN/H_2O (10 mL/4.5 mL, v/v) for 16 h to give the desired product **3a** (390 mg, 91%) as pale yellow crystals following recrystallization from boiling ether. m.p. 207-208 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 12.50 (s, 1H, $CO_2\underline{H}$), 8.46 (d, J = 7.5 Hz, 1H), 7.75-7.55 (m, 5H), 7.15 (d, J = 2.5 Hz, 1H), 6.93 (d, J = 8.9 Hz, 1H), 6.68 (dd, J = 9.0 Hz, J = 2.5 Hz, 1H), 4.22 (quint., J = 7.3 Hz, 1H, C$\underline{H}$CH$_3$), 3.76 (s, 3H, OMe), 3.55 (d, J = 2.7 Hz, 2H, C$\underline{H}_2$CONH), 2.21 (s, 3H, CH_3, *indole*), 1.28 (d, J = 7.3 Hz, 3H, CHC$\underline{H}_3$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 174.2, 169.3, 167.9, 155.6, 137.6, 135.1, 134.3, 131.2, 130.9, 130.2, 129.1, 114.5, 114.4, 111.4, 101.9, 55.4, 47.6, 30.8, 17.4, 13.4; Anal Calcd for $C_{22}H_{21}ClN_2O_5$: C, 61.61; H, 4.94; N, 6.53. Found: C, 61.32; H, 4.76; N, 6.13.

(2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl)-L-phenylalanine (3b). Prepared following general procedure Ia from L-phenylalanine (166 mg, 1.00 mmol) in H_2O (1 mL) and Et_3N (0.35 mL). Indomethacin-Bt **2a** (455 mg, 0.99 mmol), MeCN (5 mL) and H_2O (1.5 mL) were added to the stirring solution and the reaction mixture was stirred in the dark at r.t. for 16 h to yield the desired compound, **3b** (276 mg, 55%) as white powder following recrystallization from boiling ether. m.p. 182-185 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 12.76 (s, 1H, $CO_2\underline{H}$), 8.35 (d, J = 8.1 Hz, 1H), 7.67-7.61 (m, 4H), 7.19-7.14 (m, 5H), 7.11 (d, J = 2.5 Hz, 1H), 6.95 (d, J = 9.0 Hz, 1H), 6.70 (dd, J = 8.9 Hz, J = 2.5 Hz, 1H), 4.44 (td, J = 9.2 Hz, J = 4.7 Hz, 1H, C$\underline{H}$CH$_2$Ph), 3.76 (s, 3H, OMe), 3.52 (s, 2H, C$\underline{H}_2$CONH), 3.07 (dd, J = 13.8 Hz, J = 4.7 Hz, 1H, C$\underline{H}$HPh), 2.90 (dd, J = 13.8 Hz, J = 9.2 Hz, 1H, CH$\underline{H}$Ph), 2.11 (s, 3H, CH_3, indole); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 173.0, 169.5, 167.8, 155.6, 137.5, 135.1, 134.3, 131.1, 130.9, 130.2, 129.1, 129.0, 128.1, 126.3, 114.4, 114.2, 111.4, 101.9, 55.4, 53.6, 36.7, 30.7, 13.3; Anal Calcd for $C_{28}H_{25}ClN_2O_5$: C, 66.60; H, 4.99; N, 5.55. Found: C, 66.30; H, 4.93; N, 5.21.

(2-((2,3-Dimethylphenyl)amino)benzoyl)-L-valine (3c). Prepared following general procedure Ib for the preparation of NSAID-amino acid bioconjugates with L-valine (118 mg, 1.00 mmol) in H_2O (2 mL) and Et_3N (0.35 mL). Mefenamic acid-Bt **2b** (335 mg, 0.98 mmol), MeCN (5 mL) and H_2O (1 mL) were added to the stirring solution and the reaction mixture was stirred at r.t. for 16 h to yield the desired compound, **3c** (228 mg, 86%) as orange foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.13 (*br.* s, 2H), 7.55 (dd, J = 7.9, 1.6 Hz, 1H), 7.25 (ddd, J = 8.6, 7.2, 1.5 Hz, 1H), 7.17 (dd, J = 8.0, 1.5 Hz, 1H), 7.09 (t, J = 7.7 Hz, 1H), 6.98 (dd, J = 7.1, 1.2 Hz, 1H), 6.91 (dd, J = 8.5, 1.1 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 6.74 (ddd, J = 8.2, 7.2, 1.2 Hz, 1H), 4.83 (dd, J = 8.5, 4.8 Hz, 1H), 2.44-2.36 (m, 1H), 2.33 (s, 3H), 2.20 (s, 3H), 1.09 (t, J = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 176.5, 169.7, 147.3, 139.2, 138.0, 132.6, 131.1, 127.7, 125.8, 125.7, 121.3, 116.8, 116.2, 114.9, 57.2, 31.2, 20.6, 19.0, 17.8, 13.8; HRMS (ESI-TOF) *m/z* for $C_{20}H_{23}N_2O_3^-$ [M-H]$^-$ calcd. 339.1714, found 339.1718.

(2-((2,3-Dimethylphenyl)amino)benzoyl)-L-phenylalanine (3d). Prepared following general procedure Ib for the preparation of NSAID-amino acid bioconjugates with L-phenylalanine (165 mg, 1.00 mmol) in H_2O (3 mL) and Et_3N (0.35 mL). Mefenamic acid-Bt **2b** (323 mg, 0.94 mmol), MeCN (5 mL) and H_2O (1.5 mL) were added to the stirring solution and the reaction mixture was stirred at r.t. for 16 h to yield the desired compound, **3d** (308 mg, 84%) as pale orange foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.34-7.27 (m, 3H), 7.27-7.17 (m, 3H), 7.15 (d, J = 7.6 Hz, 1H), 7.09 (t, J = 7.6 Hz, 1H), 6.99 (d, J = 7.4 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.70-6.63 (m, 2H), 5.12 (q, J = 6.1 Hz, 1H), 3.39 (dd, J = 14.0 Hz, J = 5.5 Hz, 1H), 3.27 (dd, J = 14.0 Hz, J = 6.0 Hz, 1H), 2.34 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 176.2, 169.4, 147.4, 139.2, 138.0, 135.6, 132.7, 131.2, 129.4, 128.7, 127.6, 127.3, 125.9, 125.8, 121.5, 116.8, 115.8, 114.9, 53.3, 37.4, 20.6, 13.9; HRMS (ESI-TOF) *m/z* for $C_{24}H_{23}N_2O_3^-$ [M-H]$^-$ calcd. 387.1714, found 387.1729.

(2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl)-glycine (3g). Prepared following general procedure Ia from indomethacin-Bt **2a** (342 mg, 0.75 mmol), glycine (75 mg, 1.00 mmol) and Et_3N (0.35 mL) that was stirred in the dark at r.t. in MeCN/H_2O (10 mL/4.5 mL, v/v) for 16 h to give the desired product **3g** (321 mg, 91%) as beige powder. m.p. 174-179 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 12.52 (s, 1H), 8.31 (t, J = 5.9 Hz, 1H), 7.67 (q, J = 8.5 Hz, 4H), 7.14 (d, J = 2.5 Hz, 1H), 6.96 (d, J = 9.0 Hz, 1H), 6.70 (dd, J = 9.0, 2.5 Hz, 1H), 3.77 (s, 5H, CH_2, *Gly* and CH_3, *OMe*), 3.58 (s, 2H, *Indo*), 2.23 (s, 3H, CH_3, *indole*); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 171.3, 169.9, 167.8, 155.5, 137.6, 135.2, 134.2, 131.2, 130.8, 130.3, 129.0, 114.5, 114.1, 111.4, 101.9, 55.4, 40.8, 30.8, 13.4; Anal Calcd for $C_{21}H_{19}ClN_2O_5$: C, 60.80; H, 4.62; N, 6.75. Found: C, 60.59; H, 4.51; N, 6.95.

(2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl)-γ-aminobutyric acid (3h). Prepared following general procedure IIa from γ-aminobutyric acid (107 mg, 1.04 mmol) in H_2O (1 mL) and Et_3N (0.35 mL). Indomethacin-Bt **6d** (448 mg, 0.98 mmol), MeCN (8 mL) was added to the stirring solution and the reaction mixture was stirred in the dark at r.t. for 16 h to yield the desired compound (363 mg, 84%) as white powder. m.p. 145-148 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 12.01 (s, 1H), 8.04 (t, J = 5.6 Hz, 1H), 7.66 (q, J = 8.5 Hz, 4H), 7.11 (d, J = 2.0 Hz, 1H), 6.96 (d, J = 9.0 Hz, 1H), 6.70 (dd, J = 9.0 Hz, J = 2.2 Hz, 1H), 3.76 (s, 3H), 3.49 (s, 2H), 3.07 (q, J = 6.4 Hz, 2H), 2.28-2.18 (m, 5H), 1.63 (quint., J = 7.0 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 174.2, 169.4, 167.9, 155.6, 137.6, 135.1, 134.3, 131.2, 130.9, 130.3, 129.0, 114.6, 114.4,

111.4, 101.8, 55.4, 38.1, 31.2, 31.0, 24.6, 13.4; HRMS (ESI-TOF) *m/z* for $C_{23}H_{23}{}^{35}ClN_2O_5Na^+$ $[M+Na]^+$ calcd. 465.1193, found 465.1193.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-(*tert*-butoxycarbonyl)glycyl)-amino]-2-deoxy-β-D-glucopyranose (7a). Prepared following general procedure IIa with *N*-Boc-glycine (455 mg, 2.6 mmol), EDCI (619 mg, 3.99 mmol), HOBt (527 mg) and acetylated glucosamine hydrochloride (983 mg, 2.56 mmol) to afford the desired compound, **7a** (0.94 g, 82%) as white crystals. m.p. 165-167 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.52 (*br*. s, 1H, NH), 5.76 (d, $J_{1,2}$ = 8.8 Hz, 1H, H-1), 5.25 (*app* t, *J* = 10.0 Hz, 1H, H-3), 5.18 (*br*. s, 1H, NH, *Boc*), 5.12 (*app* t, *J* = 9.7 Hz, 1H, H-4), 4.27 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 4.7 Hz, 1H, H-6a), 4.30-4.19 (m, 1H, H-2), 4.12 (dd, $J_{6b,6a}$ = 12.4 Hz, $J_{6b,5}$ = 2.2 Hz, 1H, H-6b), 3.85 (ddd, $J_{5,4}$ = 10.0 Hz, $J_{5,6a}$ = 4.6, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 3.69 (d, *J* = 5.7 Hz, 2H, CH_2, *Gly*), 2.11 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.44 (s, 9H, C(CH_3)$_3$, *Boc*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.1, 170.6, 170.0, 169.4, 169.3 (5 x C=O), 156.0 (C=O, *Boc*), 92.2 (C-1), 80.5 (*C*(CH$_3$)$_3$, *Boc*), 72.8 (C-5), 72.3 (C-3), 67.9 (C-4), 61.6 (C-6), 52.9 (C-2), 44.4 (CH_2, *Gly*), 28.3 (C(*C*H$_3$)$_3$, *Boc*), 20.8, 20.7, 20.6, 20.5 (4 x CH_3, *OAc*); Anal Calcd for $C_{21}H_{32}N_2O_{12}$: C, 50.00; H, 6.39; N, 5.55. Found: C, 50.00; H, 6.65; N, 5.32.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-(*tert*-butoxycarbonyl)-L-alanyl)-amino]-2-deoxy-β-D-glucopyranose (7b). Prepared following general procedure IIa with *N*-Boc-alanine (492 mg, 2.6 mmol), EDCI (619 mg, 3.99 mmol), HOBt (527 mg) and acetylated glucosamine hydrochloride (983 mg, 2.56 mmol) to afford the desired compound, **7b** (0.94 g, 71%) as white crystals. m.p. 209-213 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.62 (*br* s, 1H, NH), 5.75 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 5.24 (*app* t, *J* = 10.0 Hz, 1H, H-3), 5.11 (*app* t, *J* = 9.6 Hz, 1H, H-4), 5.05 (*br* s, 1H, NH, *Boc*), 4.27-4.23 (m, 2H, H-2, H-6a), 4.11 (*br* d, $J_{6b,6a}$ = 12.3 Hz, 1H, H-6b), 4.03 (quint., *J* = 7.2 Hz, 1H, C*H*CH$_3$, *Ala*), 3.85 (ddd, $J_{5,4}$ = 9.8 Hz, $J_{5,6a}$ = 4.6 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 2.09 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.01 (s, 6H, 2 x CH_3), 1.41 (s, 9H, C(CH_3)$_3$, *Boc*), 1.25 (d, *J* = 6.8 Hz, 3H, CHCH_3, *Ala*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 173.2, 170.8, 170.6, 169.5, 169.3 (5 x C=O), 155.3 (C=O, *Boc*), 92.4 (C-1), 80.1 (*C*(CH$_3$)$_3$, *Boc*), 72.8 (C-5), 72.0 (C-3), 68.0 (C-4), 61.7 (C-6), 52.7 (C-2), 50.3 (*C*HCH$_3$, *Ala*), 28.3 (C(*C*H$_3$)$_3$, *Boc*), 20.8, 20.7, 20.5 (4 x CH_3, *OAc*), 18.3 (CH_3, *Ala*); Anal Calcd for $C_{22}H_{34}N_2O_{12}$: C, 50.96; H, 6.61; N, 5.40. Found: C, 50.83; H, 6.81; N, 5.08.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-(*tert*-butoxycarbonyl)-L-valyl)-amino]-2-deoxy-β-D-glucopyranose (7c). Prepared following general procedure IIa with *N*-Boc-valine (289 mg, 1.3 mmol), EDCI (317 mg, 1.7 mmol), HOBt (262 mg) and acetylated glucosamine hydrochloride (592 mg, 1.5 mmol) to afford the desired compound, 7c (0.55 g, 76%) as white crystals. m.p. 193-196 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.47 (d, $J_{NH,2}$ = 9.5 Hz, 1H, NH), 5.73 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 5.24 (t, *J* = 10.1 Hz, 1H, H-3), 5.11 (t, *J* = 9.6 Hz, 1H, H-4), 4.96 (d, *J* = 8.9 Hz, 1H, NH, *Boc*), 4.31 (*app* dd, 1H, $J_{2,3}$ =10.2 Hz, $J_{NH,2}$ = 9.5 Hz, H-2), 4.27 (dd, $J_{6a,6b}$ = 12.6 Hz, $J_{6a,5}$ = 4.7 Hz, 1H, H-6a), 4.12 (dd, $J_{6b,6b}$ = 12.6 Hz, $J_{6b,5}$ = 2.2 Hz, 1H, H-6b), 3.84 (*app* ddd, $J_{5,4}$ = 10.2 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,6b}$ = 2.3 Hz, 2H, H-5 and C*H*NHBoc), 2.06-2.05 (m, 1H, C*H*(CH$_3$)$_2$, *Val*), 2.081 (s, 3H, CH_3), 2.077 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 1.43 (s, 9H, C(CH_3)$_3$, *Boc*), 0.90 (d, *J* = 6.8 Hz, 3H, CH_3, *Val*), 0.83 (d, *J* = 6.8 Hz, 3H, CH_3, *Val*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 172.2, 170.7, 170.6, 169.4, 169.3 (5 x C=O), 155.7 (C=O, *Boc*), 92.4 (C-1), 80.0 (*C*CH$_3$, *Boc*), 72.7 (C-5), 71.8 (C-3), 68.2 (C-4), 61.7 (C-6), 60.1 (*C*HNHBoc), 52.5 (C-2), 30.4 (*C*H(CH$_3$)$_2$, *Val*), 28.2 (C(*C*H$_3$)$_3$, *Boc*), 20.7, 20.6, 20.50, 20.49 (4 xCH$_3$, *OAc*), 19.1, 19.0 (2 x CH_3, *Val*); Anal Calcd for $C_{24}H_{38}N_2O_{12}$: C, 52.74; H, 7.01; N, 5.13. Found: C, 53.03; H, 6.80; N, 4.65.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl)-amino]-2-deoxy-β-D-glucopyranose (7d). Prepared following general procedure IIa with *N*-Boc-phenylalanine (349 mg, 1.3 mmol), EDCI (309 mg, 1.6 mmol), HOBt (263 mg) and acetylated glucosamine hydrochloride (507 mg, 1.3 mmol) to afford the desired compound, **7d** (0.70 g, 71%) as white crystals. m.p. 219-225 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.31-7.23 (m, 3H, ArH, *Phe*), 7.17 (d, *J* = 7.2 Hz, 2H, ArH, *Phe*), 6.32 (d, $J_{NH,2}$ = 9.2 Hz, 1H, NH), 5.71 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 5.19 (*app* t, *J* = 9.9 Hz, 1H, H-3), 5.12 (*app* t, *J* = 9.5 Hz, 1H, H-4), 4.67 (d, *J* = 6.1 Hz, 1H, NH, *Boc*), 4.30-4.20 (m, 3H, H-2, H-6a, C*H*NHBoc), 4.13 (dd, $J_{6b,6a}$ = 12.5 Hz, $J_{6b,5}$ = 2.3 Hz, 1H, H-6b), 3.81 (ddd, $J_{5,4}$ = 9.8 Hz, $J_{5,6a}$ = 4.5, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 3.16 (dd, *J* = 14.1, 6.0 Hz, 1H, C*H*HPh, *Phe*), 2.90 (dd, *J* = 13.7, 7.3 Hz, 1H, CH*H*Ph, *Phe*), 2.11 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.96 (s, 3H, CH_3), 1.39 (s, 9H, C(CH_3)$_3$, *Boc*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.8, 170.7, 170.6, 169.4, 169.3 (5 x C=O), 155.3 (C=O, *Boc*), 136.3 (C_q, *Ar*), 129.1, 128.6, 126.9 (CH, *Ar*), 92.5 (C-1), 80.5 (*C*(CH$_3$)$_3$, *Boc*), 72.8 (C-5), 71.7 (C-3), 67.9 (C-4), 61.6 (C-6), 55.7 (*C*HNHBoc), 53.0 (C-2), 37.3 (*C*H$_2$Ph, *Phe*), 28.2 (C(*C*H$_3$)$_3$, *Boc*), 20.9, 20.7, 20.53, 20.47 (4 x CH_3, *OAc*); Anal Calcd for $C_{28}H_{38}N_2O_{12}$: C, 56.56; H, 6.44; N, 4.71. Found; C, 56.49; H, 6.80; N, 4.53.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-(carbobenzyloxy)glycyl)-amino]-2-deoxy-β-D-glucopyranose (7e). Prepared following general procedure IIa with *N*-Cbz-glycine (272 mg, 1.30 mmol), EDCI (607 mg, 1.67 mmol), HOBt (272 mg) and acetylated glucosamine hydrochloride **5** (486 mg, 1.27 mmol) to afford the desired compound, **7e** (0.40 g, 63%) as white crystals following recrystallization from EtOH/CH_2Cl_2 (9:1, v/v). m.p. 168-172 °C (lit. m.p. 165 °C);[34] ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.37-7.35 (m, 5H, ArH, *Cbz*), 6.58 (d, *J* = 9.3 Hz, 1H, NH), 5.79 (d, $J_{1,2}$ = 8.8 Hz, 1H, H-1), 5.50 (*br* s, 1H, NH, *Cbz*), 5.26 (dd, *J* = 10.6, 9.4 Hz, 1H, H-3), 5.14 (*app* t, *J* = 9.7 Hz, H-4), 5.13 (s, 2H, CH_2, *Cbz*), 4.31-4.25 (m, 1H, H-2), 4.28 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 4.4 Hz, 1H, H-6a), 4.14 (*app* d, $J_{6b,6a}$ = 12.2 Hz, 1H, H-6b), 3.87-3.83 (m, 1H, H-5), 3.77 (t, *J* = 6.1 Hz, 2H, CH_2, *Gly*), 2.12 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.04 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.2, 170.6, 169.5, 169.2 (5 x C=O), 156.6 (C=O, Cbz), 135.9, 128.6, 128.3, 128.1 (Ar, *Cbz*), 92.2 (C-1), 72.8 (C-5), 72.3 (C-3), 67.8 (C-4), 67.4 (CH_2, *Cbz*), 61.6 (C-6), 53.0 (C-

2), 44.7 (*C*H$_2$, *Gly*), 20.8, 20.7, 20.6, 20.5 (4 x CH$_3$, *OAc*); Anal Calcd for $C_{24}H_{30}N_2O_{12}$: C, 53.53; H, 5.62; N, 5.20. Found: C, 53.48; H, 5.77; N, 4.99.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-(carbobenzyloxy)-L-alanyl)-amino]-2-deoxy-*β*-D-glucopyranose (7f). Prepared following general procedure IIa with *N*-Cbz-alanine (290 mg, 1.30 mmol), EDCI (315 mg, 1.64 mmol), HOBt (245 mg) and GlcN **5** (490 mg, 1.28 mmol) to afford the desired compound, **7f** (0.41 g, 57%) as white crystals. m.p. 172-175°C, (lit. m.p. 172 °C);[34] ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.39-7.33 (m, 5H, ArH, *Cbz*), 6.31 (d, $J_{NH,2}$ = 9.5 Hz, 1H, NH), 5.74 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 5.21 (*app* t, *J* = 10.0 Hz, 1H, H-3), 5.16-5.06 (m, 4H, H-4, NH and CH$_2$, *Cbz*), 4.28 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 4.7 Hz, 1H, H-6a), 4.27-4.23 (m, 1H, H-2), 4.14 (dd, $J_{6b,6a}$ = 12.5 Hz, $J_{6b,5}$ = 2.2 Hz, 1H, H-6b), 4.10 (t, *J* = 7.0 Hz, 1H, C*H*CH$_3$, *Ala*), 3.81 (ddd, $J_{5,4}$ = 9.4 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 2.10 (s, 3H, CH$_3$), 2.08 (s, 3H, CH$_3$), 2.05 (s, 6H, 2 x CH$_3$), 1.31 (d, *J* = 7.1 Hz, 3H, CH$_3$, *Ala*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 172.7, 170.9, 170.6, 169.6, 169.2 (5 x C=O), 155.7 (C=O, *Cbz*), 136.0, 128.4, 128.1, 127.9 (Ar, *Cbz*), 92.3 (C-1), 72.7 (C-5), 71.9 (C-3), 68.0 (C-4), 66.9 (CH$_2$, *Cbz*), 61.7 (C-6), 52.7 (C-2), 50.8 (*C*HCH$_3$, *Ala*), 20.7, 20.59, 20.53, 20.46 (4 x CH$_3$, *OAc*), 18.1 (CH$_3$, *Ala*); Anal Calcd for $C_{25}H_{32}N_2O_{12}$: C, 54.34; H, 5.84; N, 5.07. Found: C, 54.46; H, 6.13; N, 4.83.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-(carbobenzyloxy)-L-valyl)-amino]-2-deoxy-*β*-D-glucopyranose (7g). Prepared following general procedure IIa with *N*-Cbz-valine (338 mg, 1.35 mmol), EDCI (320 mg, 1.67 mmol), HOBt (250 mg) and acetylated glucosamine hydrochloride (497 mg, 1.30 mmol) to afford the desired compound, **7g** (0.52 g, 69%) as white crystals. m.p. 218-221 °C; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.37-7.31 (m, 5H, ArH, *Cbz*), 6.43 (d, $J_{NH,2}$ = 9.5 Hz, 1H, NH), 5.76 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 5.27 (*app* t, *J* = 10.1 Hz, 1H, H-3), 5.19 (d, *J* = 8.3 Hz, 1H, NH, *Val*), 5.14-5.08 (m, 3H, H-4 and CH$_2$, *Cbz*), 4.32 (*app* dd, *J* = 10.0 Hz, *J* = 9.6 Hz, H-2), 4.28 (dd, $J_{6a,6b}$ = 12.7 Hz, $J_{6a,5}$ = 4.6 Hz, 1H, H-6a), 4.12 (*app* d, $J_{6b,6a}$ = 12.3 Hz, 1H, H-6b), 3.89-3.82 (m, 2H, H-5 and C*H*NHCO, *Val*), 2.12-2.07 (m, 1H, C*H*(CH$_3$)$_2$, *Val*), 2.09 (s, 3H, CH$_3$), 2.04 (s, 6H, 2 x CH$_3$), 2.03 (s, 3H, CH$_3$), 0.92 (d, *J* = 6.7 Hz, 3H, CH$_3$, *Val*), 0.86 (d, *J* = 6.8 Hz, 3H, *Val*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.7, 171.0, 170.7, 169.4, 169.3 (5 x C=O), 156.3 (C=O, *Cbz*), 135.9, 128.6, 128.3, 128.1 (4 x Ar, *Cbz*), 92.5 (C-1), 72.9 (C-5), 71.8 (C-3), 67.9 (C-4), 67.3 (CH$_2$, *Cbz*), 61.6 (C-6), 60.9 (*C*HNHCO, *Val*), 52.7 (C-2), 30.1 (*C*H(CH$_3$)$_2$, *Val*), 20.73, 20.72, 20.65, 20.6 (4 x CH$_3$, *OAc*), 19.2, 17.5 (2 x CH$_3$, *Val*); Anal Calcd for $C_{27}H_{36}N_2O_{12}$: C, 55.86; H, 6.25; N, 4.83. Found: C, 55.61; H, 6.04; N, 4.49.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-(carbobenzyloxy)-L-phenylalanyl)-amino]-2-deoxy-*β*-D-glucopyranose (7h). Prepared following general procedure IIa with *N*-Cbz-phenylalanine (405 mg, 1.35 mmol), EDCI (320 mg, 1.67 mmol), HOBt (250 mg) and acetylated glucosamine hydrochloride (497 mg, 1.30 mmol) to afford the desired compound, **7h** (545 mg, 67%) as white crystals. m.p. 207-210°C, (lit. m.p. 208-209 °C);[29] ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.38-7.31 (m, 3H, *ArH*), 7.31-7.20 (m, 5H, *ArH*), 7.13 (d, 2H, *ArH*), 6.48 (d, $J_{NH,2}$ = 6.8 Hz, 1H, NH), 5.75 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 5.23 (t, *J* = 10.0 Hz, 1H, H-3), 5.13 (*app* t, *J* = 9.6 Hz, 1H, H-4), 5.08 (d, *J* = 8.2 Hz, 1H, NH, *Phe*), 5.03 (s, 2H, CH$_2$, *Cbz*) 4.33 (q, *J* = 7.3 Hz, 1H, C*H*CH$_2$Ph, *Phe*), 4.30-4.22 (m, 2H, H-2 and H-6a), 4.12 (dd, $J_{6b,6a}$ = 12.5 Hz, $J_{6b,5}$ = 2.2 Hz, 1H, H-6b), 3.81 (*app* d, *J* = 9.5 Hz, 1H, H-5), 3.14 (dd, *J* = 14.4 Hz, *J* = 5.9 Hz, 1H, C*H*HPh, *Phe*), 2.88 (dd, *J* = 13.8 Hz, *J* = 7.8 Hz, 1H, CH*H*Ph, *Phe*), 2.09 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.6, 171.0, 170.6, 169.4, 169.3 (5 x C=O), 155.9 (C=O, *Cbz*), 136.0, 135.7, 129.0, 128.8, 128.5, 128.3, 128.2, 127.1 (8 x Ar, *Phe and Cbz*), 92.5 (C-1), 72.8 (C-5), 71.7 (C-3), 67.8 (C-4), 67.4 (CH$_2$, *Cbz*), 61.6 (C-6), 56.2 (*C*HCH$_2$Ph, *Phe*), 53.1 (C-2), 37.2 (CH$_2$, *Phe*), 20.8, 20.7, 20.59, 20.55 (4 x CH$_3$, *OAc*); Anal Calcd for $C_{31}H_{36}N_2O_{12}$: C, 59.23; H, 5.77; N, 4.46. Found; C, 59.47; H, 5.80; N, 4.30.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-((2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl)-L-alanyl)-amino]-2-deoxy-*β*-D-glucopyranose (6a).
Prepared following general procedure III with indomethacin (194 mg, 0.54 mmol), EDCI (124 mg, 0.65 mmol), HOBt (102 mg) and 1,3,4,6-tetra-*O*-acetyl-2-[(*N*-L-alanyl)-amino]-2-deoxy-β-D-glucopyranose (300 mg, 0.54 mmol) to afford the desired compound (330 mg, 80%) a single rotamer as yellow solid. m.p. 205-207°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.69 (d, *J* = 8.0 Hz, 2H, ArH, *ArCl*), 7.50 (d, *J* = 8.1 Hz, 2H, ArH, *ArCl*), 7.02-6.93 (m, 2H, ArH), 6.72 (dd, *J* = 9.1, 2.4 Hz, 1H, ArH), 6.57 (d, $J_{NH,2}$ = 9.3 Hz, 1H, NH), 6.22 (d, *J* = 7.0 Hz, 1H, NH, *Ala*), 5.57 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 5.14 (*app* t, $J_{3,2}$ = 10.1 Hz, $J_{3,4}$ = 9.7 Hz, 1H, H-3), 5.09 (*app* t, *J* = 9.5 Hz, 1H, H-4), 4.34 (quint., *J* = 7.1 Hz, 1H, C*H*CH$_3$, *Ala*), 4.24 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 4.5 Hz, 1H, H-6a), 4.19-4.14 (m, 1H, H-2), 4.09 (dd, $J_{6b,6a}$ = 12.7 Hz, $J_{6b,5}$ = 2.6 Hz, 1H, H-6b), 3.85 (s, 3H, CH$_3$, *OMe*), 3.67-3.62 (m, 3H, H-5 and CH$_2$, *Indo*), 2.36 (s, 3H, CH$_3$, *indole*), 2.08 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.21 (d, *J* = 6.9 Hz, 3H, CHC*H*$_3$, *Ala*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 172.2, 170.8, 170.6, 170.1, 169.34, 169.25, 168.3 (7 x C=O), 156.3, 139.5, 136.4, 133.6, 131.2, 131.0, 130.3, 129.2, 115.2, 112.4, 112.1, 101.1 (12 x Ar, *Indo*), 92.4 (C-1), 72.7 (C-5), 72.1 (C-3), 67.7 (C-4), 61.6 (C-6), 55.8 (CH$_3$, *OMe*), 53.0 (C-2), 49.3 (*C*HCH$_3$), 32.0 (CH$_2$, *Indo*), 29.7, 20.7, 20.6, 20.5 (4 x CH$_3$, *OAc*), 18.3 (CH*C*H$_3$, *Ala*), 13.4 (CH$_3$, *indole*); HRMS (ESI-TOF) *m/z* for $C_{36}H_{40}{}^{35}ClN_3O_{13}Na$ $[M+Na]^+$ calcd. 780.2142, found 780.2147.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-((2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl)-L-phenylalanyl)-amino]-2-deoxy-*β*-D-glucopyranose (6b). Prepared following general procedure IIb with indomethacin-L-Phe-OH (112 mg, 0.26 mmol), EDCI (236 mg, 1.23 mmol), HOBt (201 mg) and acetylated glucosamine hydrochloride (98 mg, 0.26 mmol) to afford the desired compound, **6b** (0.10 g, 48%) a mixture of rotamers, in a 2:1 ratio, as white crystals. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.66 (2 x d, *J* = 8.4 Hz, 2H, ArH, *ArCl*), 7.50 (2 x d, *J* = 8.4 Hz, 2H, ArH, *ArCl*), 7.14-7.08 (m, 3H, ArH), 7.01, 6.96 (2 x d, *J* = 8.8 Hz, 1H, ArH), 6.89 (d, *J* = 6.2 Hz, 2H, ArH), 6.84 (d, *J* = 7.2 Hz, 1H, ArH), 6.76-6.73 (m, 1H, ArH), 6.37,

6.35 (2 x d, $J_{NH,2}$ = 9.1 Hz, 1H, NH), 5.96, 5.83 (d, *J* = 7.0 Hz, 1H, NH, *Phe*), 5.67, 5.60 (2 x d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 5.22 (*app* t, *J* = 10.0 Hz, 0.34H, H-3), 5.12 (2 x t, *J* = 9.6 Hz, 1H, H-4), 4.91 (*app* t, *J* = 9.9 Hz, 0.66H, H-3), 4.51 (dq, *J* = 14.1 Hz, *J* = 6.8 Hz, 1H, C*H*CH_2Ph, *Phe*), 4.28 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{6a,5}$ = 4.0 Hz, 1H, H-6a), 422-4.12 (m, 2H, H-2 and H-6b), 3.83 (s, 3H, CH_3, *OMe*), 3.81-3.78 (m, 1H, H-5), 3.67, 3.63 (2 x d, *J* = 17.3 Hz, 1H, C*H*H, *Indo*), 3.53, 3.51 (2 x d, *J* = 17.1 Hz, 1H, CH*H*, *Indo*), 2.98 (dd, *J* = 14.1 Hz, *J* = 5.3 Hz, 1H, C*H*HPh, *Phe*), 280-2.73 (m, 1H, CH*H*Ph, *Phe*), 2.18 (s, 3H, CH_3, *Indole*), 2.12, 2.10, 2.06, 2.06, 2.04, 2.02, 1.90 (8 x s, 12H, 4 x CH_3, *OAc*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.3, 171.0, 170.93, 170.85, 170.6, 170.4, 169.4, 169.3, 169.21, 169.17, 168.15, 168.1 (C=O), 156.3, 139.6, 139.5, 136.5, 136.4, 135.7, 135.5, 133.64, 133.55, 131.2, 131.1, 130.2, 130.1, 129.21, 129.19, 128.9, 128.7, 128.6, 127.1, 127.0, 115.4, 115.3, 112.1, 112.0, 101.2, 101.0 (Ar, *Indo and Phe*), 92.6, 92.2 (2 x C-1), 72.83, 72.78, 72.8 (2 X C-5 and 1 x C-3), 71.7 (C-3), 67.7, 67.6 (2 x C-4), 61.6 (C-6), 55.90, 55.85 (2 x CH_3, *OMe*), 54.4, 54.3 (2 x *C*HCH_2Ph, *Phe*), 53.4, 53.2 (2 x C-2), 36.74, 36.67 (2 x CH_2Ph, *Phe*), 31.9, 31.8 (2 x CH_2, *Indo*), 29.7, 20.91, 20.87, 20.71, 20.65, 20.58, 20.56 (CH_3), 13.1, 13.0 (2 x CH_3, *Indole*); HRMS (ESI-TOF) *m/z* for $C_{42}H_{44}{}^{35}ClN_3O_{13}Na^+$ $[M+Na]^+$ calcd. 856.2455, found 856.2468.

3,4,6-Tri-*O*-acetyl-2-[(*N*-((2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl)-L-phenylalanyl)-amino]-2-deoxy-β-D-glucopyranose (6b'). Prepared following general procedure III with indomethacin (194 mg, 0.54 mmol), EDCI (152 mg, 1.23 mmol), HOBt (102 mg, 0.76 mmol) and 3,4,6-tri-*O*-acetyl-2-[(*N*-L-phenylalanyl)-amino]-2-deoxy-β-D-glucopyranose (244 mg, 0.54 mmol) to afford the desired compound, **6b'** (230 mg, 53%) as a yellow solid. m.p. 167-170 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.63 (d, *J* = 8.2 Hz, 2H, ArH, *ArCl*), 7.49 (d, *J* = 8.3 Hz, 2H, ArH, *ArCl*), 7.09 (dt, *J* = 14.1, 6.8 Hz, 3H), 6.95 (d, *J* = 9.1 Hz, 1H), 6.86 (d, *J* = 2.5 Hz, 1H), 6.83 (d, *J* = 6.9 Hz, 2H), 6.73 (td, *J* = 9.3, 2.2 Hz, 2H), 6.06 (d, *J* = 7.6 Hz, 1H, NH, *Phe*), 5.30 (*app* t, *J* = 9.6 Hz, 1H, H-3), 5.12 (*app* d, *J* = 9.8 Hz, 1H, H-4), 5.09 (d, $J_{1,2}$ = 3.1 Hz, 1H, H-1), 4.70 (td, *J* = 7.8 Hz, *J* = 5.5 Hz, 1H, C*H*CH_2, *Phe*), 4.24-4.17 (m, 3H, H-2, H-5 and H-6a), 4.08 (dd, $J_{6b,6a}$ = 12.1 Hz, $J_{6b,5}$ = 2.1 Hz, 1H, H-6b), 3.80 (s, 3H), 3.66 (d, *J* = 17.5 Hz, 1H, C*H*H, *Indo*), 3.53 (d, *J* = 17.3 Hz, 1H, CH*H*, *Indo*), 2.96 (dd, *J* = 14.0 Hz, *J* = 5.5 Hz, 1H, C*H*HPh, *Phe*), 2.78 (dd, *J* = 14.0 Hz, *J* = 8.0 Hz, 1H, CH*H*Ph, *Phe*), 2.19 (s, 3H, CH_3, *Indole*), 2.09 (s, 3H, *OAc*), 2.02 (s, 3H, *OAc*), 1.96 (s, 3H, CH_3, *OAc*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.4, 171.3, 170.8, 170.7, 169.4, 168.2, 156.1, 139.5, 136.5, 135.6, 133.5, 131.1, 131.0, 129.2, 128.8, 128.5, 127.0, 115.0, 112.0, 111.7, 101.4, 91.2 (C-1), 70.4 (C-3), 68.2 (C-4), 67.6 (C-5), 62.1 (C-6), 55.8 (CH_3, *OMe*), 54.0 (*C*HCH_2, *Phe*), 52.7 (C-2), 37.5 (CH_2Ph, *Phe*), 31.8 (CH_2, *Indo*), 20.7, 20.6 (3 x CH_3), 13.0 (CH_3, *indole*); HRMS (ESI-TOF) *m/z* for $C_{40}H_{42}{}^{35}ClN_3O_{12}Na$ $[M+Na]^+$ calcd. 814.2349, found 814.2330.

3,4,6-Tri-*O*-acetyl-2-[(*N*-(2-((2,3-dimethylphenyl)amino)benzoyl)-L-valyl)-amino]2-deoxy-β-D-glucopyranose (6c'). Prepared following general procedure III with mefenamic acid (80 mg, 0.33 mmol), EDCI (72 mg, 0.42 mmol), HOBt (72 mg) and 3,4,6-tri-*O*-acetyl-2-[(*N*-L-valyl)-amino]-2-deoxy-β-D-glucopyranose (144 mg, 0.36 mmol) to afford the desired compound, **6c'** (0.11 g, 52%) as a beige foam. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 9.31 (s, 1H, NH, *Mef*), 8.24 (d, *J* = 8.9 Hz, 1H, NH, *Val*), 8.10 (d, $J_{NH,2}$ = 8.9 Hz, 1H, NH), 7.71 (dd, *J* = 7.8, 1.6 Hz, 1H, *ArH*), 7.27-7.24 (m, 2H, *ArH and OH*), 7.10-7.02 (m, 2H, *ArH*), 6.92 (dd, *J* = 6.0 Hz, *J* = 2.8 Hz, 1H, *ArH*), 6.83 (d, *J* = 8.3 Hz, 1H, *ArH*), 6.75 (t, *J* = 7.5 Hz, 1H, *ArH*), 5.23 (dd, *J* = 11.1 Hz, *J* = 9.3 Hz, 1H, H-3), 5.01 (t, $J_{1,2}$ = $J_{1,OH}$ = 4.0 Hz, 1H, H-1), 4.85 (*app* t, *J* = 9.3 Hz, 1H, H-4), 4.42 (t, *J* = 8.2 Hz, 1H, C*H*NHCO, *Val*), 4.19-4.10 (m, 2H, H-5 and H-6a), 4.09-4.05 (m, 1H, H-2), 4.01-3.97 (m, 1H, H-6b), 2.26 (s, 3H), 2.09 (s, 3H), 2.07-2.01 (m, 1H, C*H*$(CH_3)_2$, *Val*), 2.01 (s, 3H), 1.96 (s, 3H), 1.80 (s, 3H), 0.87 (d, *J* = 6.4 Hz, 6H, CH(C*H*$_3)_2$, *Val*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.5, 170.1, 169.5, 169.4, 168.8 (5 x C=O), 146.0, 139.3, 137.7, 132.1, 129.3, 129.1, 125.8, 125.0, 119.6, 117.2, 116.9, 113.93 (12 x Ar, *Mef*), 90.8 (C-1), 70.0 (C-3), 69.2 (C-4), 66.5 (C-5), 62.3 (C-6), 58.5 C*H*NHCO, *Val*), 51.4 (C-2), 30.4 (*C*H$(CH_3)_2$, *Val*), 20.6, 20.4, 20.34, 20.29 (4 x CH_3, *3 x OAc and 1 x Mef*), 19.2, 18.5 (2 x CH_3, *Val*), 13.5 (CH_3, *Mef*); HRMS (ESI-TOF) *m/z* for $C_{32}H_{42}N_3O_{10}^+$ $[M+H]^+$ calcd. 628.2865, found 628.2864.

3,4,6-Tri-*O*-acetyl-2-[(*N*-(2-((2,3-dimethylphenyl)amino)benzoyl)-L-phenylalanyl)-amino]2-deoxy-β-D-glucopyranose (6d'). Prepared following general procedure III with mefenamic acid (130 mg, 0.54 mmol), EDCI (124 mg, 0.65 mmol), HOBt (102 mg, 0.76 mmol) and 3,4,6-tri-*O*-acetyl-2-[(*N*-L-phenylalanyl)-amino]-2-deoxy-β-D-glucopyranose (244 mg, 0.54 mmol) to afford the desired compound, **6d'** (240 mg, 65%) as an off white solid. m.p. 114-121°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.93 (s, 1H, NH, *Mef*), 7.51 (dd, *J* = 7.8, 1.5 Hz, 1H, *ArH*), 7.49-7.33 (m, 7H, *ArH*), 7.31-7.22 (m, 1H, *ArH*), 7.16 (dd, *J* = 6.5, 2.2 Hz, 1H, *ArH*), 7.07 (d, *J* = 7.5 Hz, 1H, NH, *Phe*), 7.02 (d, $J_{NH,2}$ = 9.0 Hz, 1H, NH), 7.00 (d, *J* = 8.4 Hz, 1H, *ArH*), 6.83 (t, *J* = 7.5 Hz, 1H, *ArH*), 5.53 (*app* t, *J* = 10.1 Hz, 1H, H-3), 5.45 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 5.33 (*app* t, *J* = 9.8 Hz, 1H, H-4), 5.06 (q, *J* = 7.0 Hz, 1H, C*H*CH_2, *Phe*), 4.57 (*br*. s, 1H, OH), 4.48 (ddd, $J_{2,3}$ = 10.7 Hz, $J_{2,NH}$ = 9.0 Hz, $J_{2,1}$ = 3.4 Hz, 1H, H-2), 4.41 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{6a,5}$ = 4.2 Hz, 1H, H-6a), 4.37 (ddd, $J_{5,4}$ = 10.3 Hz, $J_{5,6a}$ = 4.2 Hz, $J_{5,6b}$ = 2.0 Hz, 1H, H-5), 4.26 (dd, $J_{6b,6a}$ = 12.1 Hz, $J_{6b,5}$ = 2.1 Hz, 1H, H-6b), 3.42 (dd, *J* = 13.9 Hz, *J* = 5.9 Hz, 1H, C*H*HPh, *Phe*), 3.24 (dd, *J* = 14.0 Hz, *J* = 7.6 Hz, 1H, CH*H*Ph, *Phe*), 2.51 (s, 3H), 2.31 (s, 3H), 2.28 (s, 3H), 2.23 (s, 3H), 2.15 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.6, 171.2, 170.8, 169.7, 169.5 (5 x C=O), 147.2, 139.3, 138.0, 136.1, 132.7, 131.1, 129.2, 128.7, 127.8, 127.1, 126.0, 125.8, 121.4, 117.1, 116.1, 115.1 (16 x Ar, *Mef and Phe*), 91.3 (C-1), 70.5 (C-3), 68.3 (C-4), 67.5 (C-5), 62.0 (C-6), 54.5 (*C*HCH_2, *Phe*), 52.7 (C-2), 38.0 (CH_2Ph, *Phe*), 20.71, 20.70, 20.69, 20.6, 13.8 (5 x CH_3); HRMS (ESI-TOF) *m/z* for $C_{36}H_{41}N_3O_{10}Na$ $[M+Na]^+$ calcd. 698.2684, found 698.2695.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-(2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)-L-alanyl)-amino]2-

deoxy-β-D-glucopyranose (6e). Prepared following general procedure III with diclofenac (150 mg, 0.51 mmol), EDCI (117 mg, 0.61 mmol), HOBt (96 mg, 0.71 mmol) and **1**,3,4,6-tetra-*O*-acetyl-2-[(*N*-L-alanyl)-amino]2-deoxy-β-D-glucopyranose (280 mg, 0.51 mmol) to afford the desired compound, **6e** (220 mg, 57%), a mixture of rotamers, as white solid. NMR data for rotamer I: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.45 (d, J = 8.0 Hz, 2H, ArH), 7.21 (d, J = 7.5 Hz, 1H, ArH), 7.16 (t, J = 7.9 Hz, 1H, ArH), 7.11 (d, J = 8.1 Hz, 1H, ArH), 7.09 (s, 1H, ArH), 6.96 (t, J = 7.4 Hz, 1H, ArH), 6.51 (d, J = 8.1 Hz, 1H, NH, *Dic*), 6.38 (d, $J_{NH,2}$ = 9.5 Hz, 1H, NH), 6.19 (d, J = 6.3 Hz, 1H, NH, *Ala*), 5.20 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 5.03 (*app* t, J = 9.6 Hz, 1H, H-4), 4.79 (*app* t, J = 10.1 Hz, 1H, H-3), 4.29 (quint, J = 6.8 Hz, 1H, C*H*CH$_3$, *Ala*), 4.21-4.15 (m, 2H, H-2 and H-6a), 4.06 (*app* d, $J_{6b,6a}$ = 12.4 Hz, 1H, H-6b), 3.85 (d, J = 16.5 Hz, 1H, C*H*H, *Dic*), 3.66 (d, J = 16.5 Hz, 1H, CH*H*, *Dic*), 3.35 (ddd, $J_{5,6a}$ = 10.2 Hz, $J_{5,6b}$ = 4.7 Hz, $J_{5,6b}$ = 2.3 Hz, 1H, H-5), 2.08 (s, 3H, CH$_3$, *OAc*), 2.07 (s, 3H, CH$_3$, *OAc*), 2.02 (s, 3H, CH$_3$, *OAc*), 2.01 (s, 3H, CH$_3$, *OAc*), 1.24 (d, J = 7.3 Hz, 3H, CH$_3$, *Ala*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 172.5, 171.5, 170.6, 170.5, 169.21, 169.15 (6 x C=O), 142.2, 137.0, 131.7, 130.6, 129.4, 128.5, 125.2, 122.7, 121.9, 116.9 (10 x Ar), 92. (C-1), 72.6 (C-5), 72.0 (C-3), 67.6 (C-4), 61.6 (C-6), 52.3 (C-2), 50.1 (*C*CH$_3$, *Ala*), 40.2 (CH$_2$, *Dic*), 20.8, 20.70, 20.66, 20.5 (4 x CH$_3$, *OAc*), 17.64 (CH$_3$, *Ala*); HRMS (ESI-TOF) *m/z* for $C_{31}H_{35}{}^{35}Cl_2N_3O_{11}Na$ $[M+Na]^+$ calcd. 718.1541, found 718.1576.

3,4,6-Tri-*O*-acetyl-2-[(*N*-(2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)-L-phenylalanyl)-amino]2-deoxy-β-D-glucopyranose (6f'). Prepared following general procedure III with diclofenac (160 mg, 0.54 mmol), EDCI (124 mg, 0.65 mmol), HOBt (102 mg, 0.76 mmol) and 3,4,6-tri-*O*-acetyl-2-[(*N*-L-phenylalanyl)-amino]-2-deoxy-β-D-glucopyranose (244 mg, 0.54 mmol) to afford the desired compound, **6f'** (280 mg, 71%) as white solid. m.p. 200-203 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.34 (d, J = 8.0 Hz, 2H, ArH), 7.17-7.10 (m, 4H, ArH), 7.05-6.98 (m, 4H, ArH), 6.88 (td, J = 7.4 Hz, J = 1.2 Hz, 1H, ArH), 6.78 (d, $J_{NH,2}$ = 8.9 Hz, 1H, NH), 6.50 (d, J = 7.4 Hz, 1H, NH, *Phe*), 6.47 (d, J = 8.0 Hz, 1H, ArH), 5.26 (dd, J = 10.9, 9.4 Hz, 1H, H-3), 5.11 (*app* t, J = 9.8 Hz, H-4), 5.10 (d, $J_{1,2}$ = 3.3 Hz, 1H, H-1), 4.66 (td, J = 7.6 Hz, J = 5.7 Hz, 1H, C*H*NHCO, *Phe*), 4.21 (*app* dd, J = 9.2 Hz, $J_{2,1}$ = 3.6 Hz, 1H, H-2), 4.18 (dd, $J_{6a,6b}$ = 12.2. Hz, $J_{6a,5}$ = 4.0 Hz, 1H, H-6a), 4.09 (ddd, $J_{5,4}$ = 10.2 Hz, $J_{5,6a}$ = 4.0 Hz, $J_{5,6b}$ = 2.3 Hz, 1H, H-5), 4.02 (dd, $J_{6b,6a}$ = 12.3 Hz, $J_{6b,5}$ = 2.3 Hz, 1H, H-6b), 3.70 (d, J = 15.2 Hz, 1H, C*H*H, *Dic*), 3.61 (d, J = 15.2 Hz, 1H, CH*H*, *Dic*), 3.07 (dd, J = 14.0 Hz, J = 5.7 Hz, 1H, C*H*HPh, *Phe*), 2.86 (dd, J = 13.9 Hz, J = 7.7 Hz, 1H, CH*H*Ph, *Phe*), 2.08 (s, 3H), 2.02 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 172.0, 171.44, 171.36, 170.8, 169.4 (5 x C = O), 142.76, 137.30, 135.64, 130.91, 130.52, 129.06, 128.80, 128.60, 128.15, 127.00, 124.65, 123.46, 121.52, 117.11 (14 x Ar), *Dic and Phe*), 91.1 (C-1), 70.5 (C-3), 68.1 (C-4), 67.4 (C-5), 62.0 (C-6), 54.4 (*C*HNHCO, *Phe*), 52.8 (C-2), 40.1 (CH$_2$, *Dic*), 37.6 (CH$_2$, *Phe*), 20.74, 20.72, 20.6 (3 x CH$_3$, *OAc*); HRMS (ESI-TOF) *m/z* for $C_{35}H_{37}{}^{35}Cl_2N_3O_{10}Na$ $[M+Na]^+$ calcd. 752.1746, found 752.1746.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-((2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl)-glycyl)-amino]-2-deoxy-β-D-glucopyranose (6g). Prepared following general procedure IIb with indomethacin-gly-OH (105 mg, 0.25 mmol), EDCI (64 mg, 0.33 mmol), HOBt (56 mg) and acetylated glucosamine hydrochloride (96 mg, 0.25 mmol) to afford bioconjugate **6g** (0.12 g, 65%) as yellow film. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.69 (d, J = 8.5 Hz, 2H, ArH, *ArCl*), 7.50 (d, J = 8.4 Hz, 2H, ArH, *ArCl*), 7.02 (d, J = 2.5 Hz, 1H, ArH, *indole*), 6.97 (d, J = 9.0 Hz, 1H, ArH, *indole*), 6.73 (dd, J = 9.1, 2.5 Hz, 1H, ArH, *indole*), 6.49 (d, $J_{NH,2}$ = 9.4 Hz, 1H, NH), 6.33 (t, J = 5.6 Hz, 1H, CH$_2$N*H*), 5.60 (d, $J_{1,2}$ = 8.8 Hz, 1H, H-1), 5.09 (*app* t, J = 9.5 Hz, 1H, H-4), 5.03 (*app* t, J = 9.8 Hz, 1H, H-3), 4.25 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 4.6 Hz, 1H, H-6a), 4.16 (*app* q, J = 9.4 Hz, 1H, H-2), 4.10 (dd, $J_{6b,6a}$ = 12.6 H, $J_{6b,5}$ = 2.2 Hz, 1H, H-6b), 3.85 (s, 3H, CH$_3$, *OMe*), 3.80 (dd, J = 5.6 Hz, J = 3.0 Hz, 2H, CH$_2$, Gly), 3.71-3.68 (m, 3H. H-5 and CH$_2$, *Indo*), 2.38 (s, 3H, CH$_3$, *indole*), 2.08 (s, 3H, CH$_3$, *OAc*), 2.06 (s, 3H, CH$_3$, *OAc*), 2.03 (s, 3H, CH$_3$, *OAc*), 1.99 (s, 3H, CH$_3$, *OAc*).; ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 171.0, 170.7, 170.6, 169.3, 169.2, 168.8, 168.3, 156.3, 139.5, 136.6, 133.6, 131.2, 131.1, 130.3, 129.2, 115.3, 112.3, 112.0, 101.3, 92.2 (C-1), 72.7 (C-5), 72.4 (C-3), 67.7 (C-4), 61.6 (C-6), 55.9 (CH$_3$, *OMe*), 53.1 (C-2), 43.3 (CH$_2$, *Gly*), 31.8 (CH$_2$, *Indo*), 20.8, 20.7, 20.6, 20.5 (4 x CH$_3$, *OAc*), 13.3 (CH$_3$, *indole*); HRMS (ESI-TOF) *m/z* for $C_{35}H_{38}{}^{35}ClN_3O_{13}Na^+$ $[M+Na]^+$ calcd. 766.1985, found 766.1995.

1,3,4,6-Tetra-*O*-acetyl-2-[(*N*-((2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl)-4-aminobutanoyl)-amino]-2-deoxy-β-D-glucopyranose (6h). Prepared following general procedure III with indomethacin-GABA-OH (115 mg, 0.26 mmol), EDCI (236 mg, 1.23 mmol), HOBt (189 mg) and acetylated glucosamine hydrochloride (103 mg, 0.27 mmol) to afford the desired compound (0.14 g, 70%) as yellow film. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.70 (d, J = 8.4 Hz, 2H, ArH, *ArCl*), 7.50 (d, J = 8.4 Hz, 2H, ArH, *ArCl*), 6.98 (d, J = 9.0 Hz, 1H, ArH, *indole*), 6.92 (d, J = 2.5 Hz, 1H, ArH, *indole*), 6.75 (dd, J = 9.0 Hz, J = 2.5 Hz, 1H, ArH, *indole*), 6.22 (d, $J_{NH,2}$ = 9.3 Hz, 1H, NH), 5.98 (t, J = 6.2 Hz, 1H, CH$_2$N*H*), 5.74 (d, $J_{1,2}$ = 8.8 Hz, 1H, H-1), 5.16 (*app* t, J = 9.6 Hz, 1H, H-3), 5.12 (*app* t, J = 9.3 Hz, 1H, H-4), 4.30 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 4.6 Hz, 1H, H-6a), 4.20 (*app* q, J = 9.3 Hz, 1H, H-2), 4.13 (dd, $J_{6b,6a}$ = 12.5 Hz, $J_{6b,5}$ = 2.2 Hz, 1H, H-6b), 3.86 (ddd, $J_{5,4}$ = 9.6 Hz, $J_{5,6a}$ = 4.6 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 3.84 (s, 3H, CH$_3$, *OMe*), 3.65 (s, 2H, CH$_2$, *Indo*), 3.20 (dt, J = 13.5 Hz, J = 6.8 Hz, 1H, C*H*HNH, *GABA*), 3.11 (dt, J = 13.7 Hz, J = 6.4 Hz, 1H, CH*H*NH, *GABA*), 2.37 (s, 3H, CH$_3$, *indole*), 2.10 (s, 3H, CH$_3$, *OAc*), 2.07 (s, 3H, CH$_3$, *OAc*), 2.05 (s, 3H, CH$_3$, *OAc*), 2.01-1.98 (m, 2H, C*H*$_2$CONH, *GABA*), 1.98 (s, 3H, CH$_3$, *OAc*), 1.68 (*app* quint., J = 6.9 Hz, 2H, CH$_2$, *GABA*); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C 172.4, 170.7, 170.62, 170.60, 169.2, 168.4, 156.2, 139.5, 136.4, 133.5, 131.1, 131.0, 130.3, 129.1, 115.1, 112.7, 112.0, 101.1, 92.3 (C-1), 72.7 (C-3), 72.6 (C-5), 67.9 (C-4), 61.6 (C-6), 55.8 (CH$_3$, *OMe*), 52.8 (C-2), 38.4 (*C*H$_2$NH,

GABA), 33.1 (<u>C</u>H_2CONH, *GABA*), 32.1 (CH_2, *Indo*), 25.2 (CH_2, *GABA*), 20.8, 20.7, 20.6, 20.5 (4 x CH_3, *OAc*), 13.3 (CH_3, *indole*); HRMS (ESI-TOF) *m/z* for $C_{37}H_{42}{}^{35}ClN_3O_{13}Na^+$ $[M+Na]^+$ calcd. 794.2298, found 794.2308.

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Notes and references

[a] Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, FL 32611-7200, USA; tel: +1-352-392-0554, e-mail: rajones@ufl.edu

[b] Chemistry Department, Faculty of Science, King Adbulaziz University, P. O. Box 80203, Jeddah, 21589, Saudi Arabia.

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‡ Professor Alan R. Katritzky passed away 10th February 2014.

(1) a) Conaghan, P. G. *Rheumatol. Int.* **2012**, *32*, 1491-1502; b) Harirforoosh, S.; Asghar, W.; Jamali, F. *J. Pharm. Pharm. Sci.* **2013**, *16*, 821-847.
(2) a) Kourounakis, P. N.; Tsiakitzis, K.; Kourounakis, A. P.; Galanakis, D. *Toxicology* **2000**, *144*, 205-210; b) Boschelli, D. H.; Connor, D. T.; Bornemeier, D. A.; Dyer, R. D.; Kennedy, J. A.; Kuipers, P. J.; Okonkwo, G. C.; Schrier, D. J.; Wright, C. D. *J. Med. Chem.* **1993**, *36*, 1802-1810; c) Moller, N. L.; Dietzel, K. E.; Schneider, H. T.; Brune, K. *Eur. J. Med. Chem.* **1989**, *24*, 463-469.
(3) Tenenbaum, J. *Can. J. Gastroenterol.* **1999**, *13*, 119 - 122.
(4) a) Coxib and traditional NSAID Trialist' (CNT) Collaboration *Lancet* **2013**, *382*, 769-779; b) Graham, D. J.; Campen, D.; Hui, R.; Spence, M.; Cheetham, C.; Levy, G.; Shoor, S. *Lancet* **2005**, *365*, 475-481.
(5) Jones, R. *Am. J. Med.* **2001**, *110*, 4S-7S.
(6) Gierse, J.; Nickols, M.; Leahy, K.; Warner, J.; Zhang, Y.; Cortes-Burgos, L.; Carter, J.; Seibert, K.; Masferrer, J. *Eur. J. Pharmacol.* **2008**, *588*, 93-98.
(7) Wallace, J. L. *Trends Pharmacol. Sci.* **1999**, *20*, 4-6.
(8) a) Chen, L.; Yang, G.; Grosser, T. *Prostag. Oth. Lipid M.* **2013**, *104–105*, 58-66; b) Kawada, N.; Moriyama, T.; Kitamura, H.; Yamamoto, R.; Furumatsu, Y.; Matsui, I.; Takabatake, Y.; Nagasawa, Y.; Imai, E.; Wilcox, C.; Rakugi, H.; Isaka, Y. *Clin. Exp. Nephrol.* **2012**, *16*, 25-29; c) Chakraborti, A. K.; Garg, S. K.; Kumar, R.; Motiwala, H. F.; Jadhavar, P. S. *Curr. Med. Chem.* **2010**, *17*, 1563-1593.
(9) a) Scheiman, J. M.; Hindley, C. E. *Clin. Ther.* **2010**, *32*, 667-677; b) Hart, F. D.; Huskisson, E. C. *Drugs* **1984**, *27*, 232-255.
(10) Suemasu, S.; Yamakawa, N.; Ishihara, T.; Asano, T.; Tahara, K.; Tanaka, K.; Matsui, H.; Okamoto, Y.; Otsuka, M.; Takeuchi, K.; Suzuki, H.; Mizushima, T. *Biochem. Pharmacol.* **2012**, *84*, 1470-1481.
(11) a) Katritzky, A. R.; Jishkariani, D.; Narindoshvili, T. *Chem. Biol. Drug Des.* **2009**, *73*, 618-626; b) Shalaby, A. M.; El-Eraky, W. *Il Farmaco* **1997**, *52*, 83-87.
(12) Zhao, X. G.; Tao, X. Y.; Wei, D. Z.; Song, Q. X. *Eur. J. Med. Chem.* **2006**, *41*, 1352-1358.
(13) Tiwari, A. D.; Panda, S. S.; Girgis, A. S.; Sahu, S.; George, R. F.; Srour, A. M.; Starza, B. L.; Asiri, A. M.; Hall, C. D.; Katritzky, A. R. *Org. Biomol. Chem.* **2014**, *12*, 7238-7249.
(14) Galanakis, D.; Kourounakis, A. P.; Tsiakitzis, K. C.; Doulgkeris, C.; Rekka, E. A.; Gavalas, A.; Kravaritou, C.; Charitos, C.; Kourounakis, P. N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3639-3643.
(15) Dang, C.-H.; Nguyen, C.-H.; Nguyen, T.-D.; Im, C. *RSC Adv.* **2014**, *4*, 6239-6245.
(16) Dahmer, S.; Schiller, R. M. *Am. Fam. Physician* **2008**, *78*, 471-476.
(17) a) Herrero-Beaumont, G.; Ivorra, J. A.; Del Carmen Trabado, M.; Blanco, F. J.; Benito, P.; Martin-Mola, E.; Paulino, J.; Marenco, J. L.; Porto, A.; Laffon, A.; Araujo, D.; Figueroa, M.; Branco, J. *Arthritis Rheum.* **2007**, *56*, 555-567; b) Das, A., Jr.; Hammad, T. A. *Osteoarthr. Cartilage* **2000**, *8*, 343-350.
(18) Vangsness, C. T., Jr.; Spiker, W.; Erickson, J. *Arthroscopy* **2009**, *25*, 86-94.
(19) Clegg, D. O.; Reda, D. J.; Harris, C. L.; Klein, M. A.; O'Dell, J. R.; Hooper, M. M.; Bradley, J. D.; Bingham, C. O., 3rd; Weisman, M. H.; Jackson, C. G.; Lane, N. E.; Cush, J. J.; Moreland, L. W.; Schumacher, H. R., Jr.; Oddis, C. V.; Wolfe, F.; Molitor, J. A.; Yocum, D. E.; Schnitzer, T. J.; Furst, D. E.; Sawitzke, A. D.; Shi, H.; Brandt, K. D.; Moskowitz, R. W.; Williams, H. J. *N. Engl. J. Med.* **2006**, *354*, 795-808.
(20) Huskisson, E. C. *J. Int. Med. Res.* **2008**, *36*, 1161-1179.
(21) Altman, R. D.; Abadie, E.; Avouac, B.; Bouvenot, G.; Branco, J.; Bruyere, O.; Calvo, G.; Devogelaer, J. P.; Dreiser, R. L.; Herrero-Beaumont, G.; Kahan, A.; Kreutz, G.; Laslop, A.; Lemmel, E. M.; Menkes, C. J.; Pavelka, K.; Van De Putte, L.; Vanhaelst, L.; Reginster, J. Y. *Osteoarthr. Cartilage* **2005**, *13*, 13-19.
(22) a) Ibrahim, M. A.; Panda, S. S.; Birs, A. S.; Serrano, J. C.; Gonzalez, C. F.; Alamry, K. A.; Katritzky, A. R. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1856-1861; b) Panda, S. S.; Hall, C. D.; Scriven, E.; Katritzky, A. R. *Aldrichim Acta* **2013**, *46*, 43-55; c) Panda, S. S.; Ibrahim, M. A.; Kucukbay, H.; Meyers, M. J.; Sverdrup, F. M.; El-Feky, S. A.; Katritzky, A. R. *Chem. Biol. Drug Des.* **2013**, *82*, 361-366; d) Panda, S. S.; Bajaj, K.; Meyers, M. J.; Sverdrup, F. M.; Katritzky, A. R. *Org. Biomol. Chem.* **2012**, *10*, 8985-8993.
(23) a) Silva, D. J.; Wang, H. M.; Allanson, N. M.; Jain, R. K.; Sofia, M. J. *J. Org. Chem.* **1999**, *64*, 5926-5929; b) Medgyes, A.; Farkas, E.; Liptak, A.; Pozsgay, V. *Tetrahedron* **1997**, *53*, 4159-4178.
(24) Franssen, E. J. F.; Koiter, J.; Kuipers, C. A. M.; Bruins, A. P.; Moolenaar, F.; De Zeeuw, D.; Kruizinga, W. H.; Kellogg, R. M.; Meijer, D. K. F. *J. Med. Chem.* **1992**, *35*, 1246-1259.
(25) Aureli, L.; Cruciani, G.; Cesta, M. C.; Anacardio, R.; De Simone, L.; Moriconi, A. *J. Med. Chem.* **2005**, *48*, 2469-2479.
(26) Kalgutkar, A. S.; Marnett, A. B.; Crews, B. C.; Remmel, R. P.; Marnett, L. J. *J. Med. Chem.* **2000**, *43*, 2860-2870.
(27) Sahu, S.; Panda, S. S.; Asiri, A. M.; Katritzky, A. R. *Synthesis* **2013**, *45*, 3369-3374.
(28) Moser, P.; Sallmann, A.; Wiesenberg, I. *J. Med. Chem.* **1990**, *33*, 2358-2368.
(29) Giordano, C.; Gallina, C.; Consalvi, V.; Scandurra, R. *Eur. J. Med. Chem.* **1991**, *26*, 753-762.
(30) Wang, L. F.; Kong, L. Q.; Fan, L.; Yang, D. C. *Res. Chem. Intermed.* **2010**, *36*, 237-243.
(31) Boc deprotection under acidic conditions (HCl in dioxane) resulted in acyl migration, as observed by proton NMR.
(32) **2b** (1.2 eq.), DIPEA (3.5 eq.), DMF/MeCN (2:8, v/v), -15 °C to r.t.
(33) a) Liefländer, M. *Hoppe-Seyler's Z. Physiol. Chem.* **1967**, *348*, 363-370; b) Liefländer, M. *Hoppe-Seyler's Z. Physiol. Chem.* **1967**, *348*, 477-481.
(34) Bergmann, M.; Zervas, L. *Chem. Ber.* **1932**, *65*, 1192-1201.

