# Synthesis and Activity of Dipeptides, Linked to Targeting Ligands, as Specific NK Cell Enhancers

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Water soluble analogues of the lipophilic immunostimulant, octadecyl D-alanyl-L-glutamine, BCH-527, were synthesized and evaluated for the ability to stimulate natural killer (NK) cells. One of these compounds in which the octadecyl chain of BCH-527 was replaced with a shorter chain alcohol, 6-(D-alanyl-L-glutaminylamino)hexan-1-ol, **9**, displayed an in vitro stimulation of NK cells comparable to that of interleukin 2 (IL 2). However, when the hydroxyl of **9** was linked to L-fucose to yield 1-$\beta$-[6-(D-alanyl-L-glutaminylamino)hex-1-yl]-L-fucopyranose (BCH-2537, **1**), the observed stimulation of NK cells was greater than that observed with IL 2. Further evaluation of these compounds revealed that the improved in vitro activity of BCH-2537 was more pronounced in vivo. That is, while both compounds significantly increased splenic NK cells, only BCH-2537 significantly increased the activity of these cells in vivo. In terms of a structure−activity relationship, NK cell activity was sensitive to minor structural modifications. It was influenced by conservative substitutions within the dipeptide, the length of the hydrocarbon chain, and the functionality at the end of the chain. No other compound enhanced NK cell activity to the extent exhibited by BCH-2537, although a few were equipotent to **9**.

## Introduction

Over the years, octadecyl L-tyrosine has been described in the literature as an inert, insoluble carrier of immunological activation signals provided by vaccine antigens, allergens, etc.[1] More recently, we reported that an immunomodulatory "signal" could be incorporated into the insoluble carrier, as exemplified by octadecyl D-alanyl-L-glutamine; BCH-527.[2] The dipeptide signal sequence was developed from the well-known, but toxic, immunomodulator muramyl dipeptide (MDP; *N*-acetylmuramyl-L-alanyl-D-isoglutamine).[3] BCH-527 induces a significant activation of NK cells and macrophages in vivo, which is reflected by an antiviral activity against murine cytomegalovirus. However, the insolubility of BCH-527 limits its utility as a therapeutic agent. It was therefore desired to develop an analogue of BCH-527 with reasonable aqueous solubility and selectivity toward NK cell or macrophage populations.

With regard to solubility, it was recognized that obvious modifications to the BCH-527 structure, such as shortening the hydrocarbon chain, could solve the problem. However, for a range of chain length, detergency and accompanying toxicity can become an issue. Removal of the ester chain would yield a dipeptide that is expected to be cleared rapidly. Interestingly, lower alkyl esters of dipeptides have been reported to be inhibitory toward cytolytic immune cells.[4] Therefore, an approach was taken which was expected to introduce polarity into the molecule, prevent rapid degradation and clearance of the molecule, and target the dipeptide signal to NK cells and/or macrophages. That is, introduction of L-fucose at the carboxyl terminus of the dipeptide. NK cells possess receptors for monosaccharides (e.g., lectins such as NKR-P1)[5] which may permit intracellular delivery of the immunostimulant signal. Indeed, it has been reported that L-fucose enhances human NK cell activity.[6]

The importance of NK cells is illustrated by their role in immunosurveillance, and much work is underway to elucidate their mechanism of action.[7] They act as a first line of defense by the recognition and cytolysis of, for example, metastatic tumors or virus-infected cells. A number of immunostimulants, primarily interferon inducers, stimulate NK cells along with other immune cell subsets. Well-known examples include tilorone, CL 246738, 7-thia-8-oxoguanosine, loxoribine, bropirimine, and imiquimod. Nevertheless, few compounds have been reported which specifically increase NK cell activity and/or populations. Only recently have examples appeared in the literature. These include agelasphin-11, an α-galactosylceramide isolated from sponge,[8] and eisenin, a tripeptide isolated from algae.[9] Yet, compounds which specifically activate NK cells are expected to be less toxic than immunostimulants, such as interferon inducers, which stimulate multiple immune cell subsets. The advantages of a selective immune cell stimulant have recently been discussed with respect to organ transplantation.[10] In this paper, we report on the immunological activity of synthetic dipeptide compounds which selectively activate NK cells.

## Chemistry

The synthesis of 1-$\beta$-[6-(D-alanyl-L-glutaminylamino)-hex-1-yl]-L-fucopyranose (BCH-2537, compound **1**), is shown in Scheme 1. Thus a suitably N-protected glutamate ester was used as a glutamine precursor. This was necessary since the carboxamide of glutamine led

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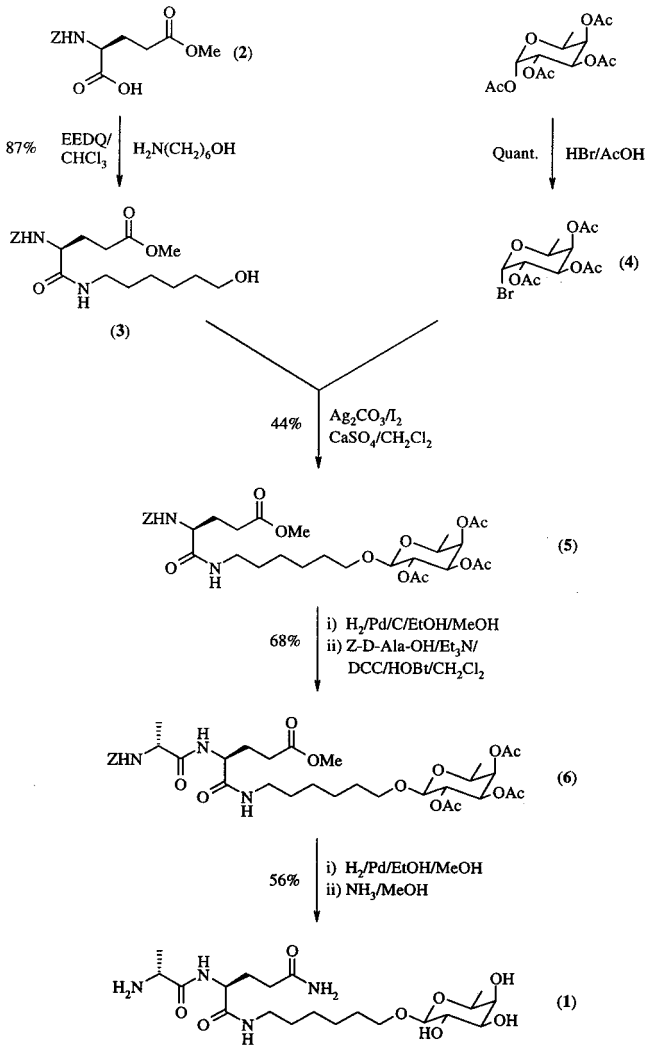
**Scheme 1:** Synthesis of 1-$\beta$-[6-(D-Alanyl-L-glutaminylamino)hex-1-yl]fucopyranose, BCH-2537, **1**




to intermediates with poor solubility and gave coupling yields which were not satisfactory. Although the benzyloxycarbonyl-protected intermediate was used in the synthesis of **1**, during analogue preparation it was found that *tert*-butoxycarbonyl protection strategy gave superior yields in the deprotection/coupling sequence. Use of a glutamate ester allowed facile conversion by aminolysis with ammonia to yield the glutamine side chain, and use of the acetyl-protected sugar allowed deprotection of this portion of the molecule in the same step. Initial syntheses used the methyl ester. However, it was found that the benzyl ester gave shorter reaction times without a decrease in product yield. For the synthesis of **1**, 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) coupling of the glutamic acid derivative **2** with unprotected 6-aminohexan-1-ol proceeded selectively, in high yield, to give the alcohol **3**. Glycosidic coupling with the protected bromofucose **4**, prepared quantitatively from the peracetate, was found to occur readily under traditional Königs–Knorr conditions. This afforded the glycopeptide **5** exclusively as the $\beta$-anomer. N-Deprotection and carbodiimide coupling gave the fully protected intermediate **6**, which was smoothly deprotected in two steps (hydrogenolysis followed by treatment with methanolic ammonia) to give compound **1**.

This strategy was followed for most other members of the series, using appropriately protected starting materials. However, the synthesis of certain analogues dictated the use of alternative synthetic routes. These analogues were prepared by standard procedures, which are described in the Experimental Section. Thus, analogues with no linker, **15**, **16**, and **19**, and the thioanalogue **10** were prepared from the amino- and thioglycosides, respectively.[11,12] Interestingly, in the case of the glucose analogue **7**, the Königs–Knorr methodology gave unacceptable yields. This compound was therefore synthesized using the trichloroacetimidate coupling procedure.[13]

## In Vitro Analysis: SAR Studies

Activation of murine NK cells is routinely measured in vitro by determination of the ability to lyze YAC-1 target cells in a conventional chromium release assay. Therefore, splenocytes were incubated for 4 h with $^{51}$Cr-labeled YAC-1 tumor cells and the released chromium determined in the lysate. With this assay, compound **1** (BCH-2537) was shown to have a direct stimulatory effect on NK cells which was consistently greater than the positive control; interleukin 2 (IL 2). Additionally, the desfucose analogue, compound **9**, consistently displayed a stimulatory effect on NK cells which was equivalent to IL 2. The assays were performed seven times ($n$ = 7), and the results are presented in Table 1. On the basis of these data, BCH-2537 was selected as a lead upon which to define a structure–activity relationship for dipeptide–linker–monosaccharide. As shown in Tables 1–4, this was undertaken by systematic modification of the monosaccharide, linker, and amino acid portions of the molecule.

**(i) Variation of the Monosaccharide.** The in vitro data for modification of the monosaccharide portion of the molecule is shown in Table 1, entries 1–9. The data indicates a preference for L-fucose. Substitution by D-glucose, **7**, results in complete loss of activity. Moderate activity is observed with the D-mannose derivative **8**. This suggests that it is not the physiochemical properties of the sugar, such as increased polarity, which are important. That is, there is a specific monosaccharide–cell surface receptor interaction. Omission of the sugar to give alcohol **9** yielded a compound with activity which was surpassed only by **1**. Hence, the incorrect sugar configuration functions as an antagonist. Even substitution of L-fucose for the thioanalogue **10** resulted in significantly weaker stimulation, although the potency was similar. Simple substitution of the hydroxyl function of **9** with chlorine, **11**, or the carboxylate, **12**, resulted in the loss of all activity. However, activity was seen with an amino replacement, **13**, but the analogue was not very potent. Capping of alcohol **9** with an *O*-acetyl group, **14**, also resulted in the complete loss of activity.

**(ii) Variation of the Linker.** It was next decided to vary the length and type of the linker between the D-alanyl-L-glutamine dipeptide and the monosaccharide, as shown in Table 1, entries 10–18. Omission of the linker from **1** to give compound **15** resulted in a loss of approximately half of the activity, but little change in

**Table 1.** In Vitro Natural Killer Cell Activity of D-Alanyl-L-glutaminyl−Linker−Sugars and Analogues Relative to Interleukin 2

D-Ala-L-Gln-linker-R

| entry | linker | R | compd | activity[a] | concentration[b] |
|---|---|---|---|---|---|
| 1 | $NH(CH_2)_6O$ | L-fucose | BCH-2537 **1** | +++++ | $10^{-12}$−$10^{-7}$ |
| 2 | $NH(CH_2)_6O$ | D-glucose | **7** | 0 | |
| 3 | $NH(CH_2)_6O$ | D-mannose(2-*O*-Ac)[c] | **8** | ++++ | $10^{-4}$ |
| 4 | $NH(CH_2)_6O$ | H | **9** | ++++ | $10^{-12}$−$10^{-4}$ |
| 5 | $NH(CH_2)_6S$ | L-fucose | **10** | ++ | $10^{-12}$−$10^{-8}$ |
| 6 | $NH(CH_2)_6$ | Cl | **11** | 0 | |
| 7 | $NH(CH_2)_5$ | $CO_2H$ | **12** | 0 | |
| 8 | $NH(CH_2)_6$ | $NH_2$ | **13** | ++++ | $10^{-4}$ |
| 9 | L-Ala-D-Gln- $NH(CH_2)_6OAc$ | | **14** | 0 | |
| 10 | NH | $\beta$-L-fucose | **15** | +++ | $10^{-12}$−$10^{-4}$ |
| 11 | NH | α-L-fucose | **16** | 0 | |
| 12 | OH | | **17** | 0 | |
| 13 | D-Ala-L-glutarimide | | **18** | 0 | |
| 14 | NH | $\beta$-D-mannose | **19** | ++ | $10^{-12}$−$10^{-5}$ |
| 15 | $NH(CH_2)_{12}O$ | L-fucose | **20** | + | $10^{-8}$−$10^{-4}$ |
| 16 | $NH(CH_2)_{12}O$ | D-mannose(2-*O*-Ac)[c] | **21** | ++ | $10^{-12}$−$10^{-8}$ |
| 17 | $NH(CH_2)_{12}O$ | H | **22** | ++ | $10^{-10}$−$10^{-4}$ |
| 18 | NH-1,4-$C_6H_4(CH_2)_2O$ | L-fucose | **23** | 0 | |

[a] All compounds were tested in the concentration range $10^{-4}$−$10^{-12}$ M. Reported concentrations represent the peak or the range of peak activity. Each active compound was tested two to five times. Inactive compounds were tested once. Peak activities are reported, relative to positive IL 2 control. Therefore, with IL 2 control as 100% stimulation, then 0 = 0−20% of IL 2; + = 21−40% of IL 2; ++ = 41−60% of IL 2; +++ = 61−80% of IL 2; ++++ = 81−100% of IL 2; +++++ = 101−120% of IL 2. [b] Quoted over the range of activity observed. [c] The 2-*O*-acetyl function and other protecting groups could not be removed under an array of conditions without degradation of the product.

**Table 2.** In Vitro Natural Killer Cell Activity of Dipeptide-Aminohex-1-ylfucopyranoses Relative to Interleukin 2

X-Y-$NH(CH_2)_6$O-L-fucose

| entry | X | Y | compd | activity[a] | concentration[b] |
|---|---|---|---|---|---|
| 1 | D-Ala | L-Gln | **1** | +++++ | $10^{-12}$−$10^{-7}$ |
| 2 | L-Ala | L-Gln | **24** | +++ | $10^{-12}$−$10^{-5}$ |
| 3 | D-Val | L-Gln | **25** | ++ | $10^{-12}$−$10^{-4}$ |
| 4 | L-amino-Ala | L-Gln | **26** | +++ | $10^{-12}$−$10^{-4}$ |
| 5 | | L-Gln | **27** | + | $10^{-8}$−$10^{-4}$ |
| 6 | Ac-D-Ala | L-Gln | **28** | 0 | |
| 7 | Ac-L-Ala | L-Gln | **29** | 0 | |
| 8 | Ac | L-Gln | **30** | 0 | |
| 9 | $\beta$-Ala | L-Gln | **31** | 0 | |
| 10 | L-Ala | D-Gln | **32** | 0 | |
| 11 | D-Ala | L-Glu | **33** | ++ | $10^{-12}$−$10^{-4}$ |
| 12 | L-amino-Ala | L-Glu | **34** | ++ | $10^{-7}$ |
| 13 | | L-pyro-Glu | **40** | 0 | |
| 14 | Me | L-pyro-Glu | **41** | 0 | |
| 15 | D-Ala | Gly | **42** | 0 | |
| 16 | D-Ala | L-Orn | **43** | 0 | |
| 17 | D-Ala | L-Asn | **44** | 0 | |
| 18 | L-Ala | L-Pro | **45** | 0 | |
| 19 | L-Ile | L-Pro | **46** | 0 | |

[a] All compounds were tested in the concentration range $10^{-4}$−$10^{-12}$ M. Reported concentrations represent the peak or the range of peak activity. Each active compound was tested two to five times. Inactive compounds were tested once. Peak activities are reported, relative to positive IL 2 control. Therefore, with IL 2 control as 100% stimulation, then 0 = 0−20% of IL 2; + = 21−40% of IL 2; ++ = 41−60% of IL 2; +++ = 61−80% of IL 2; ++++ = 81−100% of IL 2; +++++ = 101−120% of IL 2. [b] Quoted over the range of activity observed.

potency. However, a change in the stereochemistry of the glycosidic linkage to yield the α-L-fucose analogue **16** resulted in complete loss of activity. A similar abrogation of activity was observed with the dipeptide **17** (no linker, no sugar) or the carboximide of **17**, compound **18**. The D-mannose equivalent of **15**, compound **19**, displayed a loss of activity relative to its parent **8** and relative to **15**. Extension of the linker to a 12-carbon chain, compounds **20**, **21**, and **22**, led to a significant loss of activity, regardless of the sugar present. Introduction of an aromatic ring into the linker of **1**, to give compound **23**, also abolished activity. Taken together, these data suggest that a flexible hexamethylene chain provides an optimum spatial arrangement between the dipeptide and monosaccharide.

**(iii) Variation of the Dipeptide.** Numerous analogues of BCH-2537 were synthesized which contained variations of the dipeptide signal, as shown in Table 2. Activity is significantly affected by variations in the D-alanyl-L-glutamine sequence. For example, conservative substitution of D-alanine with other α-amino acids gave analogues with moderate activity, compounds **24**−**26**. Note that this includes the L-alanine diastereomer **24**. Interestingly, even with the removal of the first amino acid to give **27**, weak activity remains. However, acetylation of alanine, compounds **28** and **29**, or replacement of alanine with an acetyl group, compound **30**, abolished activity. Activity is also lost when alanine is replaced with $\beta$-alanine, compound **31**. These data illustrate the requirement for an appropriately placed primary amino function or positive charge.

Very little change is tolerated at the L-glutamine position. In contrast to D-alanine, the enantiomeric D-glutamine resulted in an inactive compound **32**. Replacement with L-glutamate resulted in compounds **33** and **34** which displayed moderate activity. All other changes, compounds **40**−**46**, afforded inactive analogues. For example, compound **44**, which contained the glutamine homologue asparagine, was devoid of activity. L-Alanyl-L-proline and L-isoleucyl-L-proline analogues, compounds **45** and **46**, were examined because of the structural similarity to alanylglutamine and its occurrence at the *N*-terminus of cytokine immunostimulants.[14]

Analogues were also examined in which the dipeptide was varied and the fucose portion was replaced with

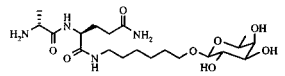
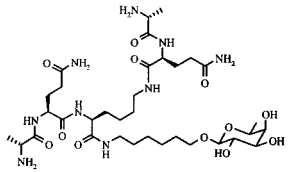
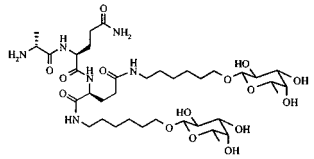
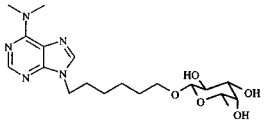
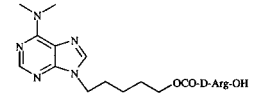
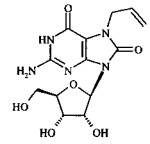
**Table 3.** In Vitro Natural Killer Cell Activity of Signal−Aminohex-1-yl−Sugar Relative to Interleukin 2

X-Y-NH$(CH_2)_6$O-R

| entry | X-Y | R | compd | activity[a] | concentration[b] |
|---|---|---|---|---|---|
| 1 | D-Ala-L-Gln | L-fucose | **1** | +++++ | $10^{-12}$−$10^{-7}$ |
| 2 | D-Ala-L-Gln | D-mannose(2-*O*-Ac) | **7** | ++++ | $10^{-4}$ |
| 3 | L-Ala-L-Gln | D-mannose(2-*O*-Ac) | **47** | ++++ | $10^{-10}$ |
| 4 | Ac-D-Ala-L-Gln | D-mannose(2-*O*-Ac) | **48** | +++ | $10^{-7}$−$10^{-4}$ |
| 5 | L-Gln | D-mannose(2-*O*-Ac) | **49** | ++++ | $10^{-5}$ |
| 6 | L-pyro-Glu | D-mannose(2-*O*-Ac) | **50** | +++ | $10^{-12}$−$10^{-7}$ |
| 7 | cyclo(D-Ala-L-Glu) | D-mannose(2-*O*-Ac) | **51** | 0 | |
| 8 | L-Ala-L-Gln | H | **52** | ++ | $10^{-12}$−$10^{-4}$ |
| 9 | L-Ala-D-Gln | H | **53** | ++ | $10^{-12}$−$10^{-8}$ |
| 10 | L-Gln | H | **54** | 0 | |
| 11 | L-pyro-Glu | H | **55** | +++ | $10^{-12}$−$10^{-5}$ |
| 12 | L-Ala-L-Pro | H | **56** | 0 | |
| 13 | L-Ile-L-Pro | H | **57** | + | $10^{-12}$−$10^{-6}$ |

[a] All compounds were tested in the concentration range of $10^{-4}$−$10^{-12}$ M. Reported concentrations represent the peak or the range of peak activity. Each active compound was tested two to five times. Inactive compounds were tested once. Peak activities are reported, relative to positive IL 2 control. Therefore, with IL 2 control as 100% stimulation, then 0 = 0−20% of IL 2; + = 21−40% of IL 2; ++ = 41−60% of IL 2; +++ = 61−80% of IL 2; ++++ = 81−100% of IL 2; +++++ = 101−120% of IL 2. [b] Quoted over the range of activity observed.

**Table 4.** In Vitro Natural Killer Cell Activity of Other Fucose Analogues of BCH-2537, 1, Relative to Interleukin 2

| | Structure | Compd | Activity[a] | Concentration[b] |
|---|---|---|---|---|
| 1 |  | **1** | +++++ | $10^{-12}$-$10^{-7}$ |
| 2 |  | **35** | + | $10^{-4}$ |
| 3 |  | **36** | 0 | – |
| 4 |  | **37** | ++++ | $10^{-12}$-$10^{-6}$ |
| 5 |  | BCH-1393<br>**38** | 0 | – |
| 6 |  | **39** | 0 | – |

[a] All compounds were tested in the concentration range $10^{-4}$−$10^{-12}$ M. Reported concentrations represent the peak or the range of peak activity. Each active compound was tested two to five times. Inactive compounds were tested once. Peak activities are reported, relative to positive IL 2 control. Therefore, with IL 2 control as 100% stimulation, then 0 = 0−20% of IL 2; + = 21−40% of IL 2; ++ = 41−60% of IL 2; +++ = 61−80% of IL 2; ++++ = 81−100% of IL 2; +++++ = 101−120% of IL 2. [b] Quoted over the range of activity observed.

D-mannose or removed to yield the corresponding alcohol. This is presented in Table 3, compounds **47**−**57**. In all cases the analogue exhibited less activity than **1**. However, some activity was maintained through a greater variation of dipeptide signal than seen with the L-fucose series.

**(iv) Other Analogues.** Table 4 shows the results of further investigation of the activity of analogues of BCH-2537, **1**. These compounds represent an extension of the general dipeptide−linker−monosaccharide structure. First, analogues were synthesized which contained either two dipeptide signal sequences, **35**, or two fucose monosaccharides, **36**. The goal was to exploit the known clustering of cell surface receptors as a primary event in cell−cell contact and cell signaling processes. Indeed, individual protein−carbohydrate (receptor−ligand) interactions are generally weak. To enhance the strength of cell surface binding, protein−saccharide interactions often occur via multivalent presentation in order to achieve the necessary avidity.[15] Therefore, while these initial results were disappointing, the length of the linker or distance between the bivalent groups may not be appropriate for a synergistic binding interaction.

Additionally, the hybrid molecule **37** was synthesized in which the L-fucose sugar is linked to the purine portion of the cytotoxic T-lymphocyte (CTL) activator 6-(*N*,*N*-dimethylamino)purin-9-yl pentoxycarbonyl-D-arginine; BCH-1393,[16] **38**. This expanded the specific CTL activity of BCH-1393 to include a significant NK cell stimulation. In view of the recently described commonality of CTL and NK cells and their collective role in immune surveillance,[17] compound **37** exhibits an interesting in vitro immune profile.

Finally, the activity of BCH-2537, **1**, was compared to the well-described NK cell activator 7-allyl-8-oxoguanosine, **39** (loxoribine).[18] The lack of activity of loxoribine in our direct-stimulation (4 h incubation) assay is consistent with the indirect activation of NK cells via the induction of interferon. Demonstration of the activity of loxoribine in the chromium release assay requires a 14 h incubation of the NK cells with YAC-1 target cells.

**(v) In Vitro Immune Profile of 1.** The in vitro activity of BCH-2537, **1**, was both potent and selective for NK cells. The latter was evidenced by a lack of activity in B- and T-cell mitogenic proliferation assays, macrophage phagocytosis and respiratory burst, and the

**Table 5.** Summary of the in Vitro Immunological Analysis of BCH-2537, 1

| assay[a] | cell source[b] | activity[c] |
|---|---|---|
| NK cell cytolytic activity (YAC-1 target) | murine splenocytes | +++++ |
| NK cell cytolytic activity (K 562 target) | human PBML | ++++ |
| phagocytosis | murine whole blood | NE |
| respiratory burst | murine whole blood | NE |
| phagocytosis | human PBML | NE |
| respiratory burst | human PBML | NE |
| cytotoxic T lymphocyte activity | murine splenocytes | + |
| mixed lymphocyte reaction | murine splenocytes | NE |
| T cell proliferation (PHA, ConA) | murine splenocytes | NE |
| B cell proliferation (PWM, LPS) | murine splenocytes | NE |

[a] Experimental protocol for murine NK cell assay is described in the Experimental Section, while the protocol for human NK cell assay is given in ref 22. Protocol for measurement of macrophage/monocyte phagocytosis and respiratory burst is given in ref 23. Protocol for CTL/MLR assay is given in ref 16. Protocol for mitogen-induced lymphocyte (T and B cell) assay is given in ref 22. [b] PBML = peripheral blood mononuclear leukocytes: obtained from heparinized blood by density gradient centrifugation on Ficoll-Hypaque. Splenocytes: obtained as described in the Experimental Section. [c] NE = no effect. For NK cell and CTL assays, activity is reported relative to IL 2 control. Therefore, with IL 2 control as 100% stimulation, then 0 = 0−20% of IL 2; + = 21−40% of IL 2; ++ = 41−60% of IL 2; +++ = 61−80% of IL 2; ++++ = 81−100% of IL 2; +++++ = 101−120% of IL 2.

mixed lymphocyte reaction. However, compound **1** weakly enhances cytotoxic T-cells. A summary of the in vitro analysis of BCH-2537, **1**, is presented in Table 5.

### In Vivo Activity of BCH-2537, 1

In view of the significant in vitro NK cell activity of BCH-2537, **1**, this compound was selected for in vivo evaluation. Also, because of the similar activity of the desfucose analogue **9**, and its relatively easier synthesis, it was also selected for a more limited in vivo evaluation. It was possible that the small difference in activity between BCH-2537 and **9** would become insignificant upon in vivo analysis. Therefore, two immunophenotyping experiments were undertaken with normal immune status mice. The first experiment was performed in the presence or absence of BCH-2537 to determine the increase or decrease in immune cell subset populations, *relative to each other*. Mice were injected intraperitoneally for four consecutive days with 5, 25, or 50 mg/kg of BCH-2537. At the end of the experiment, blood and spleens were collected for determination by flow cytometry of the effects of BCH-2537 on the immune cell subsets within these tissues. The second experiment was performed in the presence or absence of **9** or BCH-2537 in order to undertake a direct comparison of the two compounds. Mice were injected intraperitoneally for four consecutive days with 5 or 25 mg/kg of **9** or BCH-2537. Again, blood and spleens were collected at the end of the experiment for analysis by flow cytometry. A summary of the results is presented in Tables 6 and 7.

The first experiment demonstrated that BCH-2537 induces a significant increase in NK cells. In blood, 25 mg/kg of BCH-2537 resulted in a significant increase in NK cells (24%, $P \leq 0.015$). Significant increases also occur in T-helper cells (CD4+CD45+, 45%, $P \leq 0.02$) and monocytes (CD11b+, 93% to 427%, $P \leq 0.048$). In spleen, a significant increase in NK cells (12% to 23%, $P \leq 0.039$) is observed at 25 and 50 mg/kg of BCH-2537.

**Table 6.** Summary of Immunophenotyping Data for Mice Administered Four Doses of BCH-2537, **1**

| cell subsets | | 0 mg/kg | 25 mg/kg | 50 mg/kg |
|---|---|---|---|---|
| Blood Immunophenotyping BCH-2537, **1**[a] | | | | |
| CD4+CD45+ | mean | 21.3 | 32.5 | 30.9 |
| | STD | 2.8 | 5.3 | 4.8 |
| | *P* value | | 0.02 | 0.002 |
| NK+ | mean | 5.40 | 6.70 | 5.90 |
| | STD | 0.5 | 0.2 | 0.6 |
| | *P* value | | 0.015 | 0.2 |
| CD4+CD11b+ | mean | 0.75 | 3.95 | 1.45 |
| | STD | 0.4 | 2.2 | 0.3 |
| | *P* value | | 0.048 | 0.032 |
| Spleen Immunophenotyping BCH-2537, **1** | | | | |
| CD4+CD45+ | mean | 22.8 | 26.2 | 26.5 |
| | STD | 1.4 | 1.0 | 1.6 |
| | *P* value | | 0.02 | 0.008 |
| NK+CD3+ | mean | 1.90 | 2.13 | 2.33 |
| | STD | 0.2 | 0.1 | 0.2 |
| | *P* value | | 0.039 | 0.021 |

[a] Groups of four C57BL/6 mice were injected ip for four consecutive days with BCH-2537. No effect was observed on NK cells when mice were given 5 mg/kg BCH-2537.

**Table 7.** Summary of Immunophenotyping Data for Mice Administered Four Doses of Either BCH-2537, **1**, or the Desfucose Analogue **9**

| cell subsets | | 0 mg/kg | 5 mg/kg | 25 mg/kg |
|---|---|---|---|---|
| Blood Immunophenotyping BCH-2537, **1**[a] | | | | |
| CD4+CD11b+ | mean | 5.70 | 9.00 | 7.20 |
| | STD | 2.6 | 1.7 | 2.0 |
| | *P* value | | 0.049 | 0.46 |
| CD11b+ | mean | 24.7 | 38.6 | 20.6 |
| | STD | 8.6 | 3.8 | 4.6 |
| | *P* value | | 0.043 | 0.26 |
| Spleen Immunophenotyping BCH-2537, **1** | | | | |
| CD4+ | mean | 24.3 | 29.6 | 29.5 |
| | STD | 2.5 | 2.5 | 3.7 |
| | *P* value | | 0.02 | 0.15 |
| NK+ | mean | 2.13 | 3.88 | 3.90 |
| | STD | 0.7 | 0.6 | 0.7 |
| | *P* value | | 0.006 | 0.041 |
| NK+CD3+ | mean | 2.30 | 3.64 | 2.82 |
| | STD | 0.3 | 0.8 | 0.2 |
| | *P* value | | 0.027 | 0.146 |
| CD4+CD11b+ | mean | 2.14 | 4.30 | 4.34 |
| | STD | 0.6 | 1.0 | 0.9 |
| | *P* value | | 0.007 | 0.006 |
| CD11b+ | mean | 3.78 | 5.56 | 7.08 |
| | STD | 0.2 | 0.8 | 1.2 |
| | *P* value | | 0.008 | 0.015 |
| Blood Immunophenotyping, compound **9** no significant effect | | | | |
| Spleen Immunophenotyping, compound **9** | | | | |
| CD4+ | mean | 24.3 | 32.1 | 36.0 |
| | STD | 2.5 | 3.6 | 3.9 |
| | *P* value | | 0.003 | 0.005 |
| CD8+ | mean | 19.3 | 26.4 | 31.6 |
| | STD | 3.1 | 3.6 | 4.5 |
| | *P* value | | 0.023 | 0.001 |
| NK+ | mean | 2.13 | 2.76 | 3.08 |
| | STD | 0.7 | 0.1 | 0.5 |
| | *P* value | | 0.080 | 0.008 |
| NK+CD3+ | mean | 2.30 | 3.04 | 2.96 |
| | STD | 0.3 | 1.0 | 0.3 |
| | *P* value | | 0.094 | 0.004 |
| CD4+CD11b+ | mean | 2.14 | 3.00 | 3.04 |
| | STD | 0.6 | 1.1 | 0.6 |
| | *P* value | | 0.073 | 0.013 |

[a] Groups of five C57BL/6 mice were injected ip for four consecutive days with BCH-2537, **1**, or compound **9**.

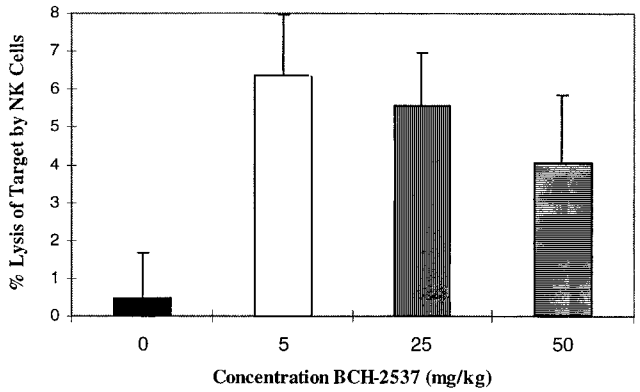



**Figure 1.** Dose−response profile of the functional ex vivo NK cell activity of BCH-2537, **1**.

This is accompanied by a significant increase in T-helper cells (13% to 14%, $P \leq 0.02$).

The second experiment with BCH-2537 confirmed a significant increase in NK cells. However, the immune cell subset profile is different. For example, there is no significant increase in NK cells in the blood. Nonetheless, in the spleen, significant increases in NK cells (60% to 83%, $P \leq 0.041$) occur which are of greater magnitude than in the first experiment. Further, these increases occur at two doses of BCH-2537; 5 and 25 mg/kg. Again, the increase in NK cells is accompanied by increases in T-helper cells (22%, $P \leq 0.02$) and monocytes (47% to 103%, $P \leq 0.015$). Additionally, the second experiment revealed that **9** also induces a significant increase in NK cells, but to a lesser extent than BCH-2537. In blood, **9** did not afford a significant increase in any immune cell subset. In spleen, however, a significant increase in NK cells (28% to 45%, $P \leq 0.008$) is observed, but the magnitude of the increase is less than with BCH-2537 and it is seen only at 25 mg/kg **9**. As with BCH-2537, significant increases occur in T-helper cells (32% to 48%, $P \leq 0.005$) and monocytes (42%, $P \leq 0.013$). Unlike BCH-2537, a significant increase also occurs in cytotoxic T-lymphocytes (37% to 64%, $P \leq 0.023$).

To fully understand the immunological effect of BCH-2537 on NK cells, an important component of the immunophenotyping experiments was to assess any changes in the functional activity of NK cells along with changes in their relative numbers. That is, to determine the ability of BCH-2537 to stimulate NK cells. Therefore, upon sacrifice of the mice at the end of the experiment, a portion of the splenic cell suspension was assayed for lytic activity by the same chromium release assay used for the in vitro determination of NK cell activity. The results from the first experiment are shown in Figure 1. NK cell activity is enhanced at all three doses of BCH-2537. Further, this increase in functional NK cell activity is significant at 5 mg/kg ($P \leq 0.002$) and 25 mg/kg ($P \leq 0.022$) BCH-2537. The second experiment confirmed this significant ($P \leq 0.010$) augmentation of NK cell activity at 5 and 25 mg/kg BCH-2537. Interestingly, the increase in NK cell activity induced by 5 and 25 mg/kg **9** is not significant. Thus, there is a significant difference in the in vivo stimulation of NK cell activity induced by BCH-2537 and the desfucose analogue **9**.

## Discussion

In this study, the determination of the in vitro NK cell activity of more than 50 water soluble analogues of the lipophilic immunomodulator octadecyl D-alanyl-L-glutamine (BCH-527) was undertaken. On the basis of these results, a lead molecule, 1-$\beta$-[6-(D-alanyl-L-glutaminylamino)hex-1-yl]-L-fucopyranose (BCH-2537, **1**), was selected for in vivo immunological evaluation. A structure−activity relationship centered about this dipeptide−linker−monosaccharide was approximated as follows:

(1) Activity is dependent upon the type and stereochemistry of the monosaccharide attached to the linker portion of the dipeptide. However, the presence of a monosaccharide is not mandatory for activity, as illustrated by the desfucose analogue **9**. Indeed, the incorrect monosaccharide can function as an antagonist. This is illustrated by the loss of activity with replacement of fucose with glucose; compound **7**. Generally, the lack of any monosaccharide abrogates activity, as illustrated by compounds **11**, **12**, and **14**.

(2) Activity is dependent upon the distance and relative flexibility or spatial freedom between the dipeptide and monosaccharide. No separation between the dipeptide and monosaccharide results in reduced activity relative to a six-carbon spacer (compounds **15**−**19**). Similarily, too large a separation also results in reduced activity (compounds **20**−**22**). Compound **23** illustrates the importance of a highly flexible linker. Additionally, compound **23** suggests that the hexamethylene linker adopts a bent configuration, which could perhaps be replaced with a shorter chain.

(3) Activity is dependent upon the sequence and stereochemistry of the dipeptide. D-Alanyl-L-glutamine, as represented by BCH-2537, is the most active compound. Conservative substitution of D-alanine resulted in loss of activity, as illustrated by compounds **24**−**26**. However, the *N*-terminal amino function must be present, **28** and **29**, to presumably provide an appropriately positioned positive charge. Very little change is tolerated for L-glutamine (compounds **32**−**34** and **40**−**46**).

The model structure which emerges from the above is one in which the *N*-terminal amino function, the glutamine carboxamide, and the linker (terminal) oxygen atom participate in attractive (e.g., hydrogen bond) interactions with receptor protein(s). Once established, these interactions may be reinforced by binding of the appropriate portion of the fucose sugar and the alanine methyl group. Alternatively, the alternating (D-alanine) stereochemistry may facilitate bending of the dipeptide. Oligopeptides composed of D-alanyl-L-glutamine have been reported to have a propensity for a bend.[19] Evidence for the importance of the linker oxygen atom is provided by comparison of the activity of the thio analogue **10** which retains moderate activity. Additionally, a specific (hydrogen bond) interaction is implied by the superior activity of compound **9** when compared to compounds **11**−**14** which also do not possess a monosaccharide. In summary, while the structure−activity data is consistent with a drug−receptor interaction, and BCH-2537 was designed to bind to a monosaccharide receptor, it is not known with which NK cell receptor(s) BCH-2537 and analogues interact.

The potent and selective in vitro NK cell activity of BCH-2537, **1**, justified selection for in vivo immunological evaluation. Indeed, it is interesting that a low molecular weight synthetic (MW = 463) could mimic the activity of interleukin 2 (MW ≈ 15 000). Immunophenotyping experiments with BCH-2537 revealed a significant increase in spleen NK cells. The magnitude of this increase, up to 83% relative to control, was greater in the second experiment. However, a significant increase in blood NK cells was observed in the first experiment. While these results may appear somewhat variable, this reflects the variability between experiments with two groups of mice. Further, the consistent increase in spleen NK cells suggests a more sustained response indicative of a greater therapeutic potential. In support of the latter was the concomitant increase in NK cell functional activity. This increase in NK cells induced by BCH-2537 was significant at 5 and 25 mg/kg concentrations of drug in both experiments. On the other hand, the desfucose analogue **9** induced a smaller increase in NK cells relative to BCH-2537. Unlike BCH-2537, this increase was only significant at one dose in the same (second) phenotyping experiment. Additionally, the stimulation of NK cells induced by compound **9** was not significant. Finally, significant increases in other immune cell subsets were also observed with BCH-2537 and the desfucose analogue **9** in spite of the selective in vitro activity. This likely reflects cellular communication processes arising from the direct stimulation of NK cells. For example, increases in monocyte/macrophage (CD11b+) populations may result from increased $\gamma$ interferon produced by activated NK cells.

In conclusion, BCH-2537, **1**, induces a significant stimulation and expansion of NK cells. Further, on the basis of this study, it appears to be well-tolerated and devoid of any apparent toxicity. Irritation was not observed at the site of injection, thereby indicating a lack of an inflammatory response. BCH-2537 is very soluble and stable in aqueous solution.[20] Therefore, BCH-2537 possesses the properties desirable for an injectable therapeutic. However, the question remains whether the effect of BCH-2537 on NK cells results in a significant therapeutic response. In an effort to address this question, a preliminary evaluation was undertaken as to the ability of BCH-2537 to inhibit the metastasis of murine B16 melanoma cells. In this model, NK cell stimulants (interferon inducers) such as loxoribine, **39**, inhibit metastasis in the lung.[21] BCH-2537 does reduce the number of lung colonies when administered by intraperitoneal injection, as shown in Table 8. Although the activity is moderate, this experiment does not represent an optimized protocol (e.g., dose schedule, route of administration). Also, most immunostimulants, such as interferon inducers, offer multiple effector mechanisms (e.g., NK, B cell, and macrophage) which result in a relatively more robust response. Therefore, specific stimulants such as BCH-2537 may offer more potential as a nontoxic adjunct in prophylactic or therapeutic regimens. Additionally, such compounds may prove valuable as tools for the delineation of the precise role of NK cells in host defense mechanisms.

**Table 8.** Summary of the Antimetastatic Effect of BCH-2537, 1, in the Lungs of Mice

| | number of lung colonies (SEM)[a] | |
|---|---|---|
| BCH-2537, **1** | exp 1 ($n$ = 8) | exp 2 ($n$ = 4) |
| 0 | 142 ± 5 | 101 ± 3 |
| 25 mg/kg | 109 ± 10 ($P$ < 0.02) | 86 ± 11 |
| 50 mg/kg | nd | 60 ± 20 |

[a] C57BL/6 mice were injected iv on day 0 with $2.5 \times 10^5$ B16 melanoma cells. Mice were injected ip with BCH-2537 on days −2, −1, 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21. All mice were sacrificed on day 24. Lungs were fixed and counted for the number of lung tumor colonies.

## Experimental Section

**Chemistry.** Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Merck silica gel (Kieselgel 60) was used for TLC and flash column chromatography (230−400 mesh). NMR spectra were recorded on a Bruker DRX-400 or a Varian VXR-300 spectrometer. Chemical shifts ($\delta$) are expressed in ppm, relative to the solvent as internal standard unless otherwise stated. Mass spectra were recorded on a Kratos MS-50 TA instrument. Analytical HPLC results were obtained using a Waters instrument with either a Hamilton PRP-1, 4.1 × 150 mm ("Hamilton") or YMC C-4 (5 $\mu$m) 120 Å, 4.6 × 250 mm ("YMC") reverse phase column. Liquid phase: A = MeOH; B = MeCN; C = 10 mM $NH_4OAc$; D = 10 mM $NH_4HCO_3$; E = 0.01% aqueous TFA, with gradients as described, flow rate of 1 mL $min^{-1}$ (0.5 mL $min^{-1}$ for TFA system), and using a UV detector at an absorption of 210 nm. Combustion analyses were carried out by Chemisar Laboratories (Guelph, ON). All amino acids and dipeptide **17** were obtained from Bachem Bioscience Inc. (King of Prussia, PA), and all other reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI), Fluka (Ronkonkoma, NY), or VWR Inc. (Montréal, PQ). All nonaqueous reactions were carried out under an inert atmosphere.

**General Procedure A: Coupling of Amino Alcohols with Amino Acids.** A solution of the suitably protected amino acid, the unprotected amino alcohol (1.2 equiv) and 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ; 1.2 equiv) in $CHCl_3$ was stirred at ambient temperature overnight. The solvent was evaporated in vacuo, and the crude product was purified by flash silica gel chromatography.

**General Procedure B: Amino Acid Coupling Procedure.** A solution of the free amine (or amine salt and 1 equiv of triethylamine) and the free acid (1.2 equiv) in dry $CH_2Cl_2$ was treated with anhydrous 1-hydroxybenzotriazole (HOBT; 1.2 equiv), and the mixture was cooled to 0 °C. The reaction was then treated with either 1,3-dicyclohexylcarbodiimide (DCC; 1.4 equiv) or 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl; 1.4 equiv) and stirred from 0 °C to ambient temperature overnight. The reaction was filtered through Celite, and the residue was washed with $CH_2Cl_2$. Combined filtrate and washings were evaporated in vacuo to give the crude product, which was purified by flash silica gel chromatography.

**General Procedure C: Coupling of Alcohols with Bromo Sugars.** The free hydroxy compound and silver(I) carbonate (1.1 equiv) were dried in the dark at ambient temperature in vacuo for 24 h prior to reaction. Freshly powdered, oven-dried calcium sulfate was placed in an oven-dried flask, which was then cooled to ambient temperature under an argon atmosphere. Solutions of the dried hydroxy compound and bromo sugar (1.1 equiv) in dry $CH_2Cl_2$ were introduced. This mixture was treated with the dried silver(I) carbonate and iodine (0.3 equiv). The reaction was wrapped in aluminum foil to exclude light and stirred at ambient temperature overnight. The reaction was filtered through Celite, and the residue was washed with $CH_2Cl_2$. Combined filtrate and washings were evaporated in vacuo to give the crude product, which was purified by flash silica gel chromatography.

**General Procedure D: Deprotection of the BOC-Protected Amino Group.** A solution of the BOC-protected compound in $Et_2O$ and EtOH (1:1) was cooled to 0 °C and was treated with toluene-4-sulfonic acid, monohydrate. Once dissolution was complete, the solvents were evaporated in vacuo, and the residue was heated at 40 °C in vacuo for 2−3 h to give the free amine, toluene-4-sulfonate salt.

**General Procedure E: Deprotection of Z-Protected Amino Group.** A solution of the Z-protected compound in denatured EtOH, or MeOH, under $N_2$, was treated with palladium on activated carbon (10% w/w, of 10% w/w Pd/C). The mixture was thoroughly degassed and then stirred at room temperature, under 1 atm of $H_2$ overnight. The mixture was diluted with MeOH and filtered through Celite. The residue was washed with MeOH, and combined filtrate and washings were evaporated in vacuo to give the free amine.

**General Procedure F: Ammonolysis.** A solution of the protected compound in MeOH was cooled to 0 °C. Anhydrous ammonia gas was then bubbled through the solution for 10 min, and the flask was sealed. The reaction was stirred at room temperature overnight, and solvent was evaporated in vacuo. The residue was purified by flash chromatography to give the desired compound.

**6-[[*N*-(Benzyloxycarbonyl)-5-*O*-methyl-(*S*)-glutamyl]-amino]hexan-1-ol, 3.** Z-L-glutamic acid, 5-methyl ester **2** (5.34 g, 18.1 mmol) and 6-aminohexanol were coupled according to general procedure A, to give, after purification, **3** as a white solid (6.18 g, 87%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.30−1.44 (4H, m, hexyl H-3 and H-4), 1.45−1.60 (4H, m, hexyl H-2 and H-5), 1.64 (1H, br s, OH), 1.87−1.96 and 2.05−2.10 (2H, 2 × m, Glu H-3), 2.36 (1H, A of $ABX_2$, *J* 17.0, 6.5 Hz, 1H of Glu H-4), 2.51 (1H, B of $ABX_2$, *J* 17.0, 7.0 Hz, 1H of Glu H-4), 3.23 (2H, dt, *J* 6.5, 6.5 Hz, hexyl H-6) 3.61 (2H, t, *J* 6.5 Hz, hexyl H-1), 3.65 (3H, s, OMe), 4.14−4.22 (1H, m, Glu H-2), 5.08 (2H, s, PhC$H_2$), 5.64 (1H, d, *J* 8.0 Hz, ZN*H*), 6.27−6.34 (1H, m, N*H*$CH_2$), 7.32 (5H, s, Ph).

**2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl Bromide, 4.** A solution of α-L-fucose tetraacetate (5.00 g, 15.0 mmol) in HBr/AcOH (45% w/v HBr; 350 mL) was stirred at room temperature, in a sealed flask (septum only), for 5 h. Acids were then removed in vacuo, and the residue was dissolved in $CH_2Cl_2$ (200 mL). This solution was treated with water (100 mL). This well-stirred mixture was treated with saturated aqueous $NaHCO_3$ solution until the pH of the aqueous phase reached 7. The layers were separated, the aqueous phase was extracted with $CH_2Cl_2$ (100 mL), and the combined organic extracts were dried ($Na_2SO_4$), filtered, and evaporated in vacuo to give **4** as a pale yellow oil (100%): TLC $R_f = 0.80$ ($SiO_2$; 3:2 hexane/EtOAc); $^1$H NMR ($CDCl_3$, 300 MHz) 1.22 (3H, d, *J* 6.5 Hz, H-6), 2.02 (3H, s, OAc), 2.12 (3H, s, OAc), 2.18 (3H, s, OAc), 4.41 (1H, qd, *J* 6.5, 1.0 Hz, H-5), 5.03 (1H, dd, *J* 10.5, 4.0 Hz, H-2), 5.37 (1H, dd, *J* 3.0, 1.0 Hz, H-4), 5.42 (1H, dd, *J* 10.5, 3.0 Hz, H-3), 6.70 (1H, d, *J* 4.0 Hz, H-1).

**1-β-[6-[[*N*-(Benzyloxycarbonyl)-5-*O*-methyl-(*S*)-glutamyl]-amino]hex-1-yl]-2,3,4-tri-*O*-acetyl-β-L-fucopyranose, 5.** Compounds **3** (4.32 g, 11.0 mmol) and **4** (12.0 mmol) were reacted according to general procedure B to give, after purification, **5** as a colorless foam (3.24 g, 44%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.18 (3H, d, *J* 6.5 Hz, fucose H-6), 1.22−1.38 (4H, m, hexyl H-3 and H-4), 1.41−1.62 (4H, m, hexyl H-2 and H-5), 1.85−1.96 (1H, m, 1H of Glu H-3), 1.86 (3H, s, OAc), 2.01 (3H, s, OAc), 2.04−2.17 (1H, m, 1H of Glu H-3), 2.14 (3H, s, OAc), 2.30−2.41 and 2.44−2.55 (2H, 2 × m, Glu H-4), 3.20 (2H, dt, *J* 7.0, 6.5 Hz, hexyl H-6), 3.41 (1H, A of $ABX_2$, *J* 8.5, 6.5 Hz, 1H of hexyl H-1), 3.64 (3H, s, OMe), 3.76 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.86 (1H, B of $ABX_2$, *J* 9.5, 6.0 Hz, 1H of hexyl H-1), 4.12−4.21 (1H, m, Glu H-2), 4.38 (1H, d, *J* 8.0 Hz, fucose H-1), 4.97 (1H, dd, *J* 10.5, 3.5 Hz, fucose H-3), 5.07 (2H, s, PhC$H_2$), 5.14 (1H, dd, *J* 10.5, 8.0 Hz, fucose H-2), 5.20 (1H, dd, *J* 3.5, 1.0 Hz, fucose H-4), 5.63 (1H, d, *J* 8.0 Hz, ZN*H*), 6.20−6.27 (1H, m, N*H*$CH_2$), 7.28−7.35 (5H, m, Ph).

**1-β-[6-[[*N*-(Benzyloxycarbonyl)-(*R*)-alanyl-5-*O*-methyl-(*S*)-glutamyl]amino]hexyl]-2,3,4-tri-*O*-acetyl-L-fucopyranose, 6.** Compound **5** (3.33 g, 5.0 mmol) was deprotected according to general method E to give the intermediate amine, which was coupled with Z-D-alanine (1.34 g, 6.0 mmol) according to general method B (using DCC). This gave, after purification, **6** as a white solid (2.52 g, 68%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.19 (3H, d, *J* 6.5 Hz, fucose H-6), 1.22−1.38 (4H, m, hexyl H-3 and H-4), 1.35 (3H, d, *J* 7.0 Hz, Ala H-3), 1.40−1.58 (4H, m, hexyl H-2 and H-5), 1.85−2.20 (2H, m, Glu H-3), 1.95 (3H, s, OAc), 2.01 (3H, s, OAc), 2.14 (3H, s, OAc), 2.28−2.58 (2H, m, Glu H-4), 3.17 (2H, dt, *J* 6.5, 6.5 Hz, hexyl H-6), 3.40 (1H, A of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 3.65 (3H, s, OMe), 3.76 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.85 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.10−4.20 and 4.34−4.42 (2H, 2 × m, Ala H-2 and Glu H-2), 4.38 (1H, d, *J* 8.0 Hz, fucose H-1), 4.98 (1H, dd, *J* 10.0, 3.5 Hz, fucose H-3), 5.05−5.16 (3H, m, C$H_2$Ph + fucose H-2), 5.19 (1H, dd, *J* 3.5, 1.0 Hz, fucose H-4), 5.39 (1H, d, *J* 6.5 Hz, ZN*H*), 6.60−6.67 (1H, m, N*H*$CH_2$), 7.06 (1H, d, *J* 7.0 Hz, Glu NH), 7.27−7.34 (5H, m, Ph).

**1-β-[6-((*R*)-Alanyl-(*S*)-glutaminylamino)hex-1-yl]-L-fucopyranose, BCH-2537, 1.** Compound **6** (177 mg, 0.17 mmol) was N-deprotected according to general method E to give the intermediate free amine as a colorless oil. This material was converted to BCH-2537, **1**, by ammonolysis according to general method F to give, after purification by flash silica gel chromatography (gradient elution of 10−20% concentrated aqueous ammonium hydroxide in propan-2-ol), compound **1** as a white solid (43 mg, 56%): mp 180−183 °C (softens 173 °C); $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.27 (3H, d, *J* 7.0 Hz, Ala H-3), 1.30−1.42 (4H, m, hexyl H-3 and H-4), 1.46−1.54 (2H, m, hexyl H-5), 1.55−1.66 (2H, m, hexyl H-2), 1.87−2.00 and 2.02−2.12 (2H, 2 × m, Gln H-3), 2.28−2.33 (2H, m, Gln H-4), 3.11−3.25 (2H, m, hexyl H-6), 3.44 (1H, dd, *J* 10.0, 7.5 Hz, fucose H-2), 3.48 (1H, q, *J* 7.0 Hz, Ala H-2), 3.51 (1H, dd, *J* 10.0, 3.5 Hz, fucose H-3), 3.54 (1H, A of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 3.64 (1H, dd, *J* 3.5, 1.0 Hz, fucose H-4), 3.65 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 4.22 (1H, d, *J* 7.5 Hz, fucose H-1), 4.28 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 16.78 (fucose C-6), 21.31 (Ala C-3), 26.73, 27.69, 29.23, 30.32, 30.69 and 32.57 (Gln C-3 and C-4 and hexyl C-2, C-3, C-4, and C-5), 40.36 (hexyl H-6), 51.51 and 54.22 (Ala C-2 and Gln C-2), 70.58 (hexyl H-1), 71.84, 72.34, 73.07 and 75.20 (fucose C-2, C-3, C-4, and C-5), 104.82 (fucose C-1), 173.56 (Gln C-5), 177.67 and 178.41 (Ala C-1 and Gln C-1); HRMS (FAB) *m*/*z* 463.27832, calculated for $MH^+$ ($C_{20}H_{39}N_4O_8^+$) 463.27679; HPLC, system A (Hamilton; 0−50% (60 min) B in C, pH 8.45), 17.4 min, 99%; system B (YMC; 0−35% (30 min) A in E), 18.4 min. Anal. ($C_{20}H_{38}N_4O_8 \cdot 0.5H_2O$) C, H, N.

**2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranose.** A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (97%; 1.00 g, 2.43 mmol) in acetone (10 mL) was treated with silver(I) carbonate (704 mg, 2.55 mmol) and water (200 μL, 11.1 mmol). The mixture was stirred in the dark at ambient temperature for 16 h. The mixture was filtered through Celite and the residue washed with $CH_2Cl_2$. Combined filtrate and washings were evaporated in vacuo to give the title compound as a white solid (820 mg, 97%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.96 (3H, s, OAc), 1.97 (3H, s, OAc), 2.02 (3H, s, OAc), 2.04 (3H, s, OAc), 3.71 (1H, ddd, *J* 10.0, 4.5, 2.5 Hz, H-5), 4.09 (1H, A of ABX, *J* 12.5, 2.5 Hz, 1H of H-6), 4.18 (1H, $D_2O$ exchangeable, d, *J* 8.0 Hz, OH), 4.19 (1H, B of ABX, *J* 12.5, 4.5 Hz, 1H of H-6), 4.70 (1H, dd, *J* 8.0, 8.0 Hz [collapses to d, *J* 8.0 Hz on addition of $D_2O$], H-1), 4.85 (1H, dd, *J* 9.5, 8.0 Hz, H-2), 5.03 (1H, dd, *J* 10.0, 9.5 Hz, H-4), 5.19 (1H, dd, *J* 9.5, 9.5 Hz, H-3).

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)trichloroacetimidate.** A solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (820 mg, 2.35 mmol) and trichloroacetonitrile (725 μL, 7.1 mmol), in dry $CH_2Cl_2$ (10 mL) was treated with oven-dried $K_2CO_3$ (553 mg, 4.00 mmol), and the reaction was stirred at ambient temperature for 64 h. The mixture was filtered through Celite and the residue washed with $CH_2Cl_2$. Combined filtrate and washings were evaporated in vacuo to give a pale brown foam, which was purified by flash silica gel

chromatography (100:1, gradient elution, 30−60% EtOAc in hexane) to give the title compound as a white foam (978 mg, 84%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.99 (3H, s, OAc), 2.01 (3H, s, OAc), 2.02 (3H, s, OAc), 2.05 (3H, s, OAc), 4.10 (1H, A of ABX, *J* 12.0, 2.0 Hz, 1H of H-6), 4.18 (1H, ddd, *J* 10.0, 4.0, 2.0 Hz, H-5), 4.25 (1H, B of ABX, *J* 12.0, 4.0 Hz, 1H of H-6), 5.10 (1H, dd, *J* 10.0, 3.5 Hz, H-2), 5.15 (1H, dd, *J* 10.0, 10.0 Hz, H-3), 5.54 (1H, dd, *J* 10.0, 10.0 Hz, H-4), 6.53 (1H, d, *J* 3.5 Hz, H-1), 8.67 (1H, s, NH).

**1-*β*-[6-[[*N*-Benzyloxycarbonyl-5-*O*-methyl-(*S*)-glutamyl]amino]hex-1-yl]-2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranose.** A solution of compound **3** (434 mg, 1.10 mmol) and *O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)trichloroacetimidate (493 mg, 1.00 mmol) in dry $CH_2Cl_2$ (40 mL) at −15 °C was treated with $BF_3.Et_2O$ (25 *μ*L, 0.20 mmol), and the reaction was stirred at −15 °C for 90 min. The reaction was quenched by addition of $NaHCO_3$ (500 mg) followed by addition of saturated aqueous $NaHCO_3$ (25 mL). The layers were separated, and the aqueous phase was further extracted with $CH_2Cl_2$ (25 mL). Combined organic extracts were dried ($Na_2SO_4$), filtered, and evaporated in vacuo. The crude material was purified by flash silica gel chromatography (100:1, gradient elution, 50−100% EtOAc in hexane) to give the title compound as a colorless oil (222 mg, 31%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.23−1.38 (4H, m, hexyl H-3 and H-4), 1.41−1.51 (2H, m, hexyl H-5), 1.51−1.61 (2H, m, hexyl H-2), 1.85−2.17 (2H, m, Glu H-3), 1.98 (3H, s, OAc), 2.00 (3H, s, OAc), 2.02 (3H, s, OAc), 2.06 (3H, s, OAc), 2.32−2.41 and 2.45−2.56 (2H, 2 × m, Glu H-4), 3.21 (2H, dt, *J* 6.5, 6.5 Hz, hexyl H-6), 3.44 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.62−3.69 (1H, m, glucose H-5), 3.65 (3H, s, OMe), 3.83 (1H, B of $ABX_2$, *J* 9.5, 6.0 Hz, 1H of hexyl H-1), 4.11−4.20 (1H, m, Glu H-2), 4.12 (1H, A of ABX, *J* 12.5, 2.5 Hz, 1H of glucose H-6), 4.24 (1H, B of ABX, *J* 12.5, 4.5 Hz, 1H of glucose H-6), 4.46 (1H, d, *J* 8.0 Hz, glucose H-1), 4.96 (1H, dd, *J* 9.5, 8.0 Hz, glucose H-2), 5.06 (1H, dd, *J* 10.0, 9.5 Hz, glucose H-4), 5.08 (2H, s, PhC$H_2$), 5.18 (1H, dd, *J* 9.5, 9.5 Hz, glucose H-3), 5.60 (1H, d, *J* 7.0 Hz, ZN*H*), 6.20−6.28 (1H, m, N*H*$CH_2$), 7.32 (5H, s, Ph).

**1-*β*-[6-[[*N*-(Benzyloxycarbonyl)-(*R*)-alanyl-5-*O*-methyl-(*S*)-glutamyl]amino]hexyl]-2,3,4,6-tetra-*O*-acetyl-D-glucopyranose.** 1-*β*-[6-[[*N*-(Benzyloxycarbonyl)-5-*O*-methyl-(S)-glutamyl]amino]hex-1-yl]-2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranose (220 mg, 0.30 mmol) was deprotected according to general method E to give the intermediate amine. This was coupled with Z-D-alanine (90 mg, 0.33 mmol) according to general method B (using EDC·HCl). This gave, after purification, the title compound as a white solid (118 mg, 54%). $^1$H NMR ($CDCl_3$, 300 MHz) 1.21−1.32 (4H, m, hexyl H-3 and H-4), 1.32 (3H, d, *J* 7.0 Hz, Ala H-3), 1.37−1.46 (2H, m, hexyl H-5), 1.46−1.55 (2H, m, hexyl H-2), 1.87−2.16 (2H, m, Glu H-3), 1.95 (3H, s, OAc), 1.97 (3H, s, OAc), 1.98 (3H, s, OAc), 2.03 (3H, s, OAc), 2.24−2.48 (2H, m, Glu H-4), 3.09−3.19 (2H, m, hexyl H-6), 3.40 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.62 (3H, s, OMe), 3.64 (1H, ddd, *J* 9.5, 4.5, 2.5 Hz, glucose H-5), 3.79 (1H, B of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 4.09 (1H, A of ABX, *J* 12.0, 2.5 Hz, 1H of glucose H-6), 4.17 (1H, qd, *J* 7.0, 7.0 Hz, Ala H-2), 4.21 (1H, B of ABX, *J* 12.0, 4.5 Hz, 1H of glucose H-6), 4.38 (1H, dt, *J* 7.5, 7.5, 5.0 Hz, Glu H-2), 4.44 (1H, d, *J* 8.0 Hz, glucose H-1), 4.92 (1H, dd, *J* 9.5, 8.0 Hz, glucose H-2), 5.01 and 5.08 (2H, AB q, *J* 12.0 Hz, PhC$H_2$), 5.03 (1H, dd, *J* 9.5, 9.5 Hz, glucose H-4), 5.16 (1H, dd, *J* 9.5, 9.5 Hz, glucose H-3), 5.63 (1H, d, *J* 7.0 Hz, ZN*H*), 6.73−6.80 (1H, m, N*H*$CH_2$), 7.19 (1H, d, *J* 7.5 Hz, Glu NH), 7.28 (5H, s, Ph).

**1-*β*-[6-((*R*)-Alanyl-(*S*)-glutaminylamino)hex-1-yl]-D-glucopyranose, 7.** 1-*β*-[6-[[*N*-(Benzyloxycarbonyl)-(*R*)-alanyl-5-*O*-methyl-(*S*)-glutamyl]amino]hexyl]-2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (118 mg, 0.15 mmol) was N-deprotected according to general method E to give the intermediate free amine as a colorless oil. This material was converted to **7** by ammonolysis according to general method F to give, after purification by flash silica gel chromatography (gradient elution of 10−20% concentrated aqueous ammonium hydroxide in propan-2-ol), compound **7** as a white solid (23 mg, 32%): TLC, $R_f$ = 0.25 ($SiO_2$; 8:2 IPA concentrated aqueous $NH_3$); mp 82−86 °C; $^1$H NMR ($D_2O$, 300 MHz, MeCN = 2.05 ppm) 1.29 (3H, d, *J* 7.0 Hz, Ala H-3), 1.29−1.40 (4H, m, hexyl H-3 and H-4), 1.45−1.53 (2H, m, hexyl H-5), 1.54−1.65 (2H, m, hexyl H-2), 1.90−2.17 (2H, m, Gln H-3), 2.35 (2H, t, *J* 7.5 Hz, Gln H-4), 3.12−3.25 (2H, m, hexyl H-6), 3.23 (1H, dd, *J* 9.0, 8.0 Hz, glucose H-2), 3.35 (1H, dd, *J* 9.0, 9.0 Hz, glucose H-4), 3.45 (1H, ddd, *J* 9.0, 6.0, 2.0 Hz, glucose H-5), 3.47 (1H, dd, *J* 9.0, 9.0 Hz, glucose H-3), 3.59 (1H, q, *J* 7.0 Hz, Ala H-2), 3.65 (1H, A of $ABX_2$, *J* 10.0, 7.5 Hz, 1H of hexyl H-1), 3.70 (1H, A of ABX, *J* 12.0, 6.0 Hz, 1H of glucose H-6), 3.90 (1H, B of $ABX_2$, *J* 10.0, 6.5 Hz, 1H of hexyl H-1), 3.90 (1H, B of ABX, *J* 12.0, 2.0 Hz, 1H of glucose H-6), 4.24 (1H, dd, *J* 8.5, 5.5 Hz, Gln H-2), 4.43 (1H, d, *J* 8.0 Hz, glucose H-1); $^{13}$C NMR ($D_2O$, 75.5 MHz, *Me*CN = 1.30 ppm) 19.93 (Ala C-3), 25.10, 26.13, 27.40, 28.56, 29.08 and 31.62 (Gln C-3 and C-4, + hexyl C-2 to C-5), 39.72 (hexyl C-6), 50.24 and 53.88 (Ala C-2 and Gln C-2), 61.21 and 70.94 (hexyl C-1 and glucose C-6), 70.10, 73.59, 76.26 and 76.34 (glucose C-2, C-3, C-4, and C-5), 102.61 (glucose C-1), 173.50 (Gln C-5), 178.14 and 178.20 (Ala C-1 and Gln C-1); HRMS (FAB) *m/z* 479.270 80, calculated for $MH^+$ ($C_{20}H_{39}N_4O_9^+$) 479.271 70; HPLC, system A (Hamilton; 0−50% (60 min) B in C, pH 7.7), 14.4 min, 93%; system B (YMC; 0−35% (30 min) A in E), 12.4 min. Anal. ($C_{20}H_{38}N_4O_9\cdot 2H_2O$) C, H, N.

**2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl Bromide.** A solution of α-D-mannose pentaacetate (1.09 g, 2.8 mmol) in HBr/AcOH (45% w/v HBr; 80 mL) was stirred at room temperature, in a sealed flask (septum only), for 4 h. Acids were then removed in vacuo, the residue was dissolved in $CH_2Cl_2$ (200 mL), and this solution was treated with water (100 mL). This well-stirred mixture was treated with saturated aqueous $NaHCO_3$ solution until the pH of the aqueous phase reached 7. The layers were separated, the aqueous phase was extracted with $CH_2Cl_2$ (100 mL), and combined organic extracts were dried ($Na_2SO_4$), filtered, and evaporated in vacuo to give the title compound as a yellow oil (100%): TLC $R_f$ = 0.50 ($SiO_2$; 3:2 hexane/EtOAc); $^1$H NMR ($CDCl_3$, 300 MHz) 1.97 (3H, s, OAc), 2.03 (3H, s, OAc), 2.06 (3H, s, OAc), 2.13 (3H, s, OAc), 4.09 (1H, A of ABX, *J* 12.5, 2.0 Hz, 1H of H-6), 4.18 (1H, ddd, *J* 10.0, 5.0, 2.0 Hz, H-5), 4.29 (1H, B of ABX, *J* 12.5, 5.0 Hz, 1H of H-6), 5.33 (1H, dd, *J* 10.0, 10.0 Hz, H-4), 5.41 (1H, dd, *J* 3.5, 1.5 Hz, H-2), 5.67 (1H, dd, *J* 10.0, 3.5 Hz, H-3), 6.26 (1H, d, *J* 1.5 Hz, H-1).

**1-*β*-[6-[[*N*-Benzyloxycarbonyl-5-*O*-methyl-(*S*)-glutamyl]amino]hex-1-yl]-2,3,4,6-tetra-*O*-acetyl-*β*-D-mannopyranose.** Compound **3** (1.00 g, 2.50 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (12 mmol) were reacted according to general procedure B to give, after purification, the title compound as a colorless oil (1.50 g, 74%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.22−1.64 (8H, m, hexyl H-2 to H-5), 1.73 (3H, s, OAc), 1.88−2.18 (2H, m, Glu H-3), 2.05 (3H, s, OAc), 2.06 (3H, s, OAc), 2.11 (3H, s, OAc), 2.32−2.57 (2H, m, Glu H-4), 3.16−3.29 (2H, m, hexyl H-6), 3.46 (2H, t, *J* 6.5 Hz, hexyl H-1), 3.64−3.72 (1H, m, mannose H-5), 3.66 (3H, s, OMe), 4.10−4.27 (3H, m, Glu H-2 and mannose H-6), 4.59 (1H, dd, *J* 4.0, 2.0 Hz, mannose H-2), 5.09 (2H, s, PhC$H_2$), 5.14 (1H, dd, *J* 10.0, 4.0 Hz, mannose H-3), 5.29 (1H, dd, *J* 10.0, 9.5 Hz, mannose H-4), 5.47 (1H, d, *J* 2.0 Hz, mannose H-1), 5.67 (1H, d, *J* 7.5 Hz, ZN*H*), 6.22−6.32 (1H, m, N*H*$CH_2$), 7.30−7.39 (5H, m, Ph).

**1-*β*-[6-[[*N*-(Benzyloxycarbonyl)-(*R*)-alanyl-5-*O*-methyl-(*S*)-glutamyl]amino]hexyl]-2,3,4,6-tetra-*O*-acetyl-D-mannopyranose.** 1-*β*-[6-[[*N*-(Benzyloxycarbonyl)-5-*O*-methyl-(S)-glutamyl]amino]hex-1-yl]-2,3,4,6-tetra-*O*-acetyl-*β*-D-mannopyranose (362 mg, 0.50 mmol) was deprotected according to general method E to give the intermediate amine, which was coupled with Z-D-alanine (134 mg, 0.60 mmol) according to general method B (using DCC). This gave, after purification, the title compound as colorless crystals (290 mg, 73%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.20−1.33 (4H, m, hexyl H-3 and H-4), 1.32 (3H, d, *J* 7.0 Hz, Ala H-3), 1.37−1.52 (4H, m, hexyl H-2 and H-5), 1.68 (3H, s, OAc), 1.82−2.15 (2H, m, Glu H-3), 2.01 (3H, s, OAc), 2.02 (3H, s, OAc), 2.07 (3H, s, OAc), 2.25−2.50 (2H, 2, Glu H-4), 3.14 (2H, dt, *J* 6.5, 6.5 Hz, hexyl H-6),

3.37−3.45 (1H, m, hexyl H-1), 3.60−3.77 (1H, m, mannose H-5), 3.62 (3H, s, OMe), 4.04−4.22 (3H, m, Glu H-2 and mannose H-6), 4.34−4.42 (1H, m, Ala H-2), 4.54 (1H, dd, *J* 4.0, 2.5 Hz, mannose H-2), 5.00 and 5.08 (2H, AB q, *J* 12.5 Hz, PhC*H*$_2$), 5.11 (1H, dd, *J* 10.0, 4.0 Hz, mannose H-3), 5.25 (1H, dd, *J* 10.0, 9.5 Hz, mannose H-4), 5.42 (1H, d, *J* 2.5 Hz, mannose H-1), 5.60 (1H, d, *J* 7.0 Hz, ZN*H*), 6.70−6.77 (1H, m, N*H*CH$_2$), 7.19 (1H, d, *J* 7.5 Hz, Glu NH), 7.25−7.32 (5H, m, Ph).

**2-*O*-Acetyl-1-*β*-[6-((*R*)-alanyl-(*S*)-glutaminylamino)hex-1-yl]-D-mannopyranose, 8.** 1-*β*-[6-[[*N*-(Benzyloxycarbonyl)-(*R*)-alanyl-5-*O*-methyl-(*S*)-glutamyl]amino]hexyl]-2,3,4,6-tetra-*O*-acetyl-D-mannopyranose (147 mg, 0.18 mmol) was N-deprotected according to general method E to give the intermediate free amine as a colorless oil. This material was converted to BCH-2549, **8**, by ammonolysis according to general method F to give, after purification by flash silica gel chromatography (gradient elution of 10−20% concentrated aqueous ammonium hydroxide in propan-2-ol), compound **8** as a white solid (33 mg, 36%): mp 79.5−82 °C; $^1$H NMR (CD$_3$OD, 300 MHz) 1.27 (3H, d, *J* 7.0 Hz, Ala H-3), 1.30−1.44 (4H, m, hexyl H-3 and H-4), 1.45−1.58 (4H, m, hexyl H-2 and H-5), 1.62 (3H, s, OAc), 1.85−1.98 and 2.01−2.14 (2H, 2 × m, Gln H-3), 2.28 (2H, dd, *J* 7.5, 7.0 Hz, Gln H-4), 3.18 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.22 (1H, ddd, *J* 9.5, 6.0, 2.5 Hz, mannose H-5), 3.45 (1H, q, *J* 7.0 Hz, Ala H-2), 3.49−3.56 (2H, m, hexyl H-1), 3.55 (1H, dd, *J* 9.5, 9.5 Hz, mannose H-4), 3.65 (1H, A of ABX, *J* 12.0, 6.0 Hz, 1H of mannose H-6), 3.69 (1H, dd, *J* 9.5, 4.0 Hz, mannose H-3), 3.84 (1H, B of ABX, *J* 12.0, 2.5 Hz, 1H of mannose H-6), 4.30 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2), 4.43 (1H, dd, *J* 4.0, 2.5 Hz, mannose H-2), 5.43 (1H, d, *J* 2.5 Hz, H-1); $^{13}$C NMR (CD$_3$OD, 75.5 MHz) 21.30 (Ala C-3), 25.83 (*Me*CO), 26.83, 27.56, 29.21, 30.26, 30.49, and 32.55 (Gln C-3 and C-4 and hexyl C-2, C-3, C-4, and C-5), 40.37 (hexyl H-6), 51.49 and 54.23 (Ala C-2 and Gln C-2), 62.83 (hexyl C-1), 68.54, 73.30, 76.92 and 81.18 (mannose C-2, C-3, C-4, and C-5), 99.07 (mannose C-1), 124.84 (mannose C-6), 173.58 (Gln C-5), 177.67 and 178.42 [2C] (Me*C*O, Ala C-1 and Glu C-1); HRMS (FAB) *m*/*z* 543.264 310, calculated for MNa$^+$ ($C_{22}H_{40}N_4NaO_{10}^+$) 543.264 214; HPLC, system A (Hamilton; 0−40% (50 min) B in C, pH 8.5), 17.9 min (solvated NH$_2$) and 23.5 min (NH$_2$ H-bonded to sugar ring [confirmed by NOESY spectroscopy]), 98% total; system B (YMC; 0−50% (30 min) A in E), 9.8 and 13.7 min. Anal. ($C_{22}H_{40}N_4O_{10}\cdot H_2O$) C, H, N.

***N*-(Benzyloxycarbonyl)-(*R*)-alanyl-1-*N*-(6-acetoxyhexyl)-5-*O*-methyl-(*S*)-glutamamide.** 1-*N*-(6-Acetoxyhexyl)-2-*N*-(benzyloxycarbonyl)-5-*O*-methyl-(*S*)-glutamamide (isolated as a biproduct during the formation of compound **5**; 437 mg, 1.00 mmol) was deprotected according to general method E to give the intermediate free amine as a colorless oil (315 mg, quant.), which was coupled with Z-D-alanine (274 mg, 1.20 mmol) according to general method B (using DCC). This gave, after purification, the title compound as a white solid (251 mg, 49%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.22−1.38 (4H, m, hexyl H-3 and H-4), 1.33 (3H, d, *J* 7.0 Hz, Ala H-3), 1.39−1.65 (4H, m, hexyl H-2 and H-5), 1.85−2.17 (2H, m, Glu H-3), 1.99 (3H, s, OAc), 2.26−2.50 (2H, m, Glu H-4), 3.16 (2H, dt, *J* 6.5, 6.5 Hz, hexyl H-6), 3.62 (3H, s, OMe), 3.99 (2H, t, *J* 6.5 Hz, hexyl H-1), 4.13−4.24 (1H, m, Ala H-2), 4.35−4.45 (1H, m, Glu H-2), 5.01 and 5.08 (2H, AB q, *J* 12.0 Hz, PhC*H*$_2$), 5.66 (1H, d, *J* 7.0 Hz, ZN*H*), 6.75−6.84 (1H, m, N*H*CH$_2$), 7.20−7.35 (6H, m, Ph and Glu NH).

**6-((*R*)-Alanyl-(*S*)-glutaminylamino)hexan-1-ol, 9.** *N*-(Benzyloxycarbonyl)-(*R*)-alanyl-1-*N*-(6-acetoxyhexyl)-5-*O*-methyl-(*S*)-glutamamide (200 mg, 0.39 mmol) was N-deprotected according to general method E to give the intermediate free amine as a colorless oil. This material was converted to compound **9** by ammonolysis according to general method F to give, after purification by flash silica gel chromatography (isocratic elution with 5% concentrated aqueous ammonium hydroxide in propan-2-ol), compound **9** as a white solid (71 mg, 70%): mp 98−101 °C; $^1$H NMR (CD$_3$OD, 300 MHz) 1.28 (3H, d, *J* 7.0 Hz, Ala H-3), 1.30−1.43 (4H, m, hexyl H-3 and H-4), 1.45−1.58 (4H, m, hexyl H-2 and H-5), 1.84−1.97 and 2.01−2.13 (2H, 2 × m, Gln H-3), 2.24−2.32 (2H, m, Gln H-4), 3.18 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.46 (1H, q, *J* 7.0 Hz, Ala H-2), 3.53 (2H, t, *J* 6.5 Hz, hexyl H-1), 4.29 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2); $^{13}$C NMR (CD$_3$OD, 75.5 MHz) 21.17 (Ala C-3), 26.59, 27.74, 29.21, 30.33, 32.55, and 33.52 (Gln C-3 and C-4 and hexyl C-2, C-3, C-4, and C-5), 40.39 (hexyl H-6), 51.46 and 54.25 (Ala C-2 and Gln C-2), 62.84 (hexyl H-1), 173.58 (Gln C-5), 177.66 and 178.16 (Ala C-1 and Glu C-1); HRMS (FAB) *m*/*z* 317.217 17, calculated for MH$^+$ ($C_{14}H_{29}N_4O_4^+$) 317.218 87; HPLC, system A (Hamilton; 0−35% (40 min) B in C, pH 6.00), 18.3 min; system B (YMC; 0−25% (30 min) A in E), 12.5 min. Anal. ($C_{14}H_{28}N_4O_4\cdot H_2O$) C, H, N; C: calcd. 50.28; found 50.71; N: calcd. 16.75; found 16.28.

**2,3,4-Tri-*O*-acetyl-1-thio-*β*-L-fucopyranoside.** A solution of 2-*S*-(2,3,4-tri-*O*-acetyl-*β*-L-fucopyranosyl)-2-pseudothiourea, hydrobromide[12] (1.00 g, 2.33 mmol) in methanol (10 mL) was treated with a solution of $K_2CO_3$ (483 mg, 3.49 mmol) in water (100 mL). The mixture was stirred at ambient temperature, under a nitrogen atmosphere for 90 min. The solution was extracted with dichloromethane (2 × 50 mL), and the combined extracts were dried ($Na_2SO_4$), filtered, and evaporated in vacuo to give the title compound as a white foam (702 mg, 98%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.20 (3H, d, *J* 6.5 Hz, H-6), 1.96 (3H, s, OAc), 2.06 (3H, s, OAc), 2.16 (3H, s, OAc), 2.45−2.90 (1H, br s, SH), 3.81 (1H, qd, *J* 6.5, 1.0 Hz, H-5), 4.47 (1H, d, *J* 9.5 Hz, H-1), 4.98 (1H, dd, *J* 10.0, 3.5 Hz, H-3), 5.13 (1H, dd, *J* 10.0, 9.5 Hz, H-2), 5.25 (1H, dd, *J* 3.5, 1.0 Hz, H-4).

**6-[[*N*-(*tert*-Butoxycarbonyl)-5-*O*-benzyl-(*S*)-glutamyl]-amino]-1-iodohexane.** A solution of triphenylphosphine (629 mg, 2.40 mmol) and iodine (609 mg, 2.40 mmol) in dry tetrahydrofuran (40 mL) was stirred at ambient temperature for 10 min. The mixture was then treated with compound **3** (873 mg, 2.00 mmol) and imidazole (163 mg, 2.40 mmol) and was stirred at ambient temperature for 20 h. Solvent was evaporated in vacuo to give the crude product, which was purified by flash silica gel chromatography (gradient elution, 20−100% EtOAc in hexane) to give the title compound as a pale yellow solid (275 mg, 25%), together with unreacted **3** (576 mg, 66%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.22−1.50 (6H, m, hexyl H-2, H-3 and H-4), 1.39 (9H, s, $^t$Bu), 1.76 (2H, tt, *J* 7.0, 7.0 Hz, hexyl H-5), 1.90 (1H, A of ABX$_2$Y, *J* 14.5, 7.5, 7.0 Hz, 1H of Glu H-3), 2.09 (1H, B of ABX$_2$Y, *J* 14.5, 7.5, 6.5 Hz, 1H of Glu H-3), 2.40 (1H, A of ABX$_2$, *J* 17.0, 7.5 Hz, 1H of Glu H-4), 2.50 (1H, B of ABX$_2$, *J* 17.0, 7.5 Hz, 1H of Glu H-4), 3.13 (2H, t, *J* 7.0 Hz, CH$_2$I), 3.18 (1H, dt, *J* 7.0, 7.0 Hz, hexyl H-6), 4.06−4.16 (1H, m, Glu H-2), 5.09 (2H, s, CH$_2$Ph), 5.38 (1H, d, *J* 7.5 Hz, BOCN*H*), 6.38−6.44 (1H, m, N*H*CH$_2$), 7.31 (5H, s, Ph).

**6-[[*N*-(*tert*-Butoxycarbonyl)-5-*O*-benzyl-(*S*)-glutamyl]-amino]hex-1-yl 2,3,4-Tri-*O*-acetyl-1-thio-*β*-L-fucopyranoside.** A solution of 2,3,4-tri-*O*-acetyl-1-thio-*β*-L-fucopyranoside (185 mg, 0.60 mmol) and 6-[[*N*-*tert*-butoxycarbonyl-5-*O*-benzyl-(*S*)-glutamyl]amino]-1-iodohexane (275 mg, 0.50 mmol) in dry $CH_2Cl_2$ (5 mL) was treated with triethylamine (85 *μ*L, 0.61 mmol). The mixture was stirred at ambient temperature for 41 h. Solvent was evaporated in vacuo to give the crude product, which was purified by flash silica gel chromatography (gradient elution, 50−70% EtOAc in hexane) to give the title compound as a colorless oil (285 mg, 78%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.14 (3H, d, *J* 6.5 Hz, fucose H-6), 1.21−1.45 (6H, m, hexyl H-2, H-3 and H-4), 1.35 (9H, s, $^t$Bu), 1.47−1.57 (2H, m, hexyl H-5), 1.78−2.15 (2H, m, Glu H-3), 1.91 (3H, s, OAc), 1.99 (3H, s, OAc), 2.10 (3H, s, OAc), 2.30−2.50 (2H, m, Glu H-4), 2.58 (1H, A of ABX$_2$, *J* 12.0, 7.0 Hz, 1H of hexyl H-1), 2.64 (1H, B of ABX$_2$, *J* 12.0, 6.5 Hz, 1H of hexyl H-1), 3.14 (1H, dt, *J* 7.0, 7.0 Hz, hexyl H-6), 3.76 (1H, q*, *J* 6.5 Hz, fucose H-5), 4.03−4.12 (1H, m, Glu H-2), 4.38 (1H, d, *J* 10.0 Hz, fucose H-2), 4.98 (1H, dd, *J* 10.0, 3.5 Hz, fucose H-3), 5.05 (2H, s, CH$_2$Ph), 5.14 (1H, dd, *J* 10.0, 10.0 Hz, fucose H-2), 5.20 (1H, d*, *J* 3.5 Hz, fucose H-4), 5.36 (1H, d, *J* 8.0 Hz, BOCN*H*), 6.34−6.41 (1H, m, N*H*CH$_2$), 7.28 (5H, s, Ph), *fucose $J_{4-5}$ unresolved.

**1-*β*-[[6-((*R*)-Alanyl-(*S*)-glutaminylamino)hex-1-yl]thio]-1-deoxy-L-fucopyranose, 10.** Using general methods D, B, D and F, 6-[[*N*-*tert*-butoxycarbonyl-5-*O*-benzyl-(*S*)-glutamyl]-amino]hex-1-yl 2,3,4-tri-*O*-acetyl-1-thio-*β*-L-fucopyranoside was

converted in four steps to compound **10** in a manner similar to that seen for the previous analogues. Compound **10** was obtained as a white solid (97 mg, 52% over four steps): mp 122−125 °C; $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.24 (3H, d, *J* 6.5 Hz, fucose H-6), 1.28 (3H, d, *J* 7.0 Hz, Ala H-3), 1.30−1.44 (4H, m, hexyl H-3 and H-4), 1.45−1.55 (2H, m, hexyl H-2), 1.56−1.67 (2H, m, hexyl H-5), 1.87−1.98 and 2.01−2.12 (2H, 2 × m, Gln H-3), 2.30 (2H, t, *J* 7.5 Hz, Gln H-4), 2.64 (1H, A of $ABX_2$, *J* 12.5, 7.0 Hz, 1H of hexyl H-1), 2.72 (1H, B of $ABX_2$, *J* 12.5, 7.0 Hz, 1H of hexyl H-1), 3.18 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.45−3.53 (3H, m, Ala H-2 and fucose H-2 and H-3), 3.67 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.69 (1H, dd, *J* 3.0, 1.0 Hz, fucose H-4), 4.29 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2), 4.30 (1H, d, *J* 9.5 Hz, fucose H-1); $^{13}$C NMR: $\delta_C$ ($CD_3OD + D_2O$, 75.5 MHz) 17.03 (fucose C-6), 21.16 (Ala C-3), 27.20, 28.96, 29.22, 29.87, 30.70, 30.93 and 32.53 (Gln C-3 and C-4, + hexyl C-1 to C-5), 40.38 (hexyl H-6), 51.30 and 54.31 (Ala C-2 and Gln C-2), 70.93, 73.00, 75.96 and 76.02 (fucose C-2, C-3, C-4, and C-5), 87.27 (fucose C-1), 173.72 (Gln C-5), 178.07 and 178.56 (Ala C-1 and Gln C-1); MS (high resolution; FAB) 479.251 90, calculated for [$MH^+$] ($C_{20}H_{39}N_4O_7S^+$) 479.253 94. HPLC, system A (Hamilton; 0−50% (60 min) B in C, pH 7.7), 18.8 min, 100%; system B (YMC; 0−50% (25 min) A in E), 18.4 min. Anal. ($C_{20}H_{39}N_4O_7S\cdot 2H_2O$) C, H, N.

**2,3,4-Tri-*O*-acetyl-*β*-L-fucopyranosyl Azide.** A solution of compound **4** (1.06 g, 3.00 mmol) in dry MeCN (3 mL) was treated with powdered $NaN_3$ (293 mg, 4.51 mmol), and the mixture was heated at reflux for 90 min and then stirred at ambient temperature for 17 h. The mixture was diluted with $CH_2Cl_2$ and filtered through Celite, and the residue was washed with $CH_2Cl_2$. Combined filtrates and washings were evaporated in vacuo, and the residue was purified by flash silica gel chromatography (100:1, gradient elution, zero to 30% EtOAc in $CH_2Cl_2$) to give the title compound as white crystals (635 mg, 67%); IR (film) $\gamma_{max}$ 1220 s (C−O), 1750 s (C═O), 2121 s ($N_3$); $^1$H NMR ($CDCl_3$, 300 MHz) 1.23 (3H, d, *J* 6.5 Hz, H-6), 1.96 (3H, s, OAc), 2.06 (3H, s, OAc), 2.17 (3H, s, OAc), 3.88 (1H, qd, *J* 6.5, 1.0 Hz, H-5), 4.56 (1H, d, *J* 8.5 Hz, H-1), 5.00 (1H, dd, *J* 10.5, 3.5 Hz, H-3), 5.12 (1H, dd, *J* 10.5, 8.5 Hz, H-2), 5.24 (1H, dd, *J* 3.5, 1.0 Hz, H-4).

**2,3,4-Tri-*O*-acetyl-*β*-L-fucopyranosylamine.** A solution of 2,3,4-tri-*O*-acetyl-*β*-L-fucopyranosyl azide (315 mg, 1.00 mmol) in EtOAc (2 mL) was treated with palladium on carbon (10% w/w, of 10% w/w Pd/C; 30 mg). The mixture was thoroughly degassed and then stirred at room temperature under 1 atm of $H_2$ for 16 h. The mixture was diluted with EtOAc and filtered through Celite. The residue was washed with EtOAc, and combined filtrates and washings were evaporated in vacuo to give the title compound as a white foam (292 mg, 100%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.14 (3H, d, *J* 6.5 Hz, H-6), 1.95 (3H, s, OAc), 2.04 (3H, s, OAc), 2.14 (3H, s, OAc), 3.76 (1H, qd, *J* 6.5, 1.0 Hz, H-5), 4.09 (1H, d, *J* 8.0 Hz, H-1), 4.96 (1H, dd, *J* 10.5, 8.0 Hz, H-2), 5.02 (1H, dd, *J* 10.5, 3.0 Hz, H-3), 5.21 (1H, dd, *J* 3.0, 1.0 Hz, H-4). $NH_2$ signal too broad to be observed.

***N*-(Benzyloxycarbonyl)-(*R*)-alanyl-(*S*)-glutamine.** A solution of (*R*)-alanyl-(*S*)-glutamine (2.17 g, 10.0 mmol) in 2.0 M aqueous sodium hydroxide (5 mL, 10.0 mmol) at 0 °C was treated alternatively with four portions each of benzyl chloroformate (1.65 mL, 11 mmol total) and 2.0 M aqueous sodium hydroxide (6.5 mL, 11 mmol total). The reaction was then allowed to warm to ambient temperature and was stirred at ambient temperature for 75 min. The resultant slurry was diluted with water, brought to pH 12 with 1 M aqueous sodium hydroxide, and washed with EtOAc (2 × 50 mL). The aqueous phase was then brought to pH 1 with 5% aqueous hydrochloric acid and extracted with EtOAc (3 × 100 mL). Combined extracts were dried ($Na_2SO_4$), filtered, and evaporated in vacuo to give the title compound as a white solid (1.71 g, 49%): $^1$H NMR (DMSO-$d_6$, 300 MHz) 1.20 (3H, d, *J* 7.0 Hz, Ala H-3), 1.68−1.80 and 1.88−1.99 (2H, 2 × m, Gln, H-3), 2.08 (2H, t, *J* 8.0 Hz, Gln H-4), 4.08 (1H, dq, *J* 7.0, 7.0 Hz, Ala H-2), 4.16 (1H, ddd, *J* 8.0, 8.0, 5.0 Hz, Gln H-2), 4.08 and 5.03 (2H, AB q, *J* 12.5 Hz, PhC$H_2$), 6.75 (1H, br s, 1H of $CONH_2$), 7.26 (1H, br s, 1H of $CONH_2$), 7.30−7.38 (6H, m, Ph and Ala NH), 8.11 (1H, d, *J* 8.0 Hz, Gln NH), 12.60 (1H, br s, $CO_2H$).

**1-*β*-[[*N*-(Benzyloxycarbonyl)-(*R*)-alanyl-(*S*)-glutaminyl]amino]-1-deoxy-2,3,4-tri-*O*-acetyl-L-fucopyranose.** 2,3,4-Tri-*O*-acetyl-*β*-L-fucopyranosylamine (292 mg, 1.00 mmol) and *N*-(benzyloxycarbonyl)-(*R*)-alanyl-(*S*)-glutamine (421 mg, 1.20 mmol) were coupled according to general method B (using DCC), except that sufficient DMF was added to allow dissolution. The title compound was obtained as a white solid (208 mg, 33%): $^1$H NMR ($CDCl_3$, 300 MHz) 1.14 (3H, d, *J* 6.5 Hz, fucose H-6), 1.37 (3H, d, *J* 7.0 Hz, Ala H-3), 1.80−2.10 (2H, m, Gln H-3), 1.85 (3H, s, OAc), 2.01 (3H, s, OAc), 2.02 (3H, s, OAc), 2.18−2.40 (2H, m, Gln H-4), 3.91 (1H, q*, *J* 6.5 Hz, fucose H-5), 4.27 (1H, qd, *J* 7.0, 7.0 Hz, Ala H-2), 4.30−4.37 (1H, m, Gln H-2), 5.08−5.20 (5H, m, PhC$H_2$ + fucose H-1, H-2 and H-3), 5.23−5.26 (1H, m, fucose H-4), 5.62 (1H, d, *J* 7.0 Hz, Ala NH), 5.80 and 5.86 (2H, 2 × br s, $CONH_2$), 7.29−7.40 (5H, m, Ph), 7.60−7.65 (2H, m, 2 × NH), *fucose $J_{4-5}$ unresolved.

**1-*β*-[(*R*)-Alanyl-(*S*)-glutaminylamino]-1-deoxy-L-fucopyranose Acetate Salt, 15.** 1-*β*-[[*N*-(Benzyloxycarbonyl)-(*R*)-alanyl-(*S*)-glutaminyl]amino]-1-deoxy-2,3,4-tri-*O*-acetyl-L-fucopyranose (205 mg, 0.33 mmol) was deprotected by ammonolysis according to general method F, followed by N-deprotection according to general method E (using $H_2O$/1,4-dioxane as solvent) to give, after purification by reverse phase HPLC (Hamilton PRP-1, isocratic C, pH 8.5), compound **15** as a very hygroscopic white solid (86 mg, 55%): $^1$H NMR ($D_2O$, 300 MHz, *Me*OH = 3.35 ppm) 1.23 (3H, d, *J* 6.5 Hz, fucose H-6), 1.53 (3H, d, *J* 7.0 Hz, Ala H-3),1.91 (6H, s, $AcO^-$ and complexed AcOH), 1.97−2.22.(2H, m, Gln H-3), 2.39 (2H, t, *J* 7.5 Hz, Gln H-4), 3.60 (1H, dd, *J* 9.5, 9.0 Hz, fucose H-2), 3.70 (1H, dd, *J* 9.5, 3.0 Hz, fucose H-3), 3.80 (1H, d*, *J* 3.0 Hz, fucose H-4), 3.88 (1H, q*, *J* 6.5 Hz, fucose H-5), 4.08 (1H, q, *J* 7.0 Hz, Ala H-2), 4.41 (1H, dd, *J* 8.5, 5.5 Hz, Gln H-2), 4.91 (1H, d, *J* 9.0 Hz, fucose H-1), *fucose $J_{4-5}$ unresolved; $^{13}$C NMR ($D_2O$, 75.5 MHz, *Me*CN = 1.30 ppm) 15.98 (fucose C-6), 17.49 (Ala C-3), 23.71 (2 × *Me*$CO_2H$), 27.40 (Gln C-3), 31.43 (Gln C-4), 49.61 and 53.73 (Ala C-2 and Gln C-2), 69.40, 71.76, 73.20, and 73.92 (fucose C-2, C-3, C-4, and C-5), 80.15 (fucose C-1), 172.60 (2 × Me$CO_2H$), 178.04 (Gln C-5), 181.84 and 177.86 (Ala C-1 and Gln C-1); HRMS (FAB) 385.168 10, calculated for [M − 2AcOH + $Na^+$] ($C_{14}H_{26}N_4NaO_7^+$) 385.169 92. HPLC, system A (Hamilton; isocratic C, pH 8.5), 4.3 min, 99%; system B (YMC; isocratic E), 4.1 min. Anal. ($C_{14}H_{26}N_4O_7.CH_3CO_2H\cdot 5H_2O$) C, H, N.

Other analogues were prepared by similar procedures:

**6-((*R*)-Alanyl-(*S*)-glutaminylamino)-1-chlorohexane, 11:** mp 158−161 °C (softens 135−140 °C); $^1$H NMR ($CD_3OD$, 300 MHz) 1.27 (3H, d, *J* 7.0 Hz, Ala H-3), 1.31−1.57 (6H, m, hexyl H-2, H-3 and H-4), 1.76 (2H, tt, *J* 6.5, 6.5 Hz, hexyl H-5), 1.91 (1H, A of $ABXY_2$, $J_{AB}$ 14.0, $J_{AX}$ 9.0, $J_{AY}$ 7.5 Hz, 1H of Gln H-3), 2.08 (1H, B of $ABXY_2$, $J_{AB}$ 14.0, $J_{BY}$ 7.5, $J_{BX}$ 5.0 Hz, 1H of Gln H-3), 2.28 (2H, t, *J* 7.5 Hz, Gln H-4), 3.18 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.45 (1H, q, *J* 7.0 Hz, Ala H-2), 3.55 (2H, t, *J* 6.5 Hz, hexyl H-1), 4.30 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 21.21 (Ala C-3), 27.16, 27.57, 29.18 and 30.19 (hexyl C-2 to C-5), 32.54 and 33.67 (Gln C-3 and C-4), 40.30 (hexyl C-6), 45.65 (hexyl C-1), 51.45 and 54.24 (Ala C-2 and Gln C-2), 173.59 (Gln C-5), 177.63 and 178.19 (Ala C-1 and Gln C-1); HRMS (FAB) 335.183 90, calculated for M[$^{35}$Cl]$H^+$ ($C_{14}H_{28}N_4O_3{}^{35}Cl^+$) 335.185,00; HPLC (Hamilton; 40−80% (50 min) A in C, pH 7.85), 14.9 min, 93%.

**6-((*R*)-Alanyl-(*S*)-glutaminylamino)hexanoic acid, hydrochloride salt, 12:** mp 127−129 °C; $^1$H NMR (DMSO-$d_6$, 300 MHz) 1.20−1.50 (6H, m, hexyl H-3, H-4 and H-5), 1.36 (3H, d, *J* 7.0 Hz, Ala H-3), 1.68−1.78 and 1.85−1.95 (2H, 2 × m, Gln H-3), 2.08 (2H, t, *J* 7.0 Hz, hexyl H-5), 2.18 (2H, t, *J* 7.0 Hz, Gln H-4), 2.96−3.06 (2H, m, hexyl H-6), 3.83−3.88 (1H, m, Ala H-2), 4.18−4.28 (1H, m, Gln H-2), 6.76 and 7.31 (2H, 2 × br s, $CONH_2$), 8.09 (1H, t, *J* 7.0 Hz, N*H*$CH_2$), 8.20 (3H, br s, $NH_3^+$) 8.66−8.63 (1H, m, Gln NH); $^{13}$C NMR (DMSO-$d_6$, 75.5 MHz) 17.65 (Ala C-3), 24.56, 26.28, 28.49, and 29.12 (hexyl C-2 to C-5), 31.66 and 33.98 (Gln C-3 and C-4), 40.23 (hexyl

C-6), 48.61 and 52.82 (Ala C-2 and Gln C-2), 169.72, 170.81, 173.76 and 174.80 (hexyl C-1, Ala C-1, Gln C-1 and C-5); HRMS (FAB) 331.196 80, calculated for $[M - Cl^-]^+$ ($C_{14}H_{27}N_4O_5^+$), HPLC (YMC; 0−25% (30 min) A in C, pH 6.00), 10.6 min, 92%.

**6-((*R*)-Alanyl-(*S*)-glutaminylamino)-1-aminohexane, 13:** mp 111−114 °C; $^1$H NMR ($CD_3OD$, 300 MHz) 1.33 (3H, d, *J* 7.0 Hz, Ala H-3), 1.35−1.47 (4H, m, hexyl H-2 and H-4), 1.52−1.59 (2H, hexyl H-5), 1.61−1.70 (2H, m, hexyl H-2), 1.89−2.00 and 2.06−2.14 (2H, 2 × m, Gln H-3), 2.30 (2H, t, *J* 7.5 Hz, Gln H-4), 2.90 (2H, t, *J* 7.5 Hz, hexyl H-1), 3.18 (1H, A of $ABX_2$, *J* 13.5, 6.5 Hz, 1H of hexyl H-6), 3.23 (1H, B of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.66 (1H, q, *J* 7.0 Hz, Ala H-2), 4.28 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 20.26 (Ala C-3), 26.70, 26.91, 28.52, 28.72, 29.07, and 29.72 (Gln C-3 and C-4 and hexyl C-2 to C-5), 32.71 (hexyl C-1), 40.04 (hexyl C-6), 51.16 and 54.70 (Ala C-2 and Gln C-2), 174.04 (Gln C-5), 176.73 and 177.96 (Ala C-1 and Gln C-1); HRMS (FAB) 316.232 60, calculated for $[MH^+]$ ($C_{14}H_{30}N_5O_3^+$) 316.234 86; HPLC (Hamilton; 0−20% (40 min) A in C, pH 7.85), 14.9 min, 92%.

**6-((*S*)-Alanyl-(*R*)-glutaminylamino)hex-1-yl acetate, 14:** mp 100−104 °C (softens ∼80 °C); $^1$H NMR ($CD_3OD$, 400 MHz) 1.29 (3H, d, *J* 7.0 Hz, Ala H-3), 1.32−1.40 (4H, m, hexyl H-3 and H-4), 1.48−1.56 (2H, m, hexyl H-5), 1.60−1.67 (2H, m, hexyl H-2), 1.87−1.96 (1H, m, 1H of Gln H-3), 2.01 (3H, s, OAc), 2.03−2.11 (1H, m, 1H of Gln H-3), 2.28 (2H, t, *J* 8.0 Hz, Gln H-4), 3.18 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.48 (1H, q, *J* 7.0 Hz, Ala H-2), 4.05 (2H, t, *J* 6.5 Hz, hexyl H-1), 4.29 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 21.17 (Ala C-3), 26.62 and 27.48 (hexyl C-3 and C-4), 27.80 (*Me*CO), 29.17, 29.57 and 30.20 (Gln C-3 and hexyl C-2 and C-5), 32.52 (Gln C-4), 40.29 (hexyl C-6), 51.47 and 54.27 (Ala C-2 and Gln C-2), 65.62 (hexyl C-1), 173.29 and 173.85 (Gln C-5 and Me*C*O), 177.92 and 178.53 (Ala C-1 and Gln C-1); HRMS (FAB) 359.230 80, calculated for $[MH^+]$ ($C_{16}H_{31}N_4O_5^+$) 359.229 43; HPLC (Hamilton; 0−50% (60 min) A in C, pH 7.9), 59.2 min, 92%.

**α-L-Fucopyranosyl [(*R*)-alanyl-(*S*)-glutaminyl]amine, acetate salt, 16:** $^1$H NMR ($D_2O$, 300 MHz, *Me*OH = 3.35 ppm) 1.18 (3H, d, *J* 6.5 Hz, fucose H-6), 1.53 (3H, d, *J* 7.0 Hz, Ala H-3), 1.91 (3H, s, $AcO^-$), 1.96−2.20 (2H, m, Gln H-3), 2.39 (2H, t, *J* 7.5 Hz, Gln H-4), 3.81 (1H, d*, *J* 3.5 Hz, fucose H-4), 3.88 (1H, dd, *J* 10.5, 3.5 Hz, fucose H-3), 3.89 (1H, q*, *J* 6.5 Hz, fucose H-5), 4.03 (1H, dd, *J* 10.5, 5.5 Hz, fucose H-2), 4.08 (1H, q, *J* 7.0 Hz, Ala H-2), 4.53 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2), 5.59 (1H, d, *J* 5.5 Hz, fucose H-1), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($D_2O$, 75.5 MHz, *Me*CN = 1.30 ppm) 16.05 and 17.42 (fucose C-6 and Ala C-3), 23.71 (*Me*$CO_2^-$), 27.67 (Gln C-3), 31.29 (Gln C-4), 49.59 and 53.36 (Ala C-2 and Gln C-2), 66.36, 68.25, 69.92 and 71.83 (fucose C-2, C-3, C-4, and C-5), 77.35 (fucose C-1), 172.32 (Me*C*$O_2^-$), 174.92 (Gln C-5), 178.01 and 181.88 (Ala C-1 and Gln C-1); HRMS (FAB) 385.168 50, calculated for $[M - AcOH + Na^+]$ ($C_{14}H_{26}N_4NaO_7^+$) 385.169 92; HPLC (Hamilton; isocratic C, pH 8.5), 3.1 min, 98%.

**2-((*R*)-Alanylamino)-(*S*)-glutarimide, 18:** mp 245−248 °C (darkens >180 °C to brown); $^1$H NMR ($D_2O$, 300 MHz, MeCN = 2.05 ppm) 1.43 (3H, d, *J* 7.0 Hz, Ala H-3), 2.05−2.24 (2H, m, imide H-3), 2.35 (2H, t, *J* 7.0 Hz, imide H-4), 4.20−4.26 (1H, m, imide H-2), 4.22 (1H, q, *J* 7.0 Hz, Ala H-2); $^{13}$C NMR ($D_2O$, 75.5 MHz, *Me*CN = 1.30 ppm) 18.72 (Ala C-3), 28.95 (imide C-3), 30.35 (imide C-4), 50.49 (Ala C-2), 54.51 (imide C-2), 170.24 and 172.15 (Ala C-1 and imide C-5), 178.66 (imide C-1); HPLC (YMC; 0−10% (20 min) B in C, pH 6.0), 20.5 min, 95%.

***β*-*O*-Mannopyranosyl [(*R*)-alanyl-(*S*)-glutaminyl]amine, 19:** mp 138−142 °C dec; $^1$H NMR ($D_2O$, 300 MHz, $Me_2$CHOH = 1.18 ppm) 1.31 (3H, *J* 7.0 Hz, Ala H-3), 1.96−2.08 and 2.12−2.22 (2H, 2 × m, Gln H-3), 2.42 (2H, t, *J* 7.5 Hz, Gln H-4), 3.48 (1H, ddd, *J* 9.5, 6.0, 2.0 Hz, mannose H-5), 3.57−3.65 (1H, m, Ala H-2), 3.61 (1H, dd, *J* 9.5, 9.5 Hz, mannose H-4), 3.73 (1H, A of ABX, *J* 12.0, 6.0 Hz, 1H of mannose H-6), 3.73 (*sic*) (1H, dd, *J* 9.5, 3.0 Hz, mannose H-3), 3.92 (1H, B of ABX, *J* 12.0, 6.0 Hz, 1H of mannose H-6), 3.98 (1H, d*, *J* 3.0 Hz, mannose H-2), 4.42 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2), 5.24 (1H, s*, mannose H-1), *mannose $J_{H1-H2}$ unresolved; $^{13}$C NMR ($D_2O$, 75.5 MHz, *Me*CN = 1.30 ppm) 20.04 (Ala C-3), 27.18 (Gln C-3), 31.55 (Gln C-4), 50.22 and 52.37 (Ala C-2 and Gln C-2), 61.29 (mannose C-6), 66.83, 70.48, 73.68, 78.25, and 78.29 (mannose C-1, C-2, C-3, C-4, and C-5), 174.13 ($CONH_2$), 178.26 and 178.60 (2 × CONH); HRMS (FAB) 379.181 10, calculated for $[MH^+]$ ($C_{14}H_{27}N_4O_8^+$) 389.182 89; HPLC (Partisil 10 SCX, 4.6 × 150 mm; 0−40% (50 min) 7 mM potassium phosphate, pH 7.0 in 250 mM potassium phosphate, pH 7.0), 12.0, 94%.

**1-*β*-[12-((*R*)-Alanyl-(*S*)-glutaminylamino)dodec-1-yl]-L-fucopyranose, 20:** mp 140−143 °C; $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.25−1.40 (19H, m, Ala H-3 and dodecyl H-3 to H-10), 1.41−1.52 (2H, m, dodecyl H-11), 1.53−1.64 (2H, m, dodecyl H-2), 1.85−2.12 (2H, m, Gln H-3), 2.30 (2H, t, *J* 7.5 Hz, Gln H-4), 3.09−3.23 (2H, m, dodecyl H-12), 3.44 (1H, dd, *J* 10.0, 7.5 Hz, fucose H-2), 3.49 (1H, q, *J* 7.0 Hz, Ala H-2), 3.52 (1H, dd, *J* 10.0, 3.0 Hz, fucose H-3), 3.54 (1H, A of $ABX_2$, *J* 9.5, 7.0 Hz, dodecyl H-1), 3.64 (1H, dd, *J* 3.0, 1.0 Hz, fucose H-4), 3.66 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.82 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, dodecyl H-1), 4.22 (1H, d, *J* 7.5 Hz, fucose H-1), 4.28 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD + D_2O$, 75.5 MHz) 16.66 (fucose C-6), 21.10 (Ala C-3), 26.66, 27.61, 28.85, 29.92, 30.04, 30.19, 30.33 [4 × $CH_2$ unresolved], 30.41 and 32.48 (Gln C-3 and C-4, + dodecyl C-2 to C-11), 40.47 ($NHCH_2$), 51.24 and 54.29 (Ala C-2 and Gln C-2), 71.23 (dodecyl C-1), 71.83, 71.95, 72.76, and 74.69 (fucose C-2, C-3, C-4, and C-5), 104.37 (fucose C-1), 173.72 (Gln C-5), 178.17 and 178.63 (Ala C-1 and Gln C-1); HRMS (FAB) 547.373 50, calculated for $[MH^+]$ ($C_{26}H_{51}N_4O_8^+$) 547.370 67; HPLC (Hamilton; 10−50% (50 min) B in C, pH 8.5), 24.4 min, 100%.

**2-*O*-Acetyl-1-*β*-[12-((*R*)-alanyl-(*S*)-glutaminylamino)-dodec-1-yl]-D-mannopyranose, 21:** mp 81−85 °C; $^1$H NMR ($CD_3OD$, 300 MHz) 1.27−1.38 (16H, m, dodecyl H-3 to H-10), 1.45−1.57 (4H, m, dodecyl H-2 and H-11), 1.49 (3H, d, *J* 7.0 Hz, Ala H-3), 1.63 (3H, s, OAc), 1.87−1.99 and 2.01−2.12 (2H, 2 × m, Gln H-3), 2.27−2.32 (2H, m, Gln H-4), 3.17 (2H, t, *J* 7.0 Hz, dodecyl H-12), 3.22 (1H, ddd, *J* 9.5, 6.0, 2.5 Hz, mannose H-5), 3.51 (1H, A of $ABX_2$, *J* 9.0, 6.5 Hz, 1H of dodecyl H-1), 3.55 (1H, B of $ABX_2$, *J* 9.0, 6.5 Hz, 1H of dodecyl H-1), 3.55 (*sic*) (1H, dd, *J* 9.5, 9.5 Hz, mannose H-4), 3.65 (1H, A of ABX, *J* 12.0, 6.0 Hz, 1H of mannose H-6), 3.68 (1H, dd, *J* 9.5, 4.0 Hz, mannose H-3), 3.84 (1H, B of ABX, *J* 12.0, 2.5 Hz, 1H of mannose H-6), 3.90 (1H, q, *J* 7.0 Hz, Ala H-2), 4.31 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2), 4.42 (1H, dd, *J* 4.0, 2.5 Hz, mannose H-2), 5.42 (1H, d, *J* 2.5 Hz, mannose H-1); $^{13}$C NMR ($CD_3$-OD, 75.5 MHz) 19.97 (Ala C-3), 25.86 (*Me*CO), 27.24, 27.98, 29.08, 30.35, 30.40 [2C], 30.44, 30.65 [4C] and 32.42 (Gln C-3 and C-4, and dodecyl C-2 to C-11), 40.55 (dodecyl C-12), 50.45 and 54.48 (Ala C-2 and Gln C-2), 62.81 (dodecyl C-1), 68.55, 73.31, 76.93, and 81.20 (mannose C-2, C-3, C-4, and C-5), 99.09 (mannose C-1), 124.85 (mannose C-6), 171.71 [2C], 173.33 and 177.48 (4 × C=O); HRMS (FAB) 605.378 50, calculated for $[MH^+]$ ($C_{28}H_{53}N_4O_{10}^+$) 605.376 16; HPLC (Hamilton; 10−50% (50 min) B in C, pH 8.5, 25.6 min.

**12-((*R*)-Alanyl-(*S*)-glutaminylamino)dodecan-1-ol, 22:** mp 164−167 °C; $^1$H NMR ($CD_3OD$, 300 MHz) 1.27−1.40 (16H, m, dodecyl H-3 to H-10), 1.44−1.56 (4H, m, dodecyl H-2 and H-11), 1.52 (3H, d, *J* 7.0 Hz, Ala H-3), 1.87−2.13 (2H, m, Gln H-3), 2.30 (2H, t, *J* 7.5 Hz, Gln H-4), 3.12−3.21 (2H, m, dodecyl H-12), 3.53 (2H, t, *J* 6.5 Hz, dodecyl H-1), 3.98 (1H, q, *J* 7.0 Hz, Ala H-2), 4.32 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 17.62 (Ala C-3), 26.95, 28.00, 29.06, 30.36, 30.43, 30.61, 30.67, 30.71 [2C], 30.74, 32.45, and 33.66 (Gln C-3 and C-4, and dodecyl C-2 to C-11), 40.56 (dodecyl C-12), 50.36 and 54.55 (Ala C-2 and Gln C-2), 63.00 (dodecyl C-1), 171.02 (Gln C-5), 173.29 and 177.46 (Ala C-1 and Gln C-1); HRMS (FAB) 401.314 20, calculated for $[MH^+]$ ($C_{20}H_{41}N_4O_4^+$) 401.312 77; HPLC (YMC; 10−50% (50 min) B in C, pH 6.0), 33.1 min.

**1-*β*-[2-[4-((*R*)-Alanyl-(*S*)-glutaminylamino)phenyl]ethyl]-L-fucopyranose, 23:** mp 107−110 °C (softens 103 °C); $^1$H NMR ($CD_3OD + D_2O$, 400 MHz) 1.28 (3H, d, *J* 6.5 Hz, fucose

H-6), 1.33 (3H, d, *J* 7.0 Hz, Ala H-3), 1.97−2.06 and 2.12−2.21 (2H, 2 × m, Gln H-3), 2.36 (2H, t, *J* 7.5 Hz, Gln H-4), 2.90 (2H, t, *J* 7.0 Hz, ethyl H-2), 3.45 (1H, dd, *J* 9.5, 7.0 Hz, fucose H-2), 3.48 (1H, dd, *J* 9.5, 3.0 Hz, fucose H-3), 3.52 (1H, q, *J* 7.0 Hz, Ala H-2), 3.61 (1H, d*, *J* 3.0 Hz, fucose H-4), 3.63 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.72 (1H, A of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of ethyl H-1), 4.01 (1H, B of $ABX_2$, *J* 9.5, 7.5 Hz, 1H of ethyl H-1), 4.23 (1H, d, *J* 7.5 Hz, fucose H-1), 4.47 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2), 7.22 (2H, d, *J* 8.5 Hz, phenylene H-2 and H-6), 7.46 (2H, d, *J* 8.5 Hz, phenylene H-3 and H-5), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD + D_2O$, 75.5 MHz) 16.61 (fucose C-6), 11.62 (Gln C-3), 32.32 (Ala C-3), 32.41 (Gln C-4), 36.34 (ethyl C-2), 51.26 and 54.75(Ala C-2 and Gln C-2), 71.70 (ethyl C-1), 71.92, 72.05, 72.81, and 74.78 (fucose C-2, C-3, C-4, and C-5), 104.56 (fucose C-1), 121.98 (phenylene C-2 and C-5), 130.57 (phenylene C-2 and C-6), 136.67 and 137.16 (phenylene C-1 and C-4), 172.41 (Gln C-5), 178.27 and 178.86 (Ala C-1 and Gln C-1); HRMS (FAB) 483.244 40, calculated for [$MH^+$] ($C_{22}H_{35}N_4O_8^+$) 483.245 48; HPLC (Hamilton; 10−60% (60 min) A in C, pH 7.85), 25.6 min, 99%.

**1-β-[6-((*S*)-Alanyl-(*S*)-glutaminylamino)hex-1-yl]-L-fucopyranose, 24:** mp 188−190 °C; $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.27 (3H, d, *J* 7.0 Hz, Ala H-3), 1.30−1.42 (4H, m, hexyl H-3 and H-4), 1.44−1.54 (2H, m, hexyl H-5), 1.55−1.65 (2H, m, hexyl H-2), 1.87−2.11 (2H, m, Gln H-3), 2.31 (2H, t, *J* 7.5 Hz, Gln H-4), 3.15 (1H, A of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.21 (1H, B of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.44 (1H, dd, *J* 10.0, 7.5 Hz, fucose H-2), 3.48 (1H, q, *J* 7.0 Hz, Ala H-2), 3.52 (1H, dd, *J* 10.0, 3.5 Hz, fucose H-3), 3.55 (1H, A of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 3.65 (1H, dd, *J* 3.5, 1.0 Hz, fucose H-4), 3.66 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.23 (1H, d, *J* 7.5 Hz, fucose H-1), 4.29 (1H, dd, *J* 9.0, 5.5 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 16.62 (fucose C-6), 21.23 (Ala C-3), 26.18, 27.23, 28.79, 29.70, 30.19, and 32.38 (Gln C-3 and C-4 and hexyl C-2, C-3, C-4, and C-5), 40.28 (hexyl C-6), 51.22 and 54.16 (Ala C-2 and Gln C-2), 71.09 (hexyl C-1), 71.82 71.89, 72.70, and 74.59 (fucose C-2, C-3, C-4, and C-5), 104.27 (fucose C-1), 173.66 (Gln C-5), 178.24 and 178.80 (Ala C-1 and Glu C-1); HRMS (FAB) *m/z* 463.275 00, calculated for $MH^+$ ($C_{20}H_{39}N_4O_8^+$) 463.276 79; HPLC (Hamilton; 0−40% (50 min) B in C, pH 8.5), 17.6 min, 100%.

**1-β-[6-((*R*)-Valyl-(*S*)-glutaminylamino)hex-1-yl]-L-fucopyranose, 25:** mp 68−71 °C; $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 0.91 (3H, d, *J* 7.0 Hz, Val H-4), 0.94 (3H, d, *J* 7.0 Hz, Val H-4′), 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.30−1.42 (4H, m, hexyl H-3 and H-4), 1.46−1.52 (2H, m, hexyl H-5), 1.55−1.66 (2H, m, hexyl H-2), 1.88−1.99 (2H, m, Val H-3 and 1H of Gln H-3), 2.03−2.12 (1H, m, 1H of Gln H-3), 2.31 (2H, t, *J* 7.5 Hz, Gln H-4), 3.13 (1H, d, *J* 6.0 Hz, Val H-2), 3.18 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.44 (1H, dd, *J* 9.5, 7.0 Hz, fucose H-2), 3.50 (1H, dd, *J* 9.5, 3.0 Hz, fucose H-3), 3.54 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3. 63 (1H, dd, *J* 3.0, 1.0 Hz, fucose H-4), 3.65 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 4.21 (1H, d, *J* 7.0 Hz, fucose H-1), 4.30 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD + D_2O$, 75.5 MHz) 16.71 (fucose C-6), 18.02 (Val C-4), 19.82 (Val C-4′), 26.43, 27.45, 28.91, 29.95, 30.41, and 32.58 (Gln C-3 and C-4, + hexyl C-2 to C-5), 33.26 (Val C-3), 40.35 (hexyl C-6), 54.29 and 61.66 (Val C-2 and Gln C-2), 70.90 (hexyl C-1), 71.82, 72.06, 72.84, and 74.84 (fucose C-2, C-3, C-4, and C-5), 104.49 (fucose C-1), 173.65 (Gln C-5), 177.35 and 177.95 (Val C-1 and Gln C-1); HRMS (FAB) 491.307 20, calculated for [$MH^+$] ($C_{22}H_{43}N_4O_8^+$) 491.308 07; HPLC (Hamilton; 0−50% (60 min) B in C, pH 7.7), 20.8 min, 94%.

**1-β-[6-([(*S*)-3-Aminoalanyl-(*S*)-glutaminyl]amino)hex-1-yl]-L-fucopyranose, monoacetate salt, 26:** $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.29−1.41 (4H, m, hexyl H-3 and H-4), 1.43−1.54 (2H, m, hexyl H-5), 1.55−1.67 (2H, m, hexyl H-2), 1.89 (3H, s, $AcO^-$), 1.91−2.03 and 2.05−2.20 (2H, 2 × m, Gln H-3), 2.32 (2H, t, *J* 7.0 Hz, Gln H-4), 3.01 (1H, A of ABX, *J* 13.0, 7.5 Hz, 1H of amino-Ala H-3), 3.12−3.21 (3H, m, hexyl H-6 and 1H of amino-Ala H-3), 3.44 (1H, dd, *J* 10.0, 7.5 Hz, fucose H-2), 3.53 (1H, dd, *J* 10.0, 3.5 Hz, fucose H-3), 3.56 (1H, A of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 3.63−3.69 (1H, m, amino-Ala H-2), 3.66 (1H, d*, *J* 3.5 Hz, fucose H-4), 3.67 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.84 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.24 (1H, d, *J* 7.5 Hz, fucose H-1), 4.32 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD + D_2O$, 75.5 MHz) 16.71 (fucose C-6), 24.11 ($MeCO_2^-$), 26.47, 27.51, 29.06, 29.97, 30.42, and 33.10 (Gln C-3 and C-4, + hexyl C-2 to C-5), 40.42 (hexyl C-6), 44.42 (amino-Ala C-3), 54.01 and 54.30 (amino-Ala C-2 and Gln C-2), 70.92 (hexyl C-1), 71.86, 72.11, 72.86, and 74.86 (fucose C-2, C-3, C-4, and C-5), 104.51 (fucose C-1), 174.90, 175.03, 176.39, and 180.53 ($MeCO_2^-$, amino-Ala C-1, Gln C-1 and Gln C-5); HRMS (FAB) 478.285 80, calculated for [M − AcOH + $H^+$] ($C_{20}H_{40}N_5O_8^+$) 478.287 69; HPLC (YMC; 0−40% (50 min) B in C, pH 7.0), 29.3 min, 99%.

**1-β-[6-((*S*)-Glutaminylamino)hex-1-yl]-L-fucopyranose acetate salt, 27:** $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.30−1.45 (4H, m, hexyl H-3 and H-4), 1.47−1.67 (4H, m, hexyl H-2 and H-5), 1.81 (3H, s, $AcO^-$), 1.94−2.11 (2H, m, Gln H-3), 2.36 (2H, t, *J* 7.5 Hz, Gln H-4), 3.14−3.27 (3H, m, hexyl H-6 and Gln H-2), 3.44 (1H, dd, *J* 9.5, 7.0 Hz, fucose H-2), 3.50 (1H, dd, *J* 9.5, 3.0 Hz, fucose H-3), 3.54 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.63 (1H, dd, *J* 3.0, 1.0 Hz, fucose H-4), 3.64 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.84 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.20 (1H, d, *J* 7.5 Hz, fucose H-1); $^{13}$C NMR ($CD_3OD + D_2O$, 75.5 MHz) 16.73 (fucose C-6), 23.76 ($MeCO_2$-), 26.48, 27.53, 29.91, 29.97, 30.42, 31.90, and 32.58 (Gln C-3 and C-4, + hexyl C-2 to C-5), 40.50 (hexyl C-6), 58.25 (Gln C-2), 70.91 (hexyl C-1), 71.87, 72.12, 72.87, and 74.88 (fucose C-2, C-3, C-4, and C-5), 104.53 (fucose C-1), 174.73 (Gln C-5), 177.60 (Gln C-1), 180.12 ($MeCO_2^-$); HRMS (FAB) 392.239 00, calculated for [M − AcOH + $H^+$] ($C_{17}H_{34}N_3O_7^+$) 392.239 69; HPLC (Hamilton; 0−40% (60 min) B in C, pH 8.5), 21.4 min, 98%.

**1-β-[6-([*N*-Acetyl-(*R*)-alanyl-(*S*)-glutaminyl]amino)hex-1-yl]-L-fucopyranose, 28:** mp 171−174 °C; $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.28−1.42 (4H, m, hexyl H-3 and H-4), 1.36 (3H, d, *J* 7.0 Hz, Ala H-3), 1.44−1.53 (2H, m, hexyl H-5), 1.54−1.64 (2H, m, hexyl H-2), 1.84−2.00 and 2.08−2.19 (2H, 2 × m, Gln H-3), 2.00 (3H, s, NAc), 2.29 (2H, t, *J* 7.0 Hz, Gln H-4), 3.14 (1H, A of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.21 (1H, B of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.44 (1H, dd, *J* 9.5, 7.0 Hz, fucose H-2), 3.50 (1H, dd, *J* 9.5, 3.5 Hz, fucose H-3), 3.53 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.62 (1H, dd, *J* 3.5, 1.0 Hz, fucose H-4), 3.64 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 4.21 (1H, d, *J* 7.0 Hz, fucose H-1), 4.24 (1H, q, *J* 7.0 Hz, Ala H-2), 4.25 (1H, dd, *J* 9.5, 4.5 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 16.68 and 17.52 (fucose C-6 and Ala C-3), 22.56 (NCO*Me*), 26.39, 27.36, 28.34, 39.87, 30.36, and 32.55 (Gln C-3 and C-4 and hexyl C-2, C-3, C-4, and C-5), 40.40 (hexyl C-6), 51.14 and 54.50 (Ala C-2 and Gln C-2), 70.99 ($CH_2O$), 71.84, 72.03, 72.82, and 74.77 (fucose C-2, C-3, C-4, and C-5), 104.43 (fucose C-1), 173.58 and 174.21 (Gln C-5 and N*C*OMe), 175.88 and 178.17 (Ala C-1 and Gln C-1); HRMS (FAB) *m/z* 505.290 20, calculated for $MH^+$ ($C_{22}H_{41}N_4O_9^+$) 505.287 35; HPLC, system A (Hamilton; 0−25% (50 min) B in C, pH 8.5), 24.6 min, 100%.

**1-β-[6-([*N*-Acetyl-(*S*)-alanyl-(*S*)-glutaminyl]amino)hex-1-yl]-L-fucopyranose, 29:** no mp observed <250 °C (darkens to brown above 165 °C); $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.30−1.41 (4H, m, hexyl H-3 and H-4), 1.36 (3H, d, *J* 7.0 Hz, Ala H-3), 1.48−1.55 (2H, m, hexyl H-5), 1.57−1.68 (2H, m, hexyl H-2), 1.86−2.00 and 2.07−2.23 (2H, 2 × m, Gln H-3), 2.01 (3H, s, NAc), 2.24−2.30 (2H, m, Gln H-4), 3.12−3.18 (2H, m, hexyl H-6), 3.44 (1H, dd, *J* 10.0, 7.5 Hz, fucose H-2), 3.51 (1H, dd, *J* 10.0, 3.0 Hz, fucose H-3), 3.55 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.64 (1H, dd, *J* 3.0, 1.0 Hz, fucose H-4), 3.66 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 4.22 (1H, d, *J* 7.5 Hz, fucose H-1), 4.24 (1H, q, *J* 7.0 Hz, Ala H-2), 4.26 (1H, dd, *J* 9.5, 4.5 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD + D_2O$, 100.6 MHz, protonated carbons only

from HMQC assignment) 16.40 (fucose C-6), 17.21 (Ala C-3), 22.18 (NCO*Me*), 25.66 and 27.41 (hexyl C-3 and C-4), 29.22 (hexyl C-5), 29.81 (hexyl C-2), 30.08 (Gln C-3), 32.72 (Gln C-4), 40.12 (hexyl C-6), 50.80 and 53.51 (Ala C-2 and Gln C-2), 70.72 (hexyl C-1), 71.48 (fucose C-2), 71.51 (fucose C-5), 72.42 (fucose C-4), 74.42 (fucose C-3), 103.93 (fucose C-1); HRMS (FAB) *m/z* 505.289 70, calculated for $MH^+$ ($C_{22}H_{41}N_4O_9^+$) 505.287 35; HPLC, system A (Hamilton; 0−25% (50 min) B in C, pH 8.5), 26.2 min, 100%.

**1-β-[6-([*N*-Acetyl-(*RS*)-glutaminyl]amino)hex-1-yl]-L-fucopyranose, 30:** mp 71−74 °C; $^1$H NMR ($CD_3OD$ + $D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.29−1.40 (4H, m, hexyl H-3 and H-4), 1.44−1.53 (2H, m, hexyl H-5), 1.55−1.66 (2H, m, hexyl H-2), 1.83−2.14 (2H, m, Gln H-3), 2.01 (3H, s, AcN), 2.25−2.32 (2H, m, Gln H-4), 3.12−3.20 (2H, m, hexyl H-6), 3.44 (1H, dd, *J* 9.5, 7.5 Hz, fucose H-2), 3.50 (1H, dd, *J* 9.5, 3.0 Hz, fucose H-3), 3.54 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.63 (1H, dd, *J* 3.0, 1.0 Hz, fucose H-4), 3.65 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.21 (1H, d, *J* 7.5 Hz, fucose H-1), 4.27 (∼0.5H, dd, *J* 9.0, 5.0 Hz, (*S*)-Gln H-2), 4.28 (∼0.5H, dd, *J* 9.0, 5.0 Hz, (*R*)-Gln H-2); $^{13}$C NMR ($CD_3OD$ + $D_2O$, 75.5 MHz) 16.68 (fucose C-6), 22.60 (*Me*CO), 26.35, 27.38, 27.45, 28.89, 29.02, 29.87, 30.34, 32.51, 33.24 (Gln C-3 and C-4, + linker C-2 to C-5 for (*R*)-Gln and (*S*)-Gln diastereomers), 40.33 and 40.41 (hexyl C-6 for (*R*)-Gln and (*S*)-Gln diastereomers), 54.03 and 54.44 ((*R*)-Gln C-2 and (*S*)-Gln C-2), 71.01 (hexyl C-1), 71.85, 72.02, 72.81 and 74.77 (fucose C-2, C-3, C-4, and C-5), 104.43 (fucose C-1), 173.81, 174.04, 174.12, 175.10, 176.80, and 178.17 (Me*C*O, Gln C-1, and Gln C-5 for (*R*)-Gln and (*S*)-Gln diastereomers); HRMS (FAB) 434.248 40, calculated for $[MH^+]$ ($C_{19}H_{36}N_3O_8^+$) 434.250 24; HPLC (Hamilton; 0−50% (60 min) B in C, pH 7.7), 18.8 min, 100% (diastereomers not resolved).

**1-β-[6-(β-Alanyl-(*S*)-glutaminylamino)hex-1-yl]-L-fucopyranose, 31:** mp 121−124 °C; $^1$H NMR ($CD_3OD$ + $D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.29−1.41 (4H, m, hexyl H-3 and H-4), 1.45−1.53 (2H, m, hexyl H-5), 1.54−1.66 (2H, m, hexyl H-2), 1.87−2.12 (2H, m, Gln H-3), 2.33 (2H, t, *J* 7.5 Hz, Gln H-4), 2.71 (2H, t, *J* 6.5 Hz, β-Ala H-2), 3.10−3.24 (4H, m, β-Ala H-3 and hexyl H-6), 3.44 (1H, dd, *J* 10.0, 7.5 Hz, fucose H-2), 3.54 (1H, dd, *J* 10.0, 3.5 Hz, fucose H-3), 3.56 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.66 (1H, d*, *J* 3.5 Hz, fucose H-4), 3.68 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.25 (1H, d, *J* 7.5 Hz, fucose H-1), 4.27 (1H, dd, *J* 9.0, 5.5 Hz, Gln H-2), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD$ + $D_2O$, 75.5 MHz) 16.76 (fucose C-6), 26.52, 27.51, 28.95, 30.03, 30.50 and 32.67 (Gln C-3 and C-4, + hexyl C-2 to C-5), 33.95 (β-Ala C-2), 37.29 (β-Ala C-3), 40.36 (hexyl C-6), 54.64 (Gln C-2), 70.81 (hexyl C-1), 71.85, 72.17, 72.92, and 74.95 (fucose C-2, C-3, C-4, and C-5), 104.57 (fucose C-1), 173.05 and 173.89 (β-Ala C-1 and Gln C-5), 178.09 (Gln C-1); HRMS (FAB) 463.278 80, calculated for $[MH^+]$ ($C_{20}H_{39}N_4O_8^+$) 463.276 79; HPLC (Hamilton; 0−50% (50 min) B in C, pH 8.4), 14.9 min, 96%.

**1-β-[6-((*S*)-Alanyl-(*R*)-glutaminylamino)hex-1-yl]-L-fucopyranose, 32:** mp 188−190 °C (darkens slowly >150 °C); $^1$H NMR ($CD_3OD$ + $D_2O$, 300 MHz) 1.24 (3H, d, *J* 6.5 Hz, fucose H-6), 1.26 (3H, d, *J* 7.0 Hz, Ala H-3), 1.29−1.40 (4H, m, hexyl H-3 and H-4), 1.51 (2H, tt, *J* 6.5, 6.5 Hz, hexyl H-5), 1.60 (2H, tt, *J* 7.0, 6.5 Hz, hexyl H-2), 1.89−2.13 (2H, m, Gln H-3), 2.33 (2H, t, *J* 7.5 Hz, Gln H-4), 3.16 (1H, A of $ABX_2$, *J* 13.0, 6.5 Hz, 1H of hexyl H-6), 3.21 (1H, B of $ABX_2$, *J* 13.0, 7.0 Hz, 1H of hexyl H-6), 3.43 (1H, dd, *J* 10.0, 8.0 Hz, fucose H-2), 3.51 (1H, q, *J* 7.0 Hz, Ala H-2), 3.57 (1H, dd, *J* 10.0, 3.5 Hz, fucose H-3), 3.60 (1H, A of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 3.69 (1H, d*, *J* 3.5 Hz, fucose H-4), 3.73 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.85 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.24 (1H, dd, *J* 9.0, 5.5 Hz, Gln H-2), 4.30 (1H, d, *J* 8.0 Hz, fucose H-1), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD$ + $D_2O$, 75.5 MHz) 16.54 (fucose C-6), 20.97 (Ala C-3), 26.02 and 27.09 (hexyl C-3 and C-4), 28.57 (Gln C-3), 29.54 and 30.04 (hexyl C-2 and C-5), 32.35 (Gln C-4), 40.29 (hexyl C-6), 51.11 and 54.28 (Ala C-2 and Gln C-2), 71.20 (hexyl C-1), 71.84 [2C], 72.67 and 74.51 (fucose C-2, C-3, C-4, and C-5), 104.26 (fucose C-1), 174.00 (Gln C-5), 178.50 and 178.95 (Ala C-1 and Gln C-1); HRMS (FAB) 463.279 30, calculated for $[MH^+]$ ($C_{20}H_{39}N_4O_8^+$) 463.276 79; HPLC (Hamilton; 10−50% (40 min) A in C, pH 7.85), 21.5 min, 96%.

**1-β-[6-((*R*)-Alanyl-(*S*)-glutamylamino)hex-1-yl]-L-fucopyranose, 33:** mp 126−129 °C (softens 117 °C); $^1$H NMR ($D_2O$ + $CD_3OD$, 300 MHz) 1.24 (3H, d, *J* 6.5 Hz, fucose H-6), 1.29−1.40 (4H, m, hexyl H-3 and H-4), 1.47−1.58 (2H, m, hexyl H-5), 1.55 (3H, d, *J* 7.0 Hz, Ala H-3), 1.59−1.65 (2H, m, hexyl H-2), 1.85−2.09 (2H, m, Glu H-3), 2.24 (2H, t, *J* 7.0 Hz, Glu H-4), 3.15−3.22 (2H, m, hexyl H-6), 3.43 (1H, dd, *J* 10.0, 8.0 Hz, fucose H-2), 3.57 (1H, dd, *J* 10.0, 3.0 Hz, fucose H-3), 3.57−3.63 (1H, m, 1H of hexyl H-1), 3.68 (1H, d* *J* 3.0 Hz, fucose H-4), 3.72 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.86 (1H, B of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 4.04 (1H, q, *J* 7.0 Hz, Ala H-2), 4.23 (1H, dd, *J* 9.0, 5.0 Hz, Glu H-2), 4.29 (1H, d, *J* 7.5 Hz, fucose H-1), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($D_2O$, 75.5 MHz, *Me*CN = 1.30 ppm) 19.79 (fucose C-6), 21.20 (Ala C-3), 29.01, 30.11, 32.26, 32.52 and 33.01 (Glu C-3 + hexyl C-2 to C-5), 37.89 (Glu C-4), 43.70 (hexyl C-6), 53.54 and 58.47 (Ala C-2 and Glu C-2), 74.85 (hexyl C-1), 74.90, 75.23, 75.74, and 77.36 (fucose C-2, C-3, C-4, and C-5), 107.02 (fucose C-1), 175.80 and 177.63 (Ala C-1 and Glu C-1), 185.56 (Glu C-5); HRMS (FAB) 486.244 80, calculated for $[MNa^+]$ ($C_{20}H_{37}N_3NaO_9^+$) 486.24274; HPLC (Hamilton; 0−50% (60 min) B in C, pH 8.45), 16.3 min.

**1-β-[6-([(*S*)-3-Aminoalanyl-(*S*)-glutamyl]amino)hex-1-yl]-L-fucopyranose, 34:** mp 175−180 °C (decomp; darkens > 150 °C); $^1$H NMR ($D_2O$, 300 MHz, *Me*OH = 3.35 ppm) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.30−1.43 (4H, m, hexyl H-3 and H-4), 1.48−1.56 (2H, m, hexyl H-5), 1.56−1.67 (2H, m, hexyl H-2), 1.91−2.11 (2H, m, Gln H-3), 2.29 (2H, t, *J* 7.5 Hz, Glu H-4), 3.16 (1H, A of ABX, *J* 13.0, 7.5 Hz, 1H of amino-Ala H-3), 3.17 (1H, A of $ABX_2$, *J* 9.0, 7.0 Hz, 1H of hexyl H-6), 3.25 (1H, B of $ABX_2$, *J* 9.0, 7.0 Hz, 1H of hexyl H-6), 3.32 (1H, B of ABX, *J* 13.0, 5.5 Hz, 1H of amino-Ala H-3), 3.45 (1H, dd, *J* 10.0, 8.0 Hz, fucose H-2), 3.63 (1H, dd, *J* 10.0, 3.5 Hz, fucose H-3), 3.65 (1H, A of $ABX_2$, *J* 10.0, 7.0 Hz, 1H of hexyl H-1), 3.74 (1H, d*, *J* 3.5 Hz, fucose H-4), 3.78 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.85 (1H, dd, *J* 7.5, 5.5 Hz, amino-Ala H-2), 3.88 (1H, B of $ABX_2$, *J* 10.0, 7.0 Hz, 1H of hexyl H-1), 4.26 (1H, dd, *J* 8.5, 5.5 Hz, Glu H-2), 4.36 (1H, d, *J* 8.0 Hz, fucose H-1), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($D_2O$, 75.5 MHz, MeOH = 49.0 ppm) 15.54 (fucose C-6), 24.76, 25.86, 27.91, 28.27, 28.80, and 33.61 (Gln C-3 and C-4, + hexyl C-2 to C-5), 39.46 and 40.03 (amino-Ala C-3 and hexyl C-6), 52.02 and 54.28 (amino-Ala C-2 and Glu C-2), 70.63 (hexyl C-1), 70.66, 71.00, 71.51, 73.13 (fucose C-2, C-3, C-4, and C-5), 102.79 (fucose C-1), 173.09 and 173.39 (amino-Ala C-1 and Glu C-1), 181.38 (Glu C-5); HRMS (FAB) 479.270 30, calculated for $[MH^+]$ ($C_{20}H_{39}N_4O_9^+$) 479.271 70; HPLC (YMC; 0−20% (40 min) B in C, pH 7.0), 26.5 min, 99%.

**1-β-[6-([2,6-*N*,*N*′-Di((*R*)-alanyl-(*S*)-glutaminyl)-(*S*)-lysyl]-amino)hex-1-yl]-L-fucopyranose, 35:** mp 107−110 °C; $^1$H NMR ($CD_3OD$ + $D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.29 (6H, d, *J* 7.0 Hz, 2 × Ala H-3), 1.31−1.43 (6H, m, Lys H-4 and hexyl H-3 and H-4), 1.45−1.55 (4H, m, Lys H-5 and hexyl H-5), 1.60 (2H, tt, *J* 6.5, 6.5 Hz, hexyl H-2), 1.64−1.83 (2H, m, Lys H-3), 1.86−2.15 (4H, m, 2 × Gln H-3), 2.30 (2H, t, *J* 7.5 Hz, Gln H-4), 2.32 (2H, t, *J* 7.5 Hz, Gln H-4), 3.09−3.25 (2H, m, hexyl H-6), 3.19 (2H, t, *J* 7.0 Hz, Lys H-6), 3.45 (1H, dd, *J* 10.0, 7.5 Hz, fucose H-2), 3.47−3.56 (4H, m, 2 × Ala H-2, fucose H-3 and 1H of hexyl H-1), 3.62 (1H, d*, *J* 3.0 Hz, fucose H-4), 3.64 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.20 (1H, d, *J* 7.5 Hz, fucose H-1), 4.25 (1H, dd, *J* 9.0, 5.5 Hz, Gln H-2, Gln′ H-2 or Lys H-2), 4.28 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2, Gln′ H-2 or Lys H-2), 4.32 (1H, dd, *J* 8.0, 6.0 Hz, Gln H-2, Gln′ H-2 or Lys H-2), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD$ + $D_2O$, 75.5 MHz) 16.69 (fucose C-6), 21.21 (2 × Ala C-3), 23.97 (Lys C-4), 26.51 and 27.51 (hexyl C-3 and C-4), 28.78 and 28.94 (Lys C-5 and 2 × Gln C-3), 29.57, 30.03, and 32.46 (Lys C-3 and hexyl C-2 and C-5), 32.46 (2 × Gln C-4), 40.03 and 40.29

(Lys C-6 and hexyl C-6), 51.38, 54.40, and 54.90 (Lys C-2, 2 × Ala C-2 and 2 × Gln C-2), 70.90 (hexyl C-1), 71.89, 72.22, 73.01, and 75.01 (fucose C-2, C-3, C-4, and C-5), 104.71 (fucose C-1), 174.05 and 174.37 (2 × Gln C-5), 178.22, 178.91 and 179.10 (Lys C-1, 2 × Ala C-1 and 2 × Gln C-1); HRMS (FAB) 790.464 400, calculated for $[MH^+]$ ($C_{34}H_{64}N_9O_{12}^+$) 790.467 444; HPLC (Hamilton; 0−40% (50 min) B in C, pH 8.35), 22.8 min, 95%.

**1-β-[6-([(*R*)-Alanyl-(*S*)-glutaminyl-5-*N*-[6-(β-L-fucopyranosyl)oxyhexyl]-(*S*)-glutaminyl]-amino)hex-1-yl]-L-fucopyranose, 36:** mp 125−128 °C; $^1$H NMR ($CD_3OD$ + $D_2O$, 300 MHz) 1.25 (6H, d, *J* 6.5 Hz, 2 × fucose H-6), 1.28−1.42 (8H, m, 2 × [hexyl H-3 and H-4]), 1.45−1.55 (4H, m, 2 × hexyl H-5), 1.49 (3H, d, *J* 7.0 Hz, Ala H-3), 1.61 (4H, tt, *J* 7.0, 7.0 Hz, 2 × hexyl H-2), 1.90−2.20 (4H, m, 2 × Gln H-3), 2.22−2.38 (4H, m, 2 × Gln H-4), 3.10−3.23 (2H, m, hexyl H-6), 3.15 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.45 (2H, dd, *J* 10.0, 7.5 Hz, 2 × fucose H-2), 3.52 (2H, dd, *J* 10.0, 3.0 Hz, 2 × fucose H-3), 3.51−3.59 (2H, m, 1H of each hexyl H-1's), 3.64 (2H, d*, *J* 3.0 Hz, 2 × fucose H-4), 3.66 (2H, q*, *J* 6.5 Hz, 2 × fucose H-5), 3.84 (2H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of each hexyl H-1's), 4.01 (1H, q, *J* 7.0 Hz, Ala H-2), 4.23 (2H, d, *J* 7.5 Hz, 2 × fucose H-1), 4.23−4.28 (1H, m, Gln H-2), 4.30 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD$ + $D_2O$, 75.5 MHz) 16.68 (2C, 2 × fucose C-6), 18.15 (Ala C-3), 26.39, 26.45, 27.40, and 27.50 (2 × [hexyl C-3 and C-4]), 28.42 and 28.97 (2 × Gln C-3), 29.89, 29.95, and 30.38 [2C] (2 × [hexyl C-2 and C-5]), 32.49 and 33.31 (2 × Gln C-4), 40.35 and 40.43 (2 × hexyl C-6), 50.50 (Ala C-2), 54.71 and 54.92 (2 × Gln C-2), 70.97 (2C, 2 × hexyl C-1), 71.90 [2C], 72.14 [2C], 72.89, 72.93 and 74.91 [2C] (2 × [fucose C-2, C-3, C-4, and C-5]), 104.61 (2C, 2 × fucose C-1), 173.41, 173.87, 174.05, 175.42 and 178.22 (Ala C-1, 2 × Gln C-1 and 2 × Gln C-5); HRMS (FAB) 837.478 400, calculated for $[MH^+]$ ($C_{37}H_{69}N_6O_{15}^+$) 837.482 0 91; HPLC (Hamilton; 0−50% (60 min) B in C, pH 8.5), 27.3 min, 95%.

**1-β-[6-[6-(Dimethylamino)purin-9-yl]hexyl]-L-fucopyranose, 37:**[16] mixture of anomers (α:β = 0.15:0.85); mp 141−145 °C; $\nu_{max}$ 214.2 and 276.0 nm ($\epsilon_1/\epsilon_2$ = 1.1); $^1$H NMR ($CD_3OD$ + $D_2O$, 300 MHz, *Me*OH = 3.35 ppm) β-anomer 1.23 (3H, d, *J* 6.5 Hz, fucose H-6), 1.26−1.35 (2H, m, hexyl H-4), 1.36−1.47 (2h, m, hexyl H-3), 1.53−1.61 (2H, m, hexyl H-2), 1.86 (2H, tt, *J* 7.0, 7.0 Hz, hexyl H-5), 3.37−3.53 (9H, m, fucose H-2 and H-3, 1H of hexyl H-1, and $NMe_2$), 3.61 (1H, d*, *J* 3.5 Hz, fucose H-4), 3.61 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.80 (1H, B of $ABX_2$, *J* 10.0, 6.5 Hz, 1H of hexyl H-1), 4.17 (1H, d, *J* 7.5 Hz, fucose H-1), 4.21 (2H, t, *J* 7.0 Hz, linker H-6), 8.04 (1H, s, purine H-8), 8.19 (1H, s, purine H-2), *fucose $J_{H4-H5}$ unresolved. For α-anomer (where different) 1.16 (d, *J* 6.5 Hz, fucose H-6), 4.76 (d, *J* 2.0 Hz, fucose H-1); $^{13}$C NMR ($CD_3OD$ + $D_2O$, 75.5 MHz) β-anomer 16.72 (fucose C-6), 26.29, 27.12, 30.32, and 30.73 (hexyl C-2 to C-5), 39.41 ($NMe_2$), 44.82 (hexyl C-6), 70.71 (hexyl C-1), 71.84, 72.12, 72.88, and 74.91 (fucose C-2, C-3, C-4, and C-5), 104.56 (fucose C-1), 120.61 (purine C-5), 141.36 (purine C-8), 150.90 (purine C-4), 152.76 (purine C-2), 155.88 (purine C-6). For α-anomer (where different) 16.58 (fucose C-6), 26.60, 27.18, 30.19, and 30.52 (hexyl C-2 and C-5), 67.50, 69.74, 71.46, and 73.39 (fucose C-2, C-3, C-4, and C-5), 69.08 (hexyl C-1), 100.19 (fucose C-1); HRMS (FAB) 410.239 50, calculated for $[MH^+]$ ($C_{19}H_{32}N_5O_5^+$) 410.240 36; HPLC (Hamilton; 50−80% (40 min) A in D, pH 7.85), 23.9 min, 99%.

**1-β-[6-((*S*)-Pyroglutamylamino)hex-1-yl]-L-fucopyranose, 40:** mp 91−92 °C (softens 81 °C); $^1$H NMR ($CD_3OD$ + $D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.30−1.46 (4H, m, hexyl H-3 and H-4), 1.47−1.56 (2H, m, hexyl H-5), 1.56−1.67 (2H, m, hexyl H-2), 1.97−2.08 (1H, m, 1H of pyro-Glu H-3), 2.28−2.53 (3H, m, pyro-Glu H-4 and 1H of pyro-Glu H-3), 3.13−3.27 (2H, m, hexyl H-6), 3.44 (1H, dd, *J* 10.0, 7.5 Hz, fucose H-2), 3.52 (1H, dd, *J* 10.0, 3.0 Hz, fucose H-3), 3.55 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.64 (1H, dd, *J* 3.0, 1.0 Hz, fucose H-4), 3.66 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.84 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.20 (1H, dd, *J* 9.5, 5.5 Hz, pyro-Glu H-2), 4.22 (1H, d, *J* 7.5 Hz, fucose H-1); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 16.77 (fucose C-6), 26.71, 26.84, 27.64, 30.23, 30.52, and 30.64 (pyro-Glu C-3 and C-4, + hexyl C-2 to C-5), 40.36 (hexyl C-6), 58.24 (pyro-Glu C-2), 70.53 (hexyl C-1), 71.80, 72.29, 73.00, and 75.14 (fucose C-2, C-3, C-4, and C-5), 104.77 (fucose C-1), 174.75 and 181.51 (pyro-Glu C-1 and C-5); HRMS (FAB) 375.215 90, calculated for $[MH^+]$ ($C_{17}H_{31}N_2O_7^+$) 375.213 13; HPLC (Hamilton; 0−40% (50 min) B in C, pH 8.5), 18.1 min, 100%.

**1-β-[6-((*N*-Methyl-(*S*)-pyroglutamyl)amino)hex-1-yl]-L-fucopyranose, 41:** mp 143−145 °C; $^1$H NMR ($D_2O$, 300 MHz, MeCN = 2.05 ppm) 1.24 (3H, d, *J* 6.5 Hz, fucose H-6), 1.30−1.41 (4H, m, hexyl H-3 and H-4), 1.50−1.56 (2H, m, hexyl H-5), 1.58−1.63 (2H, m, hexyl H-2), 1.90−2.01 (1H, m, 1H of pyro-Glu H-3), 2.32−2.58 (3H, m, pyro-Glu H-4 and 1H of pyro-Glu H-3), 2.75 (3H, s, NMe), 3.24 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.44 (1H, dd, *J* 9.5, 8.0 Hz, fucose H-2), 3.61 (1H, dd, *J* 10.0, 3.5 Hz, fucose H-3), 3.63 (1H, A of $ABX_2$, *J* 10.0, 7.0 Hz, 1H of hexyl H-1), 3.72 (1H, d*, *J* 3.5 Hz, fucose H-4), 3.76 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.87 (1H, B of $ABX_2$, *J* 10.0, 7.0 Hz, 1H of hexyl H-1), 4.23 (1H, dd, *J* 9.0, 5.0 Hz, pyro-Glu H-2), 4.34 (1H, d, *J* 8.0 Hz, fucose H-1), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD$ + $D_2O$, 75.5 MHz) 16.75 (fucose C-6), 23.94, 26.51, 27.55, 30.06, 30.50, and 30.71 (pyro-Glu C-3 and C-4 + hexyl C-2 to C-5), 29.03 (NMe), 40.41 (hexyl C-6), 64.56 (pyro-Glu C-2), 70.80 (hexyl C-1), 71.85, 72.16, 72.90, and 74.96 (fucose C-2, C-3, C-4, and C-5), 104.60 (fucose C-1), 173.62 and 178.79 (pyro-Glu C-1 and C-5); HRMS (FAB) 389.229 50, calculated for $[MH^+]$ ($C_{18}H_{33}N_2O_7^+$) 389.228 79; HPLC (Hamilton; 10−60% (60 min) A in C, pH 7.85), 28.2 min, 96%.

**1-β-[6-((*R*)-Alanylglycylamino)hex-1-yl]-L-fucopyranose, 42:** mixture of anomers (α:β = 1:4). mp 52−54 °C; $^1$H NMR ($CD_3OD$ + $D_2O$, 300 MHz) β-anomer 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.28 (3H, d, *J* 7.0 Hz, Ala H-3), 1.31−1.42 (4H, m, hexyl H-3 and H-4), 1.47−1.54 (2H, m, hexyl H-5), 1.56−1.66 (2H, m, hexyl H-2), 3.19 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.44 (1H, dd, *J* 9.5, 7.5 Hz, fucose H-2), 3.48 (1H, q, *J* 7.0 Hz, Ala H-2), 3.49 (1H, dd, *J* 9.5, 3.0 Hz, fucose H-3), 3.53 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.62 (1H, dd, *J* 3.0, 1.0 Hz, fucose H-4), 3.63 (1H, qd, *J* 6.5, 1.0 Hz, fucose H-5), 3.80 (1H, A of AB q, *J* 16.5 Hz, 1H of Gly H-2), 3.83 (1H, B of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.88 (1H, B of AB q, *J* 16.5 Hz, 1H of Gly H-2), 4.20 (1H, d, *J* 7.5 Hz, fucose H-1). For α-anomer (where different) 1.20 (d, *J* 6.5 Hz, fucose H-6), 4.75 (d, *J* 2.5 Hz, fucose H-1); $^{13}$C NMR ($CD_3OD$ + $D_2O$, 75.5 MHz) β-anomer 16.66 (fucose C-6), 21.06 (Ala C-3), 26.30, 27.31, 29.84, and 30.26 (hexyl C-2 to C-5), 40.35 (hexyl C-6), 43.35 (Gly C-2), 51.33 (Ala C-2), 71.04 (hexyl C-1), 71.83, 71.95, 72.75, and 74.68 (fucose C-2, C-3, C-4, and C-5), 104.35 (fucose C-1), 171.63 and 179.29 (Ala C-1 and Gly C-1). For α-anomer (where different) 16.54 (fucose C-6), 26.61 and 30.13 (hexyl C-2 and C-3), 67.53, 69.54, 71.26, 73.24 (fucose C-2, C-3, C-4, and C-5), 69.27 (hexyl C-1), 99.98 (fucose C-1); HRMS (FAB) 392.238 50, calculated for $[MH^+]$ ($C_{17}H_{34}N_3O_7^+$) 392.239 69; HPLC (Hamilton; 0−50% (60 min) B in D, pH 7.7), 17.7 min, 98%.

**1-β-[6-((*R*)-Alanyl-(*S*)-ornithylamino)hex-1-yl]-L-fucopyranose, 43:** mp 82−86 °C; $^1$H NMR ($CD_3OD$ + $D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.27 (3H, d, *J* 6.5 Hz, Ala H-3), 1.31−1.41 (4H, m, hexyl H-3 and H-4), 1.47−1.55 (2H, m, hexyl H-5), 1.56−1.75 (5H, m, hexyl H-2, Orn H-4 and 1H of Orn H-3), 1.77−1.89 (1H, m, 1H of Orn H-3), 2.76 (2H, t, *J* 7.0 Hz, Orn H-5), 3.18 (2H, t, *J* 6.5 Hz, hexyl H-6), 3.44 (1H, dd, *J* 10.0, 7.0 Hz, fucose H-2), 3.49 (1H, q, *J* 6.5 Hz, Ala H-2), 3.49 (1H, dd, *J* 10.0, 3.5 Hz, fucose H-3), 3.53 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.62 (1H, d*, *J* 3.0 Hz, fucose H-4), 3.63 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 4.20 (1H, d, *J* 7.0 Hz, fucose H-1), 4.27 (1H, dd, *J* 8.5, 5.5 Hz, Orn H-2), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD$ + $D_2O$, 75.5 MHz) 15.94 (fucose C-6), 20.59 (Ala C-3), 26.22, 26.25, 28.69, 29.15, and 29.21 (Orn C-3 and C-4, + hexyl C-2 to C-5 [one peak unresolved]), 39.76 and 39.95. (hexyl C-6 and Orn C-5), 50.39 and 54.22 (Ala C-2 and Orn C-2), 71.05 (hexyl C-1), 71.09, 71.42, 71.94, and 73.60 (fucose C-2, C-3, C-4, and C-5), 103.31 (fucose C-1), 174.29 and

179.53 (Ala C-1 and Orn C-1); HRMS (FAB) 449.296 70, calculated for $[MH^+]$ ($C_{20}H_{41}N_4O_7^+$) 449.297 52.

**1-*β*-[6-((*R*)-Alanyl-(*S*)-asparaginylamino)hex-1-yl]-L-fucopyranose, 44:** mp 100−103 °C; $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.28 (3H, d, *J* 7.0 Hz, Ala H-3), 1.30−1.43 (4H, m, hexyl H-3 and H-4), 1.49 (2H, tt, *J* 7.0, 7.0 Hz, hexyl H-5), 1.61 (2H, tt, *J* 7.0, 7.0 Hz, hexyl H-2), 2.56−2.74 (2H, m, Asn H-3), 3.11−3.19 (2H, m, hexyl H-6), 3.44−3.54 (1H, m, Ala H-2), 3.45 (1H, dd, *J* 10.0, 7.0 Hz, fucose H-2), 3.49 (1H, dd, *J* 10.0, 3.0 Hz, fucose H-3), 3.53 (1H, A of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 3.62 (1H, d*, *J* 3.0 Hz, fucose H-4), 3.64 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.20 (1H, d, *J* 7.0 Hz, fucose H-1), 4.71 (1H, dd, *J* 8.0, 5.5 Hz, Asn H-2), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD + D_2O$, 75.5 MHz) 16.57 (fucose C-6), 21.03 (Ala C-3), 26.21 and 27.28 (hexyl C-3 and C-4), 29.76 and 30.20 (hexyl C-2 and C-5), 36.56 (Asn C-3), 40.40 (hexyl C-6), 51.20 and 51.41 (Ala C-2 and Asn C-2), 71.10 (hexyl C-1), 71.83, 71.96, 72.75, and 74.69 (fucose C-2, C-3, C-4, and C-5), 104.42 (fucose C-1), 172.32 (Asn C-4), 176.16 and 178.68 (Ala C-1 and Asn C-1); HRMS (FAB) 449.262 50, calculated for $[MH^+]$ ($C_{19}H_{37}N_4O_8^+$) 449.261 14; HPLC (Hamilton; 0−50% (60 min) A in C, pH 7.9), 38.2 min, 94%.

**1-*β*-[6-((*S*)-Alanyl-(*S*)-prolylamino)hex-1-yl]-L-fucopyranose, 45:** mp 66−70 °C; $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.27 (3H, d, *J* 7.0 Hz, Ala H-3), 1.31−1.44 (4H, m, hexyl H-3 and H-4), 1.50 (2H, tt, *J* 7.0, 7.0 Hz, hexyl H-5), 1.62 (2H, tt, *J* 7.0, 7.0 Hz, hexyl H-2), 1.85−1.93 and 1.94−2.01 (2H, 2 × m, Pro H-4), 2.02−2.11 (1H, m, 1H of Pro H-3), 2.20 (1H, A of $ABX_2Y$, *J* 12.0, 7.5, 7.5 Hz, 1H of Pro H-3), 3.12 (1H, A of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.21 (1H, B of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.45 (1H, dd, *J* 9.5, 7.5 Hz, fucose H-2), 3.50 (1H, dd, *J* 9.5, 3.0 Hz, fucose H-3), 3.54 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.59−3.65 (1H, m, Ala H-2), 3.63 (1H, d*, *J* 3.0 Hz, fucose H-4), 3.64 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.66−3.77 (2H, m, Pro H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.20 (1H, d, *J* 7.5 Hz, fucose H-1), 4.36 (1H, dd, *J* 8.5, 5.0 Hz, Pro H-2), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD + D_2O$, 75.5 MHz) 16.66 (fucose C-6), 19.94 (Ala C-3), 25.80 and 26.40 (hexyl C-3 and C-4), 27.40 (Pro C-4), 29.97, 30.39, and 30.62 (Pro C-3 and hexyl C-2 and C-5), 40.24 (hexyl C-6), 48.39 (Pro C-5), 61.41 and 61.73 (Ala C-2 and Pro C-2), 70.97 (hexyl C-1), 71.87, 72.10, 72.90, and 74.89 (fucose C-2, C-3, C-4, and C-5), 104.61 (fucose C-1), 174.73 and 176.34 (Ala C-1 and Pro C-1)); HRMS (FAB) 432.269 20, calculated for $[MH^+]$ ($C_{20}H_{38}N_3O_7^+$) 432.270 97; HPLC (Hamilton; 0−40% (50 min) B in D, pH 7.8), 24.2 min, 99%.

**1-*β*-[6-((*S*)-Isoleucyl-(*S*)-prolylamino)hex-1-yl]-L-fucopyranose, 46:** mp 58−61 °C; $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 0.91 (3H, t, *J* 7.5 Hz, Ile H-5), 0.97 (3H, d, *J* 6.5 Hz, Ile H-4′), 1.08−1.21 (1H, m, 1H of Ile H-4), 1.25 (3H, d, *J* 6.5 Hz, fucose H-6), 1.31−1.44 (4H, m, hexyl H-3 and H-4), 1.50 (2H, tt, *J* 7.0, 7.0 Hz, hexyl H-5), 1.57−1.71 (4H, m, hexyl H-2, Ile H-3 and 1H of Ile H-4), 1.86−2.00 (2H, m, Pro H-4), 2.02−2.11 and 2.12−2.23 (2H, 2 × m, Pro H-3), 3.13 (1H, A of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.20 (1H, B of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.43−3.49 (3H, m, Ile H-2 and fucose H-2 and H-3), 3.53 (1H, A of $ABX_2$, *J* 9.5, 6.5 Hz, 1H of hexyl H-1), 3.59−3.67 (1H, m, 1H of Pro H-5), 3.61 (1H, d*, *J* 2.5 Hz, fucose H-4), 3.64 (1H, q*, *J* 6.5 Hz, fucose H-5), 3.71 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of Pro H-5), 3.83 (1H, B of $ABX_2$, *J* 9.5, 7.0 Hz, 1H of hexyl H-1), 4.19 (1H, d, *J* 6.5 Hz, fucose H-1), 4.38 (1H, dd, *J* 8.0, 5.0 Hz, Pro H-2), *fucose $J_{H4-H5}$ unresolved; $^{13}$C NMR ($CD_3OD + D_2O$, 75.5 MHz) 11.61 (Ile C-5), 16.04 (Ile C-4′), 16.69 (fucose C-6), 24.67 (Ile C-4), 25.92, 26.55 and 27.53 (Pro C-4 and hexyl C-3 and C-4), 30.15 and 30.52 [2C] (Pro C-3 and hexyl C-2 and C-5), 39.71 (Ile C-3), 40.27 (hexyl C-6), 48.88 (Pro C-5), 58.20 and 61.76 (Ile C-2 and Pro C-2), 70.87 (hexyl C-1), 71.87, 72.20, 72.96, and 75.03 (fucose C-2, C-3, C-4, and C-5), 104.73 (fucose C-1), 174.55 and 175.54 (Ile C-1 and Pro C-1); HRMS (FAB) 474.316 50, calculated for $[MH^+]$ ($C_{23}H_{44}N_3O_7^+$) 474.317 93; HPLC (Hamilton; 5−35% (40 min) B in D, pH 7.8), 25.4 min, 99%.

**2-*O*-Acetyl-1-*β*-[6-((*S*)-alanyl-(*S*)-glutaminylamino)hex-1-yl]-D-mannopyranose, 47:** $^1$H NMR ($CD_3OD$, 300 MHz) 1.27 (3H, d, *J* 7.0 Hz, Ala H-3), 1.30−1.42 (4H, m, hexyl H-3 and H-4), 1.45−1.57 (4H, m, hexyl H-2 and H-5), 1.62 (3H, s, OAc), 1.84−1.98 and 2.02−2.11 (2H, 2 × m, Gln H-3), 2.26−2.31 (2H, m, Gln H-4), 3.15−3.20 (2H, m, hexyl H-6), 3.22 (1H, ddd, *J* 9.5, 6.0, 2.5 Hz, mannose H-5), 3.45 (1H, q, *J* 7.0 Hz, Ala H-2), 3.50−3.57 (2H, m, hexyl H-1), 3.55 (1H, dd, *J* 9.5, 9.5 Hz, mannose H-4), 3.65 (1H, A of ABX, *J* 12.0, 6.0 Hz, 1H of mannose H-6), 3.70 (1H, dd, *J* 9.5, 4.0 Hz, mannose H-3), 3.84 (1H, B of ABX, *J* 12.0, 2.5 Hz, 1H of mannose H-6), 4.32 (1H, dd, *J* 9.0, 5.5 Hz, Gln H-2), 4.43 (1H, dd, *J* 4.0, 2.5 Hz, mannose H-2), 5.43 (1H, d, *J* 2.5 Hz, H-1); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 21.38 (Ala C-3), 25.83 (*Me*CO), 26.83, 27.56, 29.32, 30.26, 30.50, and 32.50 (Gln C-3 and C-4 and hexyl C-2, C-3, C-4, and C-5), 40.35 (hexyl H-6), 51.55 and 54.10 (Ala C-2 and Gln C-2), 62.83 (hexyl C-1), 68.55, 73.30, 76.93, and 81.18 (mannose C-2, C-3, C-4, and C-5), 99.08 (mannose C-1), 124.84 (mannose C-6), 173.51, 173.59, 177.27, and 178.30 (Me*C*O, Ala C-1, Gln C-1 and Gln C-5); HRMS (FAB) *m*/*z* 521.284 00, calculated for MH+, ($C_{22}H_{41}N_4O_{10}^+$) 521.282 29; HPLC (Hamilton; 10−50% (50 min) A in C, pH 8.5), 23.7 min, 100%.

**2-*O*-Acetyl-1-*β*-[6-(*N*-acetyl-(*R*)-Alanyl-(*S*)-glutaminylamino)hex-1-yl]-D-mannopyranose, 48:** $^1$H NMR ($CD_3OD$, 300 MHz) 1.26−1.42 (4H, m, hexyl H-3 and H-4), 1.34 (3H, d, *J* 7.0 Hz, Ala H-3), 1.44−1.57 (4H, m, hexyl H-2 and H-5), 1.62 (3H, s, OAc), 1.84−1.97 and 2.09−2.20 (2H, 2 × m, Gln H-3), 1.98 (3H, s, NAc), 2.23−2.31 (2H, m, Gln H-4), 3.15 (1H, A of $ABX_2$, *J* 11.5, 7.0 Hz, 1H of hexyl H-6), 3.20 (1H, B of $ABX_2$, *J* 11.5, 7.0 Hz, 1H of hexyl H-6), 3.22 (1H, ddd, *J* 9.5, 6.0, 2.5 Hz, mannose H-5), 3.47−3.56 (2H, m, hexyl H-1), 3.55 (1H, dd, *J* 9.5, 9.5 Hz, mannose H-4), 3.65 (1H, A of ABX, *J* 12.0, 6.0 Hz, 1H of mannose H-6), 3.70 (1H, dd, *J* 9.5, 4.0 Hz, mannose H-3), 3.84 (1H, B of ABX, *J* 12.0, 2.5 Hz, 1H of mannose H-6), 4.22 (1H, q, *J* 7.0 Hz, Ala H-2), 4.26 (1H, dd, *J* 9.5, 4.5 Hz, Gln H-2), 4.43 (1H, dd, *J* 4.0, 2.5 Hz, mannose H-2), 5.43 (1H, d, *J* 2.5 Hz, H-1); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 17.47 (NCO*Me*), 22.45 (Ala C-3), 25.84 (OCO*Me*), 26.84, 27.50, 28.49, 30.19, 30.47, and 32.65 (Gln C-3 and C-4 and hexyl C-2, C-3, C-4, and C-5), 40.41 (hexyl H-6), 51.16 and 54.50 (Ala C-2 and Gln C-2), 62.80 (hexyl C-1), 68.52, 73.26, 76.88, and 81.15 (mannose C-2, C-3, C-4, and C-5), 99.04 (mannose C-1), 124.80 (mannose C-6), 173.40, 173.53, 175.59, and 177.75 (2 × Me*C*O, Ala C-1, Gln C-1 and Gln C-5); HRMS (FAB) *m*/*z* 585.2767 00, calculated for $MNa^+$ ($C_{24}H_{42}N_4NaO_{11}^+$) 585.274 778; HPLC (Hamilton; 0−40% (50 min) B in C, pH 8.5), 18.7 min, 98%.

**2-*O*-Acetyl-1-*β*-[6-(*S*)-glutaminylamino)hex-1-yl]-D-mannopyranose, acetate salt, 49:** $^1$H NMR ($CD_3OD + D_2O$, 300 MHz) 1.28−1.42 (4H, m, hexyl H-3 and H-4), 1.46−1.58 (4H, m, hexyl H-2 and H-5), 1.64 (3H, s, OAc), 1.91 (3H, s, $AcO^-$), 1.97−2.14 (2H, m, Gln H-3), 2.37 (2H, t, *J* 7.5 Hz, Gln H-4), 3.13−3.31 (4H, m, hexyl H-6, mannose H-5 and Gln H-2), 3.50−3.58 (2H, m, hexyl H-1), 3.58 (1H, dd, *J* 9.5, 9.5 Hz, mannose H-4), 3.67 (1H, A of ABX, *J* 12.0, 6.0 Hz, 1H of mannose H-6), 3.76 (1H, dd, *J* 9.5, 4.0 Hz, mannose H-3), 3.84 (1H, B of ABX, *J* 12.0, 2.5 Hz, 1H of mannose H-6), 4.46 (1H, dd, *J* 4.0, 2.5 Hz, mannose H-2), 5.46 (1H, d, *J* 2.5 Hz, H-1); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 25.05 and 25.49 (*Me*$CO_2^-$ and OCO*Me*), 26.54, 27.35, 29.21, 29.85, 30.17, and 31.77 (Gln C-3 and C-4 and hexyl C-2, C-3, C-4, and C-5), 40.52 (hexyl H-6), 58.19 (Gln C-2), 62.38 (hexyl C-1), 68.20, 72.66, 76.53, and 80.83 (mannose C-2, C-3, C-4, and C-5), 98.81 (mannose C-1), 124.68 (mannose C-6), 174.61, 174.94, 177.54, and 180.35 (2 × Me*C*O, Gln C-1 and Gln C-5); HRMS (FAB) *m*/*z* 450.244 00, calculated for $MH^+$ ($C_{19}H_{36}N_3O_9^+$) 450.245 15; HPLC (Hamilton; 0−40% (50 min) B in C, pH 8.5), 22.6 min, 97%.

**2-*O*-Acetyl-1-*β*-[6-(*S*)-pyroglutamylamino)hex-1-yl]-D-mannopyranose, 50:** mp 53−55 °C; $^1$H NMR ($CD_3OD$, 300 MHz) 1.30−1.43 (4H, m, hexyl H-3 and H-4), 1.47−1.59 (4H, m, hexyl H-2 and H-5), 1.62 (3H, s, OAc), 1.97−2.08 (1H, m, 1H of pyro-Glu H-3), 2.23−2.51 (3H, m, pyro-Glu H-4 and 1H of pyro-Glu H-3), 3.20 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.21 (1H, ddd, *J* 9.5, 6.0, 2.5 Hz, mannose H-5), 3.50−3.57 (2H, m, hexyl H-1), 3.55 (1H, dd, *J* 9.5, 9.5 Hz, mannose H-4), 3.65 (1H, A of

ABX, *J* 12.0, 6.0 Hz, 1H of mannose H-6), 3.69 (1H, dd, *J* 9.5, 4.0 Hz, mannose H-3), 3.84 (1H, B of ABX, *J* 12.0, 2.5 Hz, 1H of mannose H-6), 4.14 (1H, dd, *J* 8.5, 5.0 Hz, pyro-Glu H-2), 4.42 (1H, dd, *J* 4.0, 2.5 Hz, mannose H-2), 5.43 (1H, d, *J* 2.5 Hz, mannose H-1); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 25.86 (OCO*Me*), 26.86, 26.88, 27.51, 30.27, 30.46, and 30.53 (pyro-Glu C-3 and C-4 + hexyl C-2 to C-5), 40.38 (hexyl H-6), 58.28 (pyro-Glu C-2), 62.78 (hexyl C-1), 68.52, 73.30, 76.92, and 81.20 (mannose C-2, C-3, C-4, and C-5), 99.07 (mannose C-1), 124.85 (mannose C-6), 174.80 and 181.56 (Me*C*O and pyro-Glu C-1 and C-5); HRMS (FAB) *m*/*z* 455.201 70, calculated for $MNa^+$ ($C_{19}H_{32}N_2NaO_9^+$) 455.200 56; HPLC (Hamilton; 0−40% (50 min) B in C, pH 8.5), 19.5 min, 100%.

**2-*O*-acetyl-1-*β*-[6-([3*R*, 8*S*]-1,4-diaza-3-methyl-2,5-dioxocyclooctan-8-yl]-carbonylamino]hexyl-D-mannopyranose, 51:** mp 136−139 °C; $^1$H NMR ($CD_3OD$, 300 MHz) 1.30−1.42 (4H, m, hexyl H-3 and H-4), 1.41 (3H, d, *J* 7.0 Hz, cyclooctyl C(3)Me), 1.43−1.57 (4H, m, hexyl H-2 and H-5), 1.62 (3H, s, OAc), 2.04−2.13 (2H, m, cyclooctyl H-7), 2.22−2.33 (2H, m, cyclooctyl H-6), 3.15 (2H, t, *J* 7.5 Hz, hexyl H-6), 3.18−3.25 (1H, m, mannose H-5), 3.51 (1H, A of $ABX_2$, *J* 9.0, 6.0 Hz, 1H of hexyl H-1), 3.55 (1H, B of $ABX_2$, *J* 9.0, 6.5 Hz, 1H of hexyl H-1), 3.55 (*sic*) (1H, dd, *J* 9.5, 9.5 Hz, mannose H-4), 3.65 (1H, A of ABX, *J* 12.0, 6.0 Hz, 1H of mannose H-6), 3.69 (1H, dd, *J* 9.5, 4.0 Hz, mannose H-3), 3.84 (1H, B of ABX, *J* 12.0, 2.5 Hz, 1H of mannose H-6), 4.01 (1H, td, *J* 5.5, 1.0 Hz, cyclooctyl H-8), 4.06 (1H, qd, *J* 7.0, 1.0 Hz, cyclooctyl H-3), 4.43 (1H, dd, *J* 4.0, 2.5 Hz, mannose H-2), 5.42 (1H, d, *J* 2.5 Hz, mannose H-1); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 19.21 (cyclooctyl C(3)*Me*), 25.85 (*Me*CO), 26.87, 27.60, 30.30, 30.42, 30.49, and 32.16 (cyclooctyl C-6 and C-7 and hexyl C-2, C-3, C-4, and C-5), 40.42 (hexyl H-6), 51.42 and 55.78 (cyclooctyl C-3 and C-8), 62.84 (hexyl C-1), 68.57, 73.34, 76.95, and 81.22 (mannose C-2, C-3, C-4, and C-5), 99.11 (mannose C-1), 124.29 (mannose C-6), 174.23, 174.58, 178.87, and 178.95 (Me*C*O, *C*ONHCH$_2$, cyclooctyl C-2 and C-5); HRMS (FAB) *m*/*z* 526.240 500, calculated for $MNa^+$ ($C_{22}H_{37}N_3NaO_{10}^+$) 526.237 665; HPLC (Hamilton; 0−40% (50 min) B in C, pH 8.5), 19.5 min, 89%.

**6-((*S*)-Alanyl-(*S*)-glutaminylamino)hexan-1-ol, 52:** mp 104−106 °C; $^1$H NMR ($CD_3OD$, 300 MHz) 1.28 (3H, d, *J* 7.0 Hz, Ala H-3), 1.30−1.42 (4H, m, hexyl H-3 and H-4), 1.45−1.59 (4H, m, hexyl H-2 and H-5), 1.85−1.97 and 2.01−2.12 (2H, 2 × m, Gln H-3), 2.25−2.32 (2H, m, Gln H-4), 3.18 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.46 (1H, q, *J* 7.0 Hz, Ala H-2), 3.53 (2H, t, *J* 6.5 Hz, hexyl H-1), 4.31 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 21.26 (Ala C-3), 26.59, 27.74, 29.31, 30.33, 32.50, and 33.53 (Gln C-3 and C-4 and hexyl C-2, C-3, C-4, and C-5), 40.36 (hexyl H-6), 51.50 and 54.06 (Ala C-2 and Gln C-2), 62.84 (hexyl H-1), 173.51 (Gln C-5), 177.70 and 177.97 (Ala C-1 and Glu C-1); HRMS (FAB) *m*/*z* 317.217 90, calculated for $MH^+$ ($C_{14}H_{29}N_4O_4^+$) 317.218 87; HPLC (Hamilton; 0−40% (50 min) B in C, pH 8.5), 14.9 min, 96%.

**6-((*S*)-Alanyl-(*R*)-glutaminylamino)hexan-1-ol, 53:** mp 84−87 °C; $^1$H NMR ($CD_3OD$, 300 MHz) 1.31 (3H, d, *J* 7.0 Hz, Ala H-3), 1.34−1.40 (4H, m, hexyl H-3 and H-4), 1.48−1.54 (4H, m, hexyl H-2 and H-5), 1.87−1.96 and 2.03−2.12 (2H, 2 × m, Gln H-3), 2.28 (2H, t, *J* 8.0 Hz, Gln H-4), 3.18 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.51−3.57 (1H, m, Ala H-2), 3.53 (2H, t, *J* 6.5 Hz, hexyl H-1), 4.30 (1H, dd, *J* 9.0, 5.0 Hz, Gln H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 20.76 (Ala C-3), 25.54 and 27.69 (hexyl C-3 and C-4), 29.13, 30.29, 32.50, and 33.48 (Gln C-3 and C-4 and hexyl C-2 and C-5), 40.38 (hexyl C-6), 51.34 and 54.30 (Ala C-2 and Gln C-2), 62.86 (hexyl C-1), 173.79 (Gln C-5), 177.68 and 177.87 (Ala C-1 and Gln C-1); HRMS (FAB) *m*/*z* 317.217 50, calculated for $MH^+$ ($C_{14}H_{29}N_4O_4^+$) 317.218 87; HPLC (Hamilton; 0−50% (60 min) A in D, pH 7.9), 29.8 min, 99%.

**6-((*S*)-Glutaminylamino)hexan-1-ol, 54:** mp 89−92 °C; $^1$H NMR ($CD_3OD$, 300 MHz) 1.30−1.43 (4H, m, hexyl H-3 and H-4), 1.46−1.58 (4H, m, hexyl H-2 and H-5), 1.73−1.83 and 1.85−2.01 (2H, 2 × m, Gln H-3), 2.27 (2H, dd, *J* 8.0, 7.5 Hz, Gln H-4), 3.13−3.24 (2H, m, hexyl H-6), 3.27−3.35 (1H, m, Gln H-2), 3.54 (2H, t, *J* 6.5 Hz, hexyl H-1); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 26.60, 27.79, 30.37, 32.39, 32.65, and 33.51 (Gln C-3 and C-4, and hexyl C-2 to C-5), 40.26 (hexyl C-6), 55.61 (Gln H-2), 62.82 (hexyl C-1), 175.15 (Gln C-5), 178.11 (Gln C-1); HRMS (FAB) 246.181 80, calculated for $[MH^+]$ ($C_{11}H_{24}N_3O_3^+$) 246.181 76; HPLC (Hamilton; 10−50% (50 min) A in C, pH 8.5), 9.9 min, 100%.

**6-((*S*)-Pyroglutaminylamino)hexan-1-ol, 55:** mp 170.5−171.5 °C; $^1$H NMR ($CD_3OD$, 300 MHz) 1.31−1.43 (4H, m, hexyl H-3 and H-4), 1.46−1.58 (4H, m, hexyl H-2 and H-5), 1.97−2.08 (1H, m, 1H of pyro-Glu H-3), 2.23−2.51 (3H, m, pyro Glu H-4 and 1H of pyro-Glu H-3), 3.20 (2H, t, *J* 7.0 Hz, hexyl H-6), 3.54 (2H, t, *J* 6.5 Hz, hexyl H-1), 4.14 (1H, dd, *J* 8.5, 5.0 Hz, pyro-Glu H-2); $^{13}$C NMR ($CD_3OD$ + $D_2O$, 75.5 MHz) 26.48, 26.79, 27.63, 30.18, 30.50, and 33.33 (pyro-Glu C-3 and C-4, and hexyl C-2 to C-5), 40.43 (hexyl C-6), 58.26 (pyro-Glu H-2), 62.80 (hexyl C-1), 174.87 and 181.88 (2 × CONH); HRMS (FAB) 229.154 00, calculated for $[MH^+]$ ($C_{11}H_{21}N_2O_3^+$) 229.155 21; HPLC (Hamilton; 10−40% (40 min) A in C, pH 6.0), 15.7 min, 100%.

**6-((*S*)-Alanyl-(*S*)-prolylamino)hexan-1-ol, 56:** $^1$H NMR ($CD_3OD$, 400 MHz) 1.27 (3H, d, *J* 7.0 Hz, Ala H-3), 1.32−1.44 (4H, m, hexyl H-3 and H-4), 1.47−1.59 (4H, m, hexyl H-2 and H-5), 1.86−2.01 (2H, m, Pro H-4), 2.02−2.22 (2H, m, Pro H-3), 3.10−3.26 (2H, m, hexyl H-6), 3.54 (2H, t, *J* 6.5 Hz, hexyl H-1), 3.60−3.75 (2H, m, Pro H-5), 4.37 (1H, dd, *J* 8.0, 4.5 Hz, Pro H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 20.30 (Ala C-3), 25.89, 26.52, 27.63, 30.31, 30.65, and 33.51 (Pro C-3 and C-4, + hexyl C-2 to C-5), 40.24 (hexyl C-6), 48.26 (Pro C-5), 61.42 and 61.67 (Ala C-2 and Pro C-2), 62.84 (hexyl C-1), 174.55 and 176.48 (Ala C-1 and Pro C-1); HRMS (FAB) 286.214 30, calculated for $[MH^+]$ ($C_{14}H_{28}N_3O_3^+$) 286.213 07; HPLC (Hamilton; 0−40% (50 min) B in D, pH 7.8), 21.9 min, 97%.

**6-((*S*)-Isoleucyl-(*S*)-prolylamino)hexan-1-ol, 57:** $^1$H NMR ($CD_3OD$, 300 MHz) 0.93 (3H, t, *J* 7.5 Hz, Ile H-5), 1.00 (3H, d, *J* 7.0 Hz, Ile H-4′), 1.10−1.21 (1H, m, 1H of Ile H-4), 1.32−1.40 (4H, m, hexyl H-3 and H-4), 1.48−1.57 (4H, m, hexyl H-2 and H-5), 1.58−1.66 (1H, m, 1H of Ile H-4), 1.67−1.75 (1H, m, Ile H-3), 1.85−1.99 (2H, m, Pro H-4), 2.02−2.12 and 2.13−2.20 (2H, 2 × m, Pro H-3), 3.14 (1H, A of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.19 (1H, B of $ABX_2$, *J* 13.5, 7.0 Hz, 1H of hexyl H-6), 3.52 (1H, d, *J* 6.0 Hz, Ile H-2), 3.54 (2H, t, *J* 6.5 Hz, hexyl H-1), 3.62 (1H, A of $ABX_2$, *J* 10.0, 6.5 Hz, 1H of Pro H-5), 3.71 (1H, B of $ABX_2$, *J* 10.0, 6.5 Hz, 1H of Pro H-5), 4.38 (1H, dd, *J* 8.0, 3.0 Hz, Pro H-2); $^{13}$C NMR ($CD_3OD$, 75.5 MHz) 11.61 (Ile C-5), 16.04 (Ile C-4′), 24.78 (Ile C-4), 25.98 and 26.57 (hexyl C-3 and C-4), 27.68 (Pro C-4), 30.38 and 30.59 (hexyl C-2 and C-5), 33.52 (Pro C-3), 40.02 and 40.29 (hexyl C-6 and Ile C-3), 48.80 (Pro C-4), 58.30 (Ile C-2), 61.71 (Pro C-2), 62.89 (hexyl C-1), 174.51 and 175.70 (Ile C-1 and Pro C-1); HRMS (FAB) 328.258 80, calculated for $[MH^+]$ ($C_{17}H_{34}N_3O_3^+$) 328.260 01; HPLC (Hamilton; 30−70% (50 min) A in D, pH 7.9), 31.7 min, 99%.

**Biology. NK cell activity Assay:** Splenocyte cell suspensions were prepared by homogenization of spleens from 6−8-week old female C57BL/6 (H-2$^b$) in tissue culture medium [RPMI 1640 (Gibco, Burlington, Canada) supplemented with 10% fetal calf serum (Hyclone, Logan, USA) and 2 mM L-glutamine]. The homogenate was centrifuged on a density gradient (Lympholyte M, Cedarlane, Hornby, Canada) (800*g*, 20 min), and the mononuclear leukocytes were collected, washed three times in phosphate buffer saline (PBS), and resuspended in RPMI for evaluation of cell viability by the use of trypan blue. The assay was performed as follows:

NK cell activity was assayed by incubation of $5 \times 10^5$ splenocytes for 4 h with $5 \times 10^3$ sodium chromate labeled ($^{51}CrO_4$; Amersham, Oakville, Canada) NK-sensitive YAC-1 cells. After the incubation, chromium in the lysate was quantified and the percent specific release or lysis calculated using the expression

$$\% \text{ specific lysis} = \frac{(ER - SR)}{(TR - SR)} \times 100$$

where ER = experimental release of $^{51}$Cr, ET = total release of $^{51}$Cr, and SR = spontaneous release of $^{51}$Cr.

Due to variability between individual experiments, data is compared to the positive control; human interleukin 2 (IL 2, 15 ng/mL, R and D Systems, Minneapolis, MN) and presented in a semiquantitative (+) format. Therefore, the percent specific lysis induced by IL 2 in each experiment is taken as 100% or the maximum stimulation. As such, 0−20% activation of NK cells relative to IL 2 is assigned 0; 21−40%, +; 41−60%, ++; 61−80%, +++; 81−100%, ++++; and 100% and more, +++++.

**Immunophenotyping Assay.** Female, 6−8-week old, C57BL/6 mice were injected intraperitoneally for four consecutive days with BCH-2537 at different concentrations. Mice were sacrificed on day five by cardiac puncture. Gross pathological observations were recorded at the end of the experiment. Blood and spleens were collected and the cell suspension was prepared and lysed in ACK buffer (155 mM $NH_4Cl$, 12 mM $NaHCO_3$, 0.1 mM EDTA, pH 7.3) for 5 min. The cells were washed three times in phosphate buffered saline, pH 7.4 (PBS), and resuspended in tissue culture medium. The cells were then incubated for 45 min on ice with fluorescein (FITC) or phycoerythrin (PE) conjugated anti-cell surface marker monoclonal antibodies according to the manufacturer's (Gibco/BRL, Cedarlane, Boehringer Mannheim) recommendation. The cells were then washed in PBS, fixed with 1% paraformaldehyde, and analyzed with a Coulter XL flow cytometer. Analysis of the cell subsets was undertaken by determination of standard cells surface markers which were as follows; CD3 (T-cells), TCR (T-cell receptor), CD4 (T helper), CD8 (T cytotoxic/suppressor), CD45 (tyrosine phosphatase; activation marker), CD11b (macrophage), NK (NK cells), and Ly5 (B-cells).

**Antitumor Experiment.** Female 6−8-week old C57BL/6 mice were injected intravenously on day zero with $2.5 \times 10^5$ B16F10 melanoma cells from DCTDC Tumor Repository, NCI, Frederick, MD (source of cell culture, Dr. I. J. Fidler). Animals were then injected intraperitoneally with BCH-2537 according to the treatment schedule described in Table 8.

**Statistical Analysis.** The immunophenotyping data are presented as ± standard error. The means are compared using an unpaired student's *t* test. The differences are considered significant when $P \leq 0.05$.

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